TABLE I (*Continued*)

Bands common to several of the compounds

All of the chlorophylls, but not pheo.-a: 1190–1195, 1070, 792–796

All compounds except bacteriochlorophyll: 1590–1620[a], 1545, 1350–1355, 985–995

All compounds except chlorophyll-b: 780–782

All compounds except allomerized chlorophyll-a: 832–835

Chl.-a and pheo.-a: 1560, 1495 (chl.-a) to 1505 (pheo.-a), 1310, 910[b]

Chl.- and allom chl.-a: 1330, 1245–1265, 855, 743[a]

Chl.-b and bact. chl.: 945, 885

Pheo.-a and bact. chl.: 896, 683

Chl.-a, chl.-b and pheo.-a: 1228, 1205–1210, 1060, 763 (in pheo.-a. at 770)

Chl.-a. chl.-b and allo. chl.-a: 1610[a]

Chl.-a, chl.-b and bact. chl.: 1525, 925[b]

Chl.-b, pheo.-a and bact. chl.: 752–755[d]

Bands unique to single compounds

Chl.a: 902, 800, 705[c]

Chl.-b: 1155, 700[c]

Pheo.-a: 3400; 1620 and 1590 (these may be due to split of 1610[a]); 1365, 1095, 736, 708[c]

Bact. chl.: 694,[c] 652

Allom. chl.-a: 1105, 1015, 918[b], 808, 703[c]

[a] Chl.-a, chl.-b and allomerized chl.-a have a band at 1610, which in pheo.-a appears to be split to 1590 and 1620. [b] Chl.-a has a doublet at 910 and 925; 910 is shared with pheo.-a; 925 appears also in chl.-b and bact. chl.; allom. chl.-a has a band in between, at 918; all these appear to be related. [c] These appear to be closely related: 694–708. [d] These appear to be closely related: 743–755. [e] All these bands were also found in ethyl chlorophyllide, except for 1110–30 and 720, which may have been obscured by the oil.

TABLE II

TENTATIVE BAND ASSIGNMENTS

Frequencies in cm.$^{-1}$

1740 ester groups at C_7 and C_{10}[a]

1700 ketone in ring V

1660 phytol C=C[b]

1610 semi-isolated, or possibly vinyl, C=C[c]

1460–1470, 1445 C–H bending (mainly phytol)

1380 C–CH_3 stretching (phytol)

1105 C–OCH_3 at C_{10}

985–995 vinyl C–H bending (at C_2)

780–782 methyl C–H rock (at C_3)

736 N–H rocking

[a] Although both phytol spectra show a strong band at 1730–45 cm.$^{-1}$, this apparently is due to an impurity, such as a phytol ester. [b] For some reason, relatively weak in pheo.-a, bact. chl. and allom. chl.-a; in phytol at 1675-80. [c] Note that in pheophytin-a this band appears to be split to 1590 and 1620; this might be evidence for two isomers of pheophytin having semi-isolated double bonds in different positions.

could be attributed to the N–Mg–N oscillation. Either the absorption due to this very interesting vibration is too weak to be observed, or else it lies at longer wave lengths.

Acknowledgments.—The authors are greatly indebted to Professor B. L. Crawford, Jr., (Univ. of Minnesota) for the use of the infrared spectrophotometer and to Mr. B. Zaslow for operating it, to Mr. James Johnson (Univ. of Minnesota) for a contribution of *R. rubrum*, to Professor Farrington Daniels (Univ. of Wisconsin) for a sample of ethyl chlorophyllide, and to Professor Paul Rothemund (Antioch College) for a sample and a spectrum of phytol.

MINNEAPOLIS, MINN.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

The Phototropy of Chlorophyll in Fluid Solutions[1,2]

BY ROBERT LIVINGSTON AND VICTOR A. RYAN

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Oxygen-free chlorophyll solutions in methanol undergo reversible color changes when they are illuminated. Steady illumination (at an "intensity" of about 5×10^{19} quanta absorbed per liter per second) produced the following changes (measured with bands isolated by interference filters). For chlorophyll-b, the absorption increased at λ 4030 and 5245 Å. and decreased at λ 4680, 5880 and 6450 Å. For chlorophyll-a, the absorption increased at λ 4680, 5020 and 5245 Å. and decreased at λ 4030, 4395, 5880 and 6450 Å. Similar solutions, illuminated with a flash of high intensity and short duration, undergo color changes whose temporal course can be followed by photographing a transient trace on an oscilloscope. Using scanning light of λ 4680, 4705 or 4775 Å. and a 2×10^{-5} *m* chlorophyll-b solution, a partial bleaching, which had a half-life of about 5×10^{-4} sec., was observed. When the wave length of the scanning light was λ 5245 Å., an increase in absorption was observed. The decay of this change appears to follow a rate law different from that which governs the disappearance of the bleaching observed with λ 4680 Å., etc. A similar, although less marked, phototropic response was exhibited by a chlorophyll-a solution, scanned with λ 5245 Å. Oxygen inhibits both the flash and the steady-state phototropic changes. These results are consistent with the following postulates. In addition to the short-lived, singlet fluorescent state, excited chlorophyll can exist in a metastable state (perhaps the lowest triplet state) and as a radical. The radical is formed by the reaction of the metastable molecule with a molecule of the solvent. Under steady, relatively weak, illumination only the radical and normal chlorophyll are present in detectable amounts. Both the radical and the metastable molecule are formed (in comparable concentrations) when the solution is illuminated with a flash of high intensity. The metastable molecules disappear by reacting with normal chlorophyll molecules (or possibly by a simple, first-order decay), while the radicals reform normal chlorophyll molecules by a second-order reaction.

Solutions of chlorophyll, in air-free methanol or similar solvents, undergo a reversible color change when they are illuminated. In pure methanol at ordinary temperatures, the original color of the chlorophyll returns so rapidly when the light is extinguished, that its rate of return cannot be determined by those methods which have been previously used to study the

(1) This work was made possible by the support of the Office of Naval Research (NR 051,028, Contract N6ori-212, T.O. I) to which the authors are indebted.

(2) This paper is based in part upon a dissertation submitted by V. Ryan to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1951.

effect.[3] In an attempt to determine the rate of decay of the color change, the present experiments were performed with an apparatus which had a resolving time of about 10^{-4} second. In addition, crude absorption spectra of the bleached forms of chlorophylls-a and -b, which are present under conditions of steady illumination, were determined by the use of interference filters.

Experimental Methods

A. Experiments Made with Flash Illumination.—The disappearance of the "bleached form" was observed by allowing light transmitted through the chlorophyll solution to fall on a photomultiplier, whose output was fed through a d.c. amplifier to an oscilliscope. The actinic light was furnished by three photographer's multiflash lamps, which were fired simultaneously.

Figure 1 is a simplified diagram of the mechanical arrangement. The chlorophyll solution was contained in a cylindrical cell (a), 1 cm. in diameter, fitted with plane ends 8 cm. apart. This cell was connected by a side tube to a standard Thunberg tube, through which the cell was filled and degassed. The cell was surrounded by two concentric glass cylinders. Water from a thermostat was pumped through the inner cylindrical jacket (b). The outer jacket (c) was filled with a color-filter solution or with water. Three flash lamps (d) were placed symmetrically around the chlorophyll cell. The whole assembly was contained in a highly-polished, chromium plated drum (e).

The source of the scanning light was a 6 volt, 100-watt projection lamp (f) provided with a collimating lens (g) and an interference filter (h). After passing through the chlorophyll cell, the light beam was focused upon the cathode of a photomultiplier tube (i), which had been selected (for its favorable signal-to-noise ratio) from a group of fifty 931-A tubes.

The output of the photomultiplier tube, after suitable d.c. amplification, was impressed upon the vertical deflection plates of an oscilloscope. The vertical displacement of the oscilloscope trace was calibrated in terms of the change of intensity of the incident light, using a series of neutral gray filters of known transmissivity. The high frequency response was checked with the aid of a General Radio Strobotac 631B. The time scale was established using a Hewlett–Packard audio oscillator. Under the conditions of use, there was a time lag, in the response to a signal, not greater than 0.074 millisecond and a random error in the indicated time less than ±4%.[4]

The actinic light was furnished by three GE FT-403 multiflash lamps. They were flashed by means of a Photogenic Machine Co. Type M6 power supply unit, which is rated to discharge a total of 390 watt-seconds through the three tubes. The tubes were fired simultaneously but independently by Thordarson ignition transformers. By the use of a spring-driven sequence switch, the oscilloscope sweep was started about 10^{-4} second before the flash lamps were fired. The sweep circuit was usually adjusted to move the beam across the screen in approximately 2.5 milliseconds. The oscilloscope (transient) trace was recorded photographically.

The methods of purification of the chlorophylls and of methanol and of the preparation of the deaerated chlorophyll solutions were similar to those described by Knight and Livingston.[3d] After each series of experiments, with a given solution and filter, air was admitted to the cell and a series of measurements were made with the solution saturated with air. In the following discussion these experiments are referred to as "oxygen blanks."

B. Steady-state Experiments.—In the steady-state experiments, the multiflash lamps were replaced by a 1000 watt incandescent lamp, provided with a Corning glass color filter No. 3-66, and situated in a water-cooled housing. This lamp was placed as close as was practicable to the cell containing the chlorophyll solution. To measure the relatively slight changes in transmissivity produced by the comparatively weak steady illumination from the incandescent lamp, it was necessary to increase the effective amplification of the photomultiplier–oscilloscope system. This was possible since rapid response was not requisite to the steady-state measurements, and was accomplished by a suitable modification of the circuit and by an increase in intensity of the scanning light. The scanning light was confined to narrow spectral bands by means of interference filters.

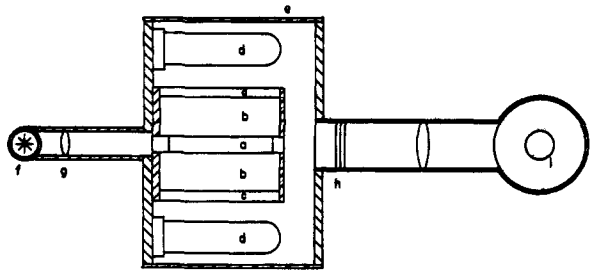


Fig. 1.—Schematic optical diagram of the flash apparatus.

The extent of the phototropic response was measured by observing the magnitude and direction of the vertical displacement of the oscilloscope trace, which occurred when the actinic light was turned on or off. Since the intensity of the scanning light was kept constant during these experiments, the ratio of the intensities of the light transmitted by the chlorophyll solution when the actinic light was on and off, is an (empirical) function of the observed displacement. This relation was determined with the aid of a set of graded neutral filters of known transmissivity.

Results of the Steady-state Measurements.—Since, under the steady-state conditions, presumably only one photo-product is present in detectable amounts

$$I = J \exp(-l)(\bar{\alpha}_1 c_1 + \bar{\alpha}_2 c_2) \qquad (1)$$

where I is the intensity of the transmitted light; J is the intensity of the light which would be transmitted if the cell contained only methanol; l is the length of the cell; $\bar{\alpha}_1$ and $\bar{\alpha}_2$ are the average extinction coefficients for chlorophyll and its photo-product, respectively; and c_1 and c_2 are the concentrations of chlorophyll and photo-product, respectively. When the actinic light is off, the intensity of the transmitted light, I^0, is

$$I^0 \cong J \exp(-l)(\bar{\alpha}_1 m) \qquad (2)$$

where m is the total or (initial) concentration of chlorophyll. Combining these equations

$$c_2 \cong \ln(I/I^0)/l(\bar{\alpha}_1 - \bar{\alpha}_2) \qquad (3)$$

The quantity I/I^0 can be computed from the observed displacement of the oscilloscope trace. $\bar{\alpha}_1$ is a function of wave length and can be obtained by graphical integration from the known extinction curves of the chlorophyll solution and the interference filter and from the emission spectrum of the filament. In general, neither c_2 nor $\bar{\alpha}_2$ are known. However, at those wave lengths where bleaching is observed

$$c_2 \geqslant \ln(I/I^0)/\bar{\alpha}_1 l \qquad (4)$$

If for each of the wave length regions used, values of $(\bar{\alpha}_1 l)^{-1} \ln(I/I^0)$ are computed, the largest value obtained should be closest to the true value of c_2 the steady-state concentration of the photo-product. This (limiting) value of c_2 can then be used to obtain a consistent set of values of $\bar{\alpha}_2$ for the other

(3) (a) D. Porret and E. Rabinowitch, *Nature*, **140**, 321 (1937); (b) R. Livingston, *J. Phys. Chem.*, **45**, 1312 (1941); (c) J. McBrady and R. Livingston, *ibid.*, **52**, 662 (1948); (d) J. Knight and R. Livingston, *ibid.*, **54**, 703 (1950).

(4) The details of the electronic equipment as well as a complete outline of the calibrating and checking methods are contained in a Doctoral Thesis submitted in December, 1951, by Victor Ryan to the Graduate Faculty of the University of Minnesota. Microfilm copies of the thesis are available from University Microfilms, Ann Arbor, Michigan. The interpretation of the experimental results which is presented here differs in several important respects from that given in the thesis.

wave lengths. Results obtained in this way are summarized in Table I.

TABLE I

SUMMARY OF AVERAGE EXTINCTION COEFFICIENTS FOR CHLOROPHYLLS-a AND -b AND THEIR PHOTO-PRODUCTS, G

λ	Chlorophyll-a $\bar{\alpha}_1$	Chlorophyll-a $\bar{\alpha}_2$	Chlorophyll-b $\bar{\alpha}_1$	Chlorophyll-b $\bar{\alpha}_2$
4030	69.3	50.8	12.7	26.4
4395	62.7	80.0		
4680	8.8	24.4	81.4	59.8
5020	3.5	20.3		
5245	3.1	13.5	3.4	21.8
5880	10.4	(0.0)	9.0	(0.0)
6450	30.6	10.4	20.7	7.6

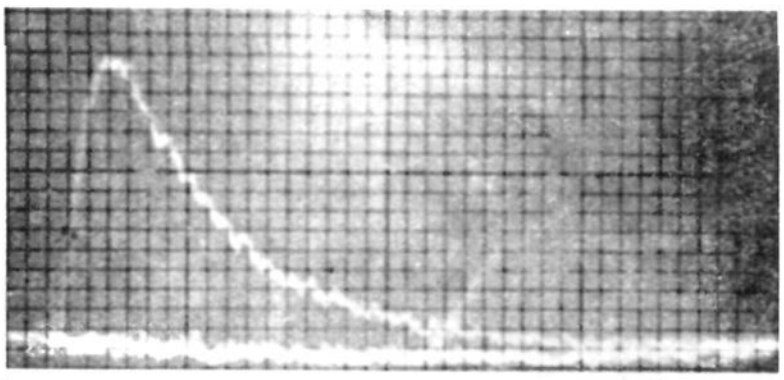
The extinction coefficients have the units of l. g.$^{-1}$ cm.$^{-1}$ and are exponents of e, the base of the natural logs. An error in the choice of c_2 would affect all of the values of $\bar{\alpha}_2$ proportionally, but would not change the shape of the absorption curve.

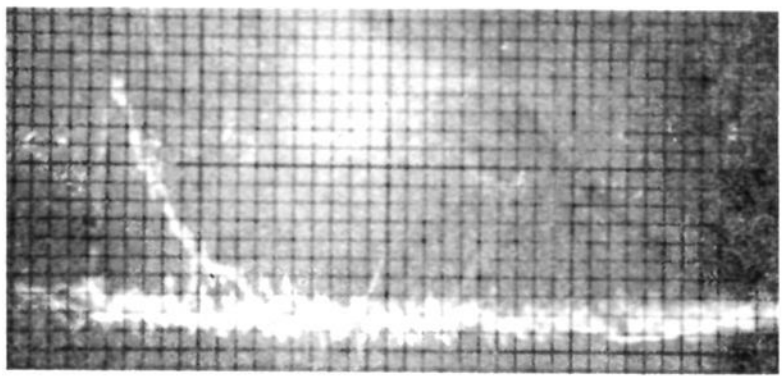
Inasmuch as the absorption is decreased in the red and greatly enhanced in the intermediate region, 5000–5300 Å., the absorption spectra of these photo-products resemble the spectra of the Molisch brown phase,[5] of the reversibly reduced form of chlorophyll[6,7] and of the photo-product formed in rigid solvents in the presence of certain quinones.[8] While the crudity of the present results makes the comparison somewhat uncertain, the absorption spectrum of the photo-product produced by steady illumination of methanolic solutions does not appear to be identical with the published spectra of these other chlorophyll intermediates.

Results of Measurements Made with Flash Illumination.—Figures 2 and 3 are reproductions of oscilloscope traces typical of experiments performed with flash illumination. Parts A of these figures were made with oxygen-free solutions, while parts B were made with the corresponding air-saturated solutions. Elapsed time is plotted as abscissa and the ordinates are proportional to changes in the intensity of the light falling upon the photomultiplier. The observed changes in intensity are due in part to variation in the transmissivity of the solution for the scanning light and in part to leakage of scattered exciting light. In an attempt to correct for the latter contribution, each series of measurements with an air-free cell was followed by a second series, using the same light filters and the same solution after saturating it with air.

The steady-state reversible photo-bleaching is completely inhibited by the presence of oxygen, even at low concentrations. Oscillograph traces, taken with the scanning light on and with the cell

A.





B.

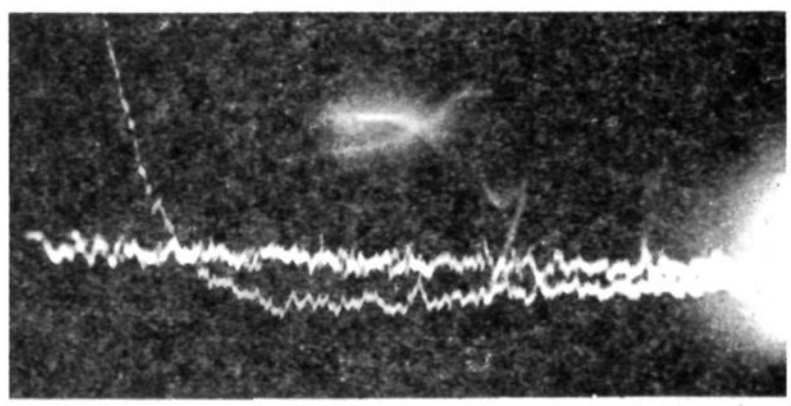
Fig. 2.—Typical oscilloscope traces taken with scanning light of average wave length of 4680 Å. (2×10^{-6} m chlorophyll-b in methanol): A, an air-free solution; B, the same solution saturated with air. The time scale is the same in Figs. A and B. The amplification factor was 2.24-fold greater for Fig. B than for Fig. A. Accordingly, the displacements from the baseline for the trace on Fig. B should be reduced by this factor.

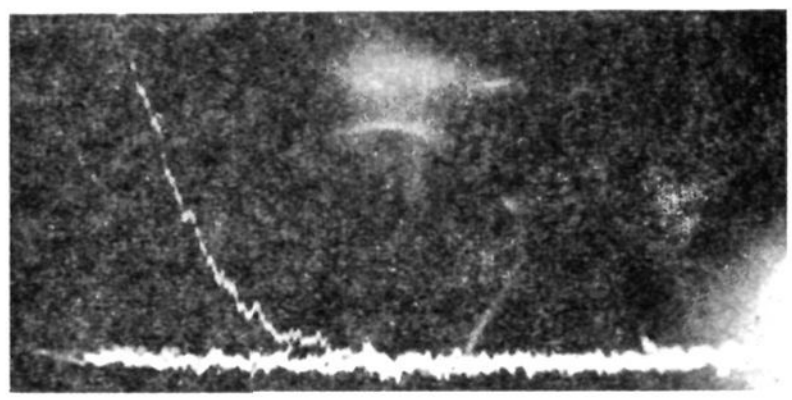
(5) B. Dunicz, T. Thomas, M. VanPee and R. Livingston, THIS JOURNAL, **73**, 3388 (1951).

(6) A. Krasnovskii, *Doklady Akad. Nauk* (*S.S.S.R.*), **60**, 421 (1948).

(7) A. Holt and E. Rabinowitch, private communication (1951).

A.





B.

Fig. 3.—Typical oscilloscope (2×10^{-6} m chlorophyll-b in methanol): traces taken with scanning light of average wave length of 5245 Å. A, an air-free solution; B, the same solution saturated with air. The scales for the ordinates and abscissas are the same in Figs. A and B.

(8) H. Linschitz and J. Rennert, *Nature*, **169**, 193 (1952).

filled with aerated chlorophyll solutions, are similar to traces taken with the scanning light off and with the cell filled with solvent. The falling part of all such traces is an exponential function of time, having a decay constant of about 7×10^3 sec.$^{-1}$. This is consistent with the expected decay of the flash, and shows that the displacement of the oscillograph trace, taken with aerated chlorophyll solutions, is due entirely to scattered light. In principle, it should be possible to correct the traces made with evacuated solutions by subtracting the values of the corresponding "oxygen blank" from them. The time scales for the oxygen blanks and for the bleaching experiments (as in Figs. 2 and 3) are identical within $\pm 2\%$. The uncertainty in the gain factors used in corresponding sets of experiments is probably less than $\pm 5\%$. Unfortunately, there is no indication on the oscilloscope traces of the instant when light was flashed. In the absence of such information, the origin of each oxygen-blank curve was chosen to make the maxima of the pair of curves coincide in time. The observed "bleaching" curve was then corrected by subtracting the corresponding intensity values of the blank run, as is illustrated in Figs. 4 and 5.[9]

The positive or negative displacement of the ordinates of the corrected oscillograph trace is a measure of the concentration of the photo-product. If it be assumed that the filtered scanning light can be treated as monochromatic and that there is only one photo-product present, equations 1, 2 and 3 can be used to represent the measurements made with flash illumination. In this application of those equations, I^0 is the intensity of the scanning light which strikes the photomultiplier a relatively long time (say 10^{-2} second) after the initiation of a flash. The significance of the other symbols is unchanged. Whether the same values of $\bar{\alpha}_2$ pertain to both the flash and steady-state experiments depends upon whether the photo-products, responsible for the observed color changes, are the same under the two different conditions of experimentation.

It follows from equation 3 that c_2 is directly proportional to log (I^0/I).

If there were only one photo-product formed, the (corrected) values of log (I^0/I) should have a maximum (or minimum) at a time corresponding approximately to the maximum intensity of the flash and should fall continuously with increasing time, approaching zero as t approaches infinity. Within the limits of experimental uncertainty, this condition appears to be satisfied (Fig. 4) by the measurements which were made with scanning light whose average wave length was 4680, 4705 or 4775 Å. It is clearly not satisfied (Fig. 5) for measurements made with λ 5245 Å.

Unless these measurements contain some unexpectedly large systematic error, two photo-products must be formed at comparable concentrations under our experimental conditions. At wave lengths in the range from 4650 to 4800 Å., the values of the extinction coefficients, of these two products, do not differ detectably, but they are smaller than the extinction coefficients of chlorophyll. At 5245 Å.

(9) There is an additional uncertainty in the evaluation of the ordinates. Depending upon the filters used, the bleached and unbleached (*i.e.*, oxygen-containing) solutions will differ in transmissivity for the scattered light. The available data do not permit a reasonable estimate of this difference, but comparison of results at different wave lengths suggests that this difference is not very important.

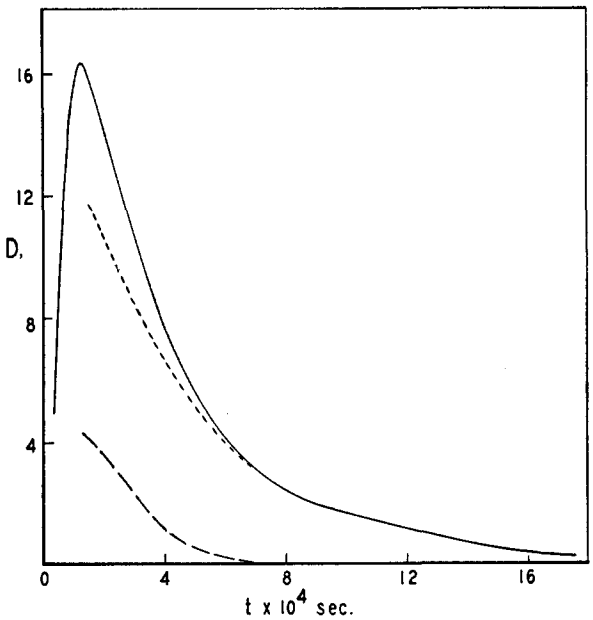


Fig. 4.—Averaged oscilloscope traces corresponding to λ 4680 Å.: solid line, averaged observed displacement for an air-free solution; dash line, averaged observed displacement for an air-saturated solution, reduced to the same scale as the solid line; dot line, displacement for an air-free solution, corrected for light leakage.

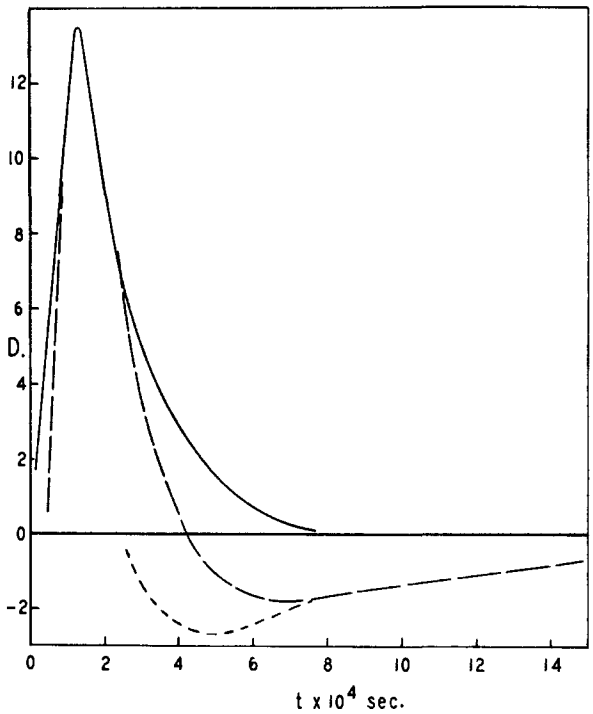


Fig. 5.—Averaged oscilloscope traces corresponding to λ 5245 Å.: dash line, averaged observed displacement for an air-free solution; solid line, averaged observed displacement for an air-saturated solution, reduced to the same scale as the dash line; dot line, displacement for an air-free solution, corrected for light leakage.

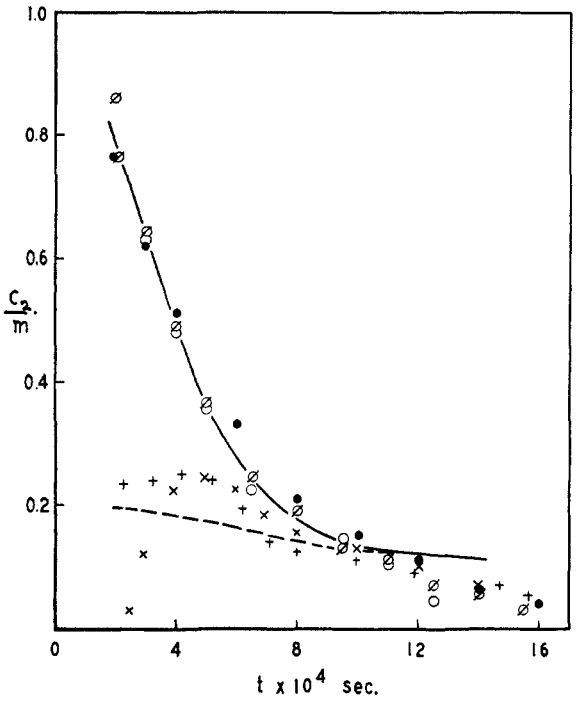


Fig. 6.—Comparison of experimental and theoretical values of c_2/m as a function of time. 2×10^{-6} m chlorophyll-b in methanol was used in all experiments: solid line, $x + y$; dotted line, y (of equations 7 and 8). The points were read from experimental curves, such as Figs. 4 and 5.

Symbol	Av. wave length, Å.	Temp., °C.	Normalizing factor
⊙	4680	24	3.04
O	4705	24	4.05
ϕ	4775	24	3.75
x	5245	22	−2.71
+	5245	13	−2.08

the longer lived product has an extinction coefficient several times greater than that of chlorophyll while the extinction coefficient of the shorter-lived product is equal to or less than that of chlorophyll. Comparison of these conclusions with the results (Table I) of the steady-state measurements on chlorophyll-b indicates that the longer-lived product observed in the flash experiments is the same as the product responsible for the changes observed under steady illumination. Analysis of the steady-state measurements[3] shows that this photo-product is a radical (or possibly an ion) formed by the reaction of an excited chlorophyll molecule with a molecule of a solvent or of an oxidizing or reducing impurity. For sake of definiteness, we shall postulate that the radical, G, is formed by the oxidation of a long-lived excited molecule, GH′, and that this species is the shorter-lived photo-product observed in the flash experiments. The following simplified mechanism is consistent with the preceding conditions.

$$h\nu + GH \longrightarrow GH^* \qquad v_1 = I \qquad (5)$$
$$GH^* \longrightarrow GH + h\nu_f \qquad v_2 = k_2(GH^*)$$
$$GH^* \longrightarrow GH' \qquad v_3 = k_3(GH^*)$$
$$GH + GH' \longrightarrow 2GH \qquad v_4 = k_4(GH)(GH')$$
$$O_{xi} + GH' \longrightarrow \dot{G} + \dot{H}O_{x_i} \qquad v_5 = k_{5i}(O_{xi})(GH')$$
$$H\dot{O}_{xi} + \dot{G} \longrightarrow GH + O_{xi} \qquad v_6 = k_{6i}(\dot{G})^2 \qquad (6)$$
$$O_2 + GH' \longrightarrow GH{\cdot}O_2 \qquad v_7 = k_7(O_2)(GH')$$
$$GH + GH{\cdot}O_2 \longrightarrow 2GH + O_2 \qquad v_8 = k_8(GH)(GH{\cdot}O_2)$$
$$GH + GH{\cdot}O_2 \longrightarrow GH + GOOH \qquad v_9 = k_9(GH)(GH{\cdot}O_2)$$

Since the first excited, singlet state, GH*, has a short life (about 7×10^{-9} sec. for chlorophyll-a and 5×10^{-9} sec. for chlorophyll-b) it cannot contribute[10] to the observed phototropic response. Step 4, the self-quenching of the long-lived GH′, is assumed, rather than a simple first-order decay, since it is consistent with the results of Knight and Livingston[3d] on the effect of chlorophyll concentration upon the extent of the steady-state bleaching. This mechanism leads to the simultaneous differential equations

$$-dx/dt = [k_4 m + k_5(O_{xi})]x - k_4 m x(x - y) \qquad (7)$$
$$-dy/dt = k_{6i} m y^2 - k_{5i}(O_{xi})x \qquad (8)$$

where m is the total or stoichiometric concentrations of chlorophyll (equal to $2 \times 10^{-6} m$) and x and y are, respectively, $(GH')/m$ and $(G)/m$. There does not appear to be a closed solution to these equations. Accordingly they were compared to the empirical data with the aid of a Reeves Electronic Analogue Computer.[11] The parameters of this solution are k_4, $k_{5i}(O_{xi})$, k_{6i}, x_0 and y_0. However, their values are subject to certain limitations. The sum, $x_0 + y_0$, is less than unity. Comparison[4] of the extinction coefficients given in Table I with the maximum values of I/I^0 found in flash experiments with λ 4680, 4705 or 4775 Å. indicates that $(x_0 + y_0) \gtrsim 0.9$. Similarly the flash measurements with λ 5245 Å. indicate that $x_0 \gtrsim 3y_0$. The quantities $k_{5i}(O_{x_i})$ and k_{6i} are fixed within an order of magnitude by the steady-state bleaching experiments. k_4 is determined only by the present measurements, but of course must be less than 10^{11}-m^{-1} sec.$^{-1}$. The following values of these parameters were obtained by fitting equations 7 and 8 to the flash data for λ 4680 Å. (Fig. 4): $x_0 = 0.72$, $y_0 = 0.21$, $k_4 = 4.5 \times 10^9 m^{-1}$ sec.$^{-1}$, $k_{5i}(O_{x_i}) = 1.5 \times 10^2$ sec.$^{-1}$ and $k_{6i}m = 2.1 \times 10^9$ sec.$^{-1}$.

As is shown by Fig. 6, these values are as consistent with the data obtained with λ 4705 and with 4775 Å. as they are with 4680 Å. The experimental data were reduced to a common basis by setting their maxima at 1.5×10^{-4} sec. and by multiplying the several sets of values of log I/I^0 by factors chosen to make the empirical and calculated values coincide at 9.5×10^{-4} sec. At times less than 5×10^{-4} sec. the intensity of the flash is still appreciable, and the calculated curve should lie above the corrected data. While the agreement is in general as good as might be expected when the experimental uncertainty is taken into account, it should be noted that the rate for the last 10 or 15% of the reactions is definitely faster than is predicted by the mechanism. Whether this discrepancy is real or is the result of some unknown systematic error cannot be decided upon available evidence.

The values for the two experiments performed with λ 5245 Å. have been made to coincide with the

(10) Sanford Weil, Ph.D. Thesis, University of Minnesota, July, 1952.

(11) We are greatly indebted to Mr. A. Acrivos of the Dept. of Chemical Engineering of the University of Minnesota who performed this operation.

other data for times greater than 10^{-3} sec. by multiplying the values of log I/I^0 by factors of -2.71 and -2.08, respectively. These adjusted data, which are plotted on Fig. 6, depart widely from the other data and the curve ($x + y$ *vs.* t) at times less than 8×10^{-4} sec. The dotted curve is a plot of y *vs.* t, calculated using the parameters selected to fit data corresponding to λ 4680 Å. The general agreement between this dotted curve and the data corresponding to λ 5245 Å. is consistent with the assumed mechanism and the special postulate that the observed increase in absorption is due entirely to the secondary photo-product, G. In other words $\bar{\alpha}_{GH'} \simeq \alpha_1$ and $\bar{\alpha}_G \simeq \alpha_2$, at λ 5245 Å. An experiment performed with light whose wave length band centered at 6810 Å. showed no change in transmissivity upon illumination, presumably indicating that none of the substances present absorb appreciably at this wave length.

A slightly modified mechanism, in which step 4 is replaced by the simple unimolecular step, GH′ → GH, $-v_{4A} = k_{4A}(GH')$, is also compatible with the results of the flash experiments. This mechanism leads to the differential equations

$$-dx/dt = [k_{4A} + k_{5i}(O_{x_i})]x$$
$$-dy/dt = k_{6i}my^2 - k_{5i}(O_{x_i})x$$

Acceptable values for the parameters corresponding to this mechanism are: $x_0 = 0.65$, $y_0 = 0.15$, $k_{4A} = 3.5 \times 10^3$ sec.$^{-1}$, $k_{5i}(O_{x_i}) = 1.5 \times 10^2$ sec.$^{-1}$ and $k_{6i} = 3.1 \times 10^9 m^{-1}$ sec.$^{-1}$. The agreement between the experimental data and the curve, derived from these equations and parameters, is somewhat inferior (especially during the first half of the reaction) to that illustrated by Fig. 6 for the preceding mechanism.

Steps 7, 8 and 9 are introduced to take into account the effect of oxygen upon the steady-state[3d] and flash reversible "bleaching" and on the slow irreversible bleaching.[3d] The bimolecular step 8, rather than a first-order dissociation of the complex GH·O_2, is indicated by Gaffron's[12] quantitative measurements of chlorophyll-sensitized autoöxidation. Values for the rate constants of these reactions, which are consistent with the constants of the preceding steps, and which appear to be compatible with all of the available pertinent data, are as follows: $k_7 = 2.7 \times 10^{10}$, $k_8 = 1.4 \times 10^{10}$ and $k_9 = 8 \times 10^5 m^{-1}$ sec.$^{-1}$.

Since all of the experiments under discussion were performed in methanol, or similar activating solutions,[13] the normal chlorophyll molecule, represented here as GH, is the one-to-one addition compound of chlorophyll and a basic solvent. The present evidence does not preclude the stabilization of an intermediate radical by the loss or gain of a proton, as has been suggested by Terenin.[14] For example, it is possible that step 5 is the result of an electron transfer followed by a rapid equilibrium

$$O_{x_i} + GH' \longrightarrow O_{x_i}^- + GH^+$$
$$MeOH + GH^+ \rightleftharpoons MeOH_2^+ + G$$

For sake of simplicity, such possibilities are not considered here.

(12) H. Gaffron, *Ber.*, **60**, 755 (1927).

(13) R. Livingston, W. Watson and J. McArdle, THIS JOURNAL, **71**, 1542 (1949); R. Livingston and S. Well, *Nature*, **170**, 750 (1952).

(14) A. Terenin, *Bull. acad. sci. U.R.S.S. ser. biol.*, 369 (1947).

MINNEAPOLIS, MINNESOTA

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A New Apparatus for Rate Studies Applied to the Photopolymerization of Methyl Methacrylate

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An apparatus is described which permits a reasonably accurate study of the rate of reaction by following refractive index changes. Certain results obtained on the polymerization of methyl methacrylate are given.

Introduction

This paper describes a new apparatus for following the rate of a reaction and presents certain data obtained some time ago in a study of the photocatalyzed polymerization of methyl methacrylate. In many bulk polymerizations, the reaction mixture passes through a viscous stage and finally to a solid. It is particularly difficult to follow the rates of reaction in these high conversion regions by dilatometric or precipitation techniques. Experimental difficulties accompanying operations with "gelled" systems make sampling and the usual kinetic measurements extremely troublesome. This equipment should be useful in following the course of any reaction accompanied by a significant change in refractive index and occurring in a homogeneous liquid system.

The apparatus described here has been termed an "oblique-line refractometer" and it has proved operable over the range of 0 to 100% conversion with methyl methacrylate. This equipment was designed to operate with actinic light; however, other modifications have been used in this Laboratory to study thermal polymerizations.

Polymerizations were carried out using benzoin or 2,2′-azo-bis-isobutyronitrile as photocatalysts with light primarily in the 3600 Å. region. As might be expected, the kinetics in such a system are identical with those of polymerizations employing thermal catalysts except for differences attributable to the method of forming chain initiating radicals. These latter differences result in several interesting effects which, however, are in agreement with the generally accepted mechanism of vinyl polymerization.[1]

(1) (a) R. G. W. Norrish and R. R. Smith, *Nature*, **150**, 336 (1942); (b) K. S. Bagdasar'yan, *J. Phys. Chem.* (*U.S.S.R.*), **21**, 25 (1947); (c) G. V. Schulz and G. Harborth, *Makromol. Chem.*, **1**, 106 (1947); (d) E. Trommsdorff, *et al.*, *ibid.*, **1**, 169 (1948); (e) M. S. Matheson, *et al.*, THIS JOURNAL, **71**, 497 (1949).