were obtained with benzene and dioxane. Ethanol, glacial acetic acid and acetonitrile were better and only occasionally showed a tendency to form a precipitate. Methanol, dimethylformamide and excess pyridine all were free from this objectionable feature.

Analytical Methods Used.—In the absence of sulfur dioxide, analyses for strong acidity as hydriodic or sulfuric acid were obtained by titration with standard 0.5 *N* sodium hydroxide to a phenolphthalein end-point. The samples were then titrated back to the brom phenol blue end-point with 0.5 *N* hydrochloric acid to obtain a measure of pyridine. This titrimetric procedure was found by control experiments to be reproducible within ±0.2% and to account for at least 99% of the pyridine and inorganic acid added. Sulfate was determined by Raschig's benzidine method[15] and iodide argentimetrically using fluorescein as an adsorption indicator,[16] in both cases removing most of the pyridine by making alkaline to phenolphthalein with sodium hydroxide and boiling for fifteen to twenty minutes before proceeding with the analysis. Free and "periodide" iodine was obtained by thiosulfate titration after acidification to a *p*H of 4. In the presence of sulfur dioxide, the sample was boiled for ten to fifteen minutes with a known excess of standard hydrochloric or sulfuric acid, depending on whether sulfate or iodide was being determined. This modified analytical scheme was applicable to spent Fischer reagent. The alkali and acid titrations of mixtures containing free or "periodide" iodine were made successfully after destroying the iodine with sodium thiosulfate. The presence of this salt and its reaction product, sodium tetrathionate, did not interfere with the double titration procedure for strong acids and pyridine. When analyzing for sulfate and iodide ions, hydrazine hydrate in neutral or slightly alkaline solution was used instead of sodium thiosulfate to reduce iodine.

Summary

1. The stoichiometric relations and mechanism of the reaction of water with Karl Fischer reagent have been investigated.

2. Crystalline intermediates and products of the reaction have been identified by optical crystallographic methods.

3. The nature of a parasitic reaction affecting the stability of the reagent has been clarified.

4. The effect of substituting other amines for pyridine and other solvents for methanol has been investigated.

WILMINGTON, DELAWARE RECEIVED JULY 11, 1939

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(15) Treadwell-Hall, "Analytical Chemistry," 8th ed., John Wiley and Sons, Inc., New York, N. Y., 1935, Vol. II, p. 660.

(16) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1936, p. 542.

[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Electro-optical Effect in Certain Viruses

BY MAX A. LAUFFER

Introduction

Kerr[1] was the first to observe that many substances become doubly refracting when placed in a strong electrical field, behaving like uniaxial crystals with optic axes in the direction of the field. As a result of the studies of Raman and Sirkar[2] and of Kitchin and Mueller,[3] it is now known that this effect in homogeneous liquids is due to the orientation of molecules. Colloidal solutions containing rod-like or plate-like particles, such as those of vanadium pentoxide, benzopurpurin, ferric oxide, and bentonite, also show the Kerr effect. It is thought that this is due to the orientation of the anisometric colloidal particles. However, the studies of Errera, Overbeek, and Sack[4] on vanadium pentoxide and benzopurpurin sols and those of Mueller[5] and of Norton[6] on bentonite sols show that the phenomenon in colloids is rather complex and not as yet encompassed by a single simple theory.

Bawden and associates[7] first reported that the particles of tobacco mosaic virus can be oriented by an electric field, *i. e.*, that the material exhibits electrical double refraction. This double refraction was reported recently by the author[8] to be positive with respect to the direction of the electric field. However, more detailed studies have substantiated this observation only in part, for it has been found that under some circumstances the electrical double refraction shown by the viruses may be negative. It is the purpose of this communication to describe further studies on the electrical double refraction of tobacco mosaic virus, the closely related aucuba mosaic of tomato virus, and latent mosaic of potato virus.

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(1) Kerr, *Phil. Mag.*, [4] **50**, 337, 446 (1875).

(2) Raman and Sirkar, *Nature*, **121**, 794 (1928).

(3) Kitchin and Mueller, *Phys. Rev.*, **32**, 979 (1928).

(4) Errera, Overbeek, and Sack, *J. chim. phys.*, **32**, 681 (1935).

(5) Mueller, *Phys. Rev.*, **55**, 508, 792 (1939).

(6) Norton, *ibid.*, **55**, 668 (1939).

(7) Bawden, Pirie, Bernal and Fankuchen, *Nature*, **138**, 1051 (1936).

(8) Lauffer and Stanley, *Chem. Rev.*, **24**, 303 (1939).

Description of the Method and the Materials.—The apparatus used for measuring double refraction resembled somewhat that described by Kunitz.[9] It consisted of a polarizing microscope equipped with a Babinet compensator and external analyzer. A mercury quartz lamp provided with filters which transmit only the mercury 5461 Å. green band was used as the source of illumination. The Kerr cell consisted of a flat-bottomed glass tube 2.5 cm. deep and 1.5 cm. in diameter containing 2 platinum electrodes 5 mm. wide and long enough to reach nearly to the bottom of the tube. In order to facilitate mounting onto the stage of the microscope, the glass tube was fitted into a hollow hard rubber cylindrical housing. The electrodes were firmly held 3 mm. apart by a second housing, which acted as a cap for the first, and were thus permitted to dip into the solution in the glass tube. As a source of potential, 120-v., 60-cycle alternating current was used, and fractional parts of this voltage were obtained by employing the principle of the potentiometer. The voltages actually applied across the terminals of the Kerr cell were measured with a Weston a. c. voltmeter. During the course of actual measurements, the light from the polarizer passed between the platinum electrodes of the Kerr cell in such a manner that the direction of the electric field made an angle of 45° with the plane of polarization of the incident light. With an experimental arrangement of the type described, good comparative data may be obtained on systems showing as much double refraction as that shown by reasonably concentrated tobacco mosaic virus solutions, but no great precision may be claimed for the absolute magnitudes of the quantities measured.

The viruses used in this study were purified by ultracentrifugation and then dissolved in distilled water. Preparation A of tobacco mosaic virus was stored for about two months in the cold at about *p*H 6.5. Preparation B was dialyzed against distilled water and stored for about six months at about *p*H 5. It was brought to neutrality before using. The aucuba mosaic virus was stored for about four months at about *p*H 6.5, and the latent mosaic virus for two months at about *p*H 6.7.[10]

Presentation and Discussion of Results.—The electrical double refraction shown by each of the 3 viruses was determined for electric fields ranging from 0 to 400 root mean square volts per cm. In the case of tobacco mosaic and aucuba mosaic viruses, these measurements were carried out for various concentrations of virus and at acidities near neutrality and near the isoelectric zones. The data are presented in Figs. 1–6. In these figures, field strengths in root mean square volts per cm. or concentration of virus in milligrams per milliliter are plotted as abscissas and displacements in wave lengths per cm. of solution as ordinates. The latter is equal to the quotient of the double refraction divided by the wave length of the light used. An inspection of Fig. 1 reveals that in relatively concentrated solutions of tobacco mosaic virus (preparation A) the electrical double refraction is negative for the lower values of the field strength, but reverses in sign and finally appears to approach a maximum for higher values of the field strength. By simply diluting the solution, the negative effect gradually disappears, until in the more dilute solutions the double refraction appears to be positive for all values of the field strength. Preparation B of tobacco mosaic virus, which had been allowed to age for six months instead of two months as in the case of A, behaved in a manner very similar to that just described, except that the transition points from positive to negative double refraction were more than twice those of the first preparation. However, as may be seen in Fig. 2, the maximum values of the positive double refraction approached by both preparations are approximately proportional to the virus concentration and are the same on a concentration basis. As is revealed by Figs. 3 and 4, the unusual type of reversal found for tobacco mosaic virus is common to all 3 of the viruses examined.

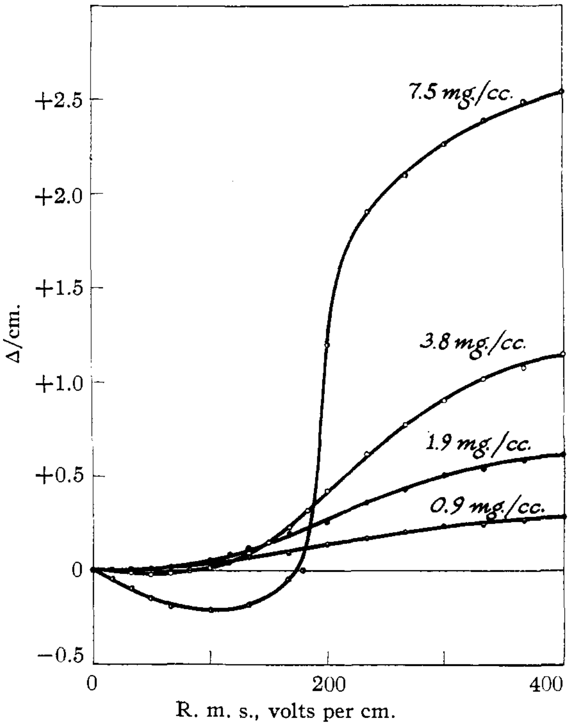

Fig. 1.—The double refraction of several concentrations of tobacco mosaic virus (preparation A) at *p*H 6.4 ± 0.2, plotted as a function of the field strength.

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[9] Kunitz, *J. Gen. Phys.*, **13**, 565 (1930). The author wishes to acknowledge his gratitutde to Dr. Kunitz for making much of the apparatus described in this publication available to him.

[10] The author wishes to acknowledge his gratitude to Dr. Loring for supplying the latent mosaic virus protein.

In a superficial way, at least, this phenomenon resembles the magnetic double refraction of ferric oxide sols, known as the Majorana effect, which has been described and studied by Cotton and Mouton.[11] However, the electrical double refraction of the viruses is perhaps more closely related to the behavior of bentonite sols as described by Mueller[5] and by Norton.[6] Norton found that the electrical double refraction of a 1% bentonite sol varied in sign and magnitude with the frequency of alternation of the electric field, it being negative for frequencies up to 630 cycles and positive at higher frequencies. Mueller extended this observation by demonstrating that, at a given frequency, a 1% bentonite sol may show negative double refraction, but that as the sol is diluted the double refraction decreases to zero and finally becomes positive. The transition point varies with the frequency. These observations were made in relatively weak electric fields. It may be seen from Fig. 1 that in fields weaker than 100 volts per cm., the phenomenon here reported seems to be identical with that just described for bentonite sols.

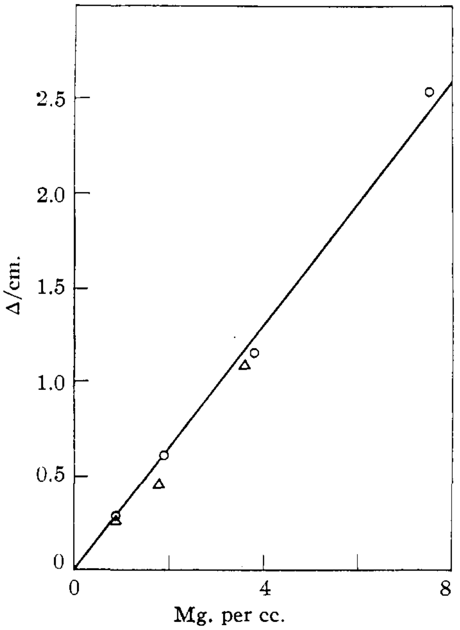

Fig. 2.—The electrical double refraction of tobacco mosaic virus in an electric field of 400 r. m. s. volts/cm., plotted as a function of the concentration of virus: ○, preparation A; △, preparation B.

A considerable body of physical evidence, recently reviewed,[12] shows that the particles of tobacco mosaic virus are rod-shaped bodies about 12–15 mμ in thickness and about 400–500 mμ in length. Recent studies with the electron microscope[13] substantiate the conclusions concerning the shape and thickness of the particles and indicate a probable length of about 300 mμ. The amount of double refraction of flow shown by all three of the viruses here studied is about the same,[14] indicating that the conclusions concerning the physical nature of the tobacco mosaic virus probably apply, in general, to the other two. The double refraction of flow of tobacco mosaic virus is always positive, and it has been shown by the author to be due to the orientation of rod-shaped particles which are themselves isotropic or nearly so.[15] In view of these facts, the elec-

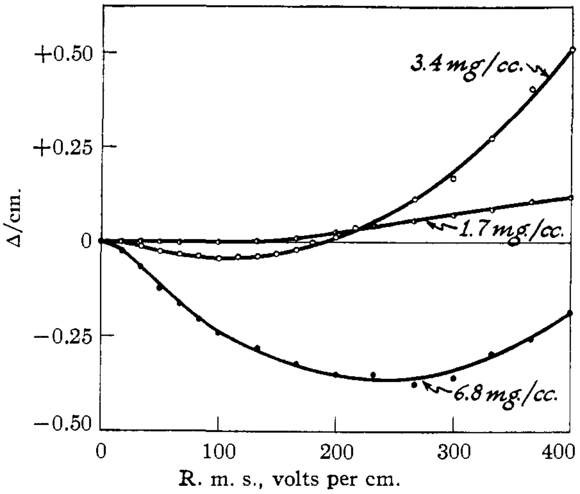

Fig. 3.—The double refraction of several concentrations of aucuba mosaic virus at pH 6.7 ± 0.1, plotted as a function of field strength.

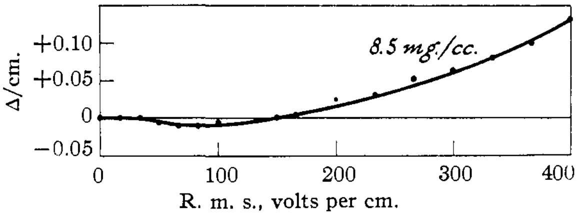

Fig. 4.—The double refraction of latent mosaic virus (concentration of 8.5 mg./cc.) at pH 6.7, plotted as a function of field strength.

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[11] Cotton and Mouton, *Ann. chim. phys.*, [8] **11**, 145, 289 (1907).

[12] Lauffer and Stanley, ref. 8. For recent papers, see also Kausche, Guggisberg, and Wissler, *Naturwissenschaften*, **27**, 303 (1939), and Robinson, *Proc. Roy. Soc.* (London), **A170**, 519 (1939); *Nature*, **143**, 923 (1939).

[13] Kausche, Pfankuch and Ruska, *Naturwissenschaften*, **27** 292 (1939).

[14] Lauffer and Stanley, *J. Biol. Chem.*, **123**, 507 (1938).

[15] Lauffer, *J. Phys. Chem.*, **42**, 935 (1938).

trical double refraction of these materials would seem to be due to the orientation of rod-shaped particles, parallel to the electrical field for the case of positive birefringence, and perpendicular to the field for the case of negative birefringence.

It would seem reasonable to assume that the positive orientation of the virus particles is due, at least in part, to a dipole moment in the direction of the long axis of the rods. In such a case, in sufficiently weak fields, the positive double refraction should be proportional to the second power of the field strength. In order to explain the negative double refraction at low field strengths, it is necessary to assume further that opposing the positive orienting tendency is some negative orienting tendency which is greater than the positive one for weak fields, but which increases less rapidly than the positive effect as the field strength is increased, and which is thereby finally overtaken by the positive effect. This condition would be satisfied if the negative double refraction varied with approximately the first power of the field strength. This would suggest some type of mechanical force, perhaps one associated with the electrokinetic properties of the viruses. However, whatever the nature of the orienting tendencies may be, by postulating a negative tendency proportional to the first power of the field strength which is opposed by a positive tendency proportional to the second power of the field strength, it is possible to explain the existence of negative double refraction in weak fields and positive double refraction in strong fields. In fact, except for the higher values of the field strength, many of the data obtained in this study can be described moderately well by equations of the form, $y = Ax^2 - Bx$. However, the change in inversion point with change of concentration of the virus solutions still remains unexplained. Hence, a consideration of other properties of virus solutions which depend upon concentration becomes necessary.

At concentrations of tobacco virus somewhat greater than the highest shown in Fig. 1, the material is spontaneously doubly refracting, *i. e.*, the particles spontaneously orient themselves parallel to each other in small doubly refracting elements of volume.[16] This means that between the particles there exist forces of attraction which should vary in some manner inversely with the interparticle distance.[17] If the positive orientation of the viruses is due largely to a permanent dipole moment in the direction of the long axis of the particles, it would have to follow the alternations of the field. In such a case, forces of attraction between the particles and actual aggregation of particles should tend to increase the relaxation time of positive orientation. From the estimates of the rotational diffusion constant of tobacco mosaic virus obtained by Mehl,[18] it follows that the critical frequency for the virus is of the same order of magnitude as the frequency here employed. Under such circumstances, an increase in relaxation time will result in a decrease in average positive orientation for a given orienting force. If we assume further that the negative orientation does not follow the alternations of the field, as it should not if it were due to mechanical causes, the negative birefringence should not be diminished by forces of attraction between particles. Hence, the net result of imposing interparticle attraction upon the system should be an apparent increase in the negative double refraction and an inversion point at a higher field strength, all of which would be due mainly to the decrease of the positive birefringence. Dilution of the solution, which results in a decrease of the interparticle attraction, should result in an apparent decrease in the negative effect and a corresponding lowering of the inversion point. As Fig. 1 shows, this is consistent with the experimental observations.

There is considerable evidence[8] which indicates that when a solution of tobacco mosaic virus is acidified to *p*H 5 there is an end to end aggregation of rod-like particles. This has the effect of decreasing the ratio of mean interparticle distance to particle length and should, therefore, result in more interaction between particles. As a matter of fact, changing a relatively concentrated sol from neutrality to *p*H 5 causes it to acquire the properties of a gel. The particles in an acidified solution, then, should have an increased relaxation time and should, therefore, show a retarded positive orientation. By comparing Fig. 5 with Fig. 1, it may be seen that bringing the acidity of a solution of tobacco mosaic virus from *p*H 6.4 to 5.1 results in a general increase of the negative effect and in an inversion point at a

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[16] Bawden and Pirie, *Proc. Roy. Soc.* (London), **B123**, 274 (1937). See also refs. 7 and 15.

[17] For a theoretical treatment of this question, see Langmuir, *J. Chem. Phys.*, **6**, 873 (1938).

[18] Mehl, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 218 (1938).

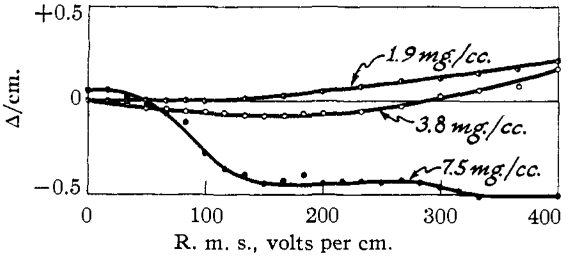

Fig. 5.—The double refraction of several concentrations of tobacco mosaic virus (preparation A) at pH 5.1 ± 0.1, plotted as a function of field strength.

higher field strength, just as could have been predicted from the foregoing considerations. By comparing Figs. 3 and 6, it may be seen that, in general, the aucuba mosaic virus responds to the addition of acid in a similar manner. The effect on the inversion point of aging of tobacco mosaic virus at pH 5, already described, may be accounted for in a similar manner, for it is known that upon long standing under such conditions the virus undergoes partially irreversible linear aggregation.

The point of view just outlined could be subjected to further tests by studying the phenomenon in viruses with alternating fields of various frequencies. If it is correct, raising the frequency should tend to result in an apparent increase of the negative effect for weak fields and in an inversion point at a higher field strength. It is recognized that many obstacles remain in the path of the complete acceptance of the proposed interpretation of the electrical double refraction of the viruses. Nevertheless, it is hoped that the observations and suggestions herein presented eventually may prove of use in understanding the nature of the electro-optical effect in colloidal solutions.

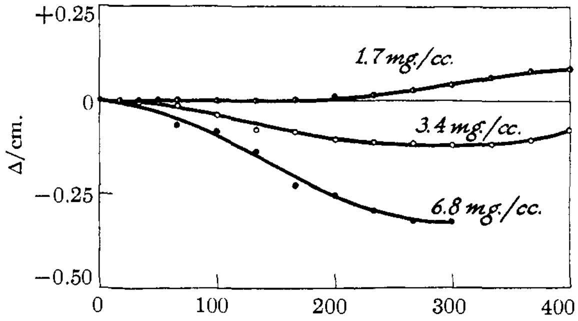

Fig. 6.—The double refraction of several concentrations of aucuba mosaic virus at pH 5.2 ± 0.1, plotted as a function of field strength.

Summary

It has been found that solutions of tobacco mosaic virus, aucuba mosaic virus, and potato latent mosaic virus exhibit the Kerr electro-optical effect. In dilute solutions in an alternating electric field of 60 cycles, the double refraction of tobacco mosaic virus appears to be positive for all values of the field strength, but for more concentrated solutions the effect becomes negative in weak electric fields, passes through an inversion point for stronger fields, becomes positive for still stronger fields, and finally attains a maximum value. The inversion point occurs at higher field strengths in more concentrated solutions. By bringing the virus nearer its isoelectric point, the negative effect is generally increased and the inversion point is shifted to stronger fields. Aging the virus has a similar effect. In an attempt to explain these observations, it was suggested that two opposing forces control the orientation of the particles. One of these may be a negative orienting effect which varies with the first power of the field strength and which does not follow the alternations of the field. Opposing it and eventually overcoming it in strong fields, there was assumed to be a positive orienting effect, perhaps due to a permanent dipole moment, an effect which varies with the second power of the field strength, but which does follow the alternations of the field, and which is, therefore, affected by interparticle attraction.

PRINCETON, N. J.

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