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THE THERMAL DECOMPOSITION OF VISUAL PURPLE

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In 1878 Ewald & Kühne measured the time necessary for solutions of visual purple to become decolourized completely when heated to various temperatures. They also investigated the effects of NaCl and of the absence of water on the thermal bleaching, and concluded that the reactions were very similar to the coagulation of albumen by heat. Since very little is known about the chemical nature of visual purple, it was decided to extend these observations by making some quantitative measurements of its thermal bleaching at various hydrogen-ion concentrations. Apart from the theoretical interest, it is of practical importance to know the amount of decomposition which may be expected during certain operations on the substance.

The experiments were carried out over the whole range of acidities within which visual purple is not very rapidly destroyed at room temperatures. The velocity of decomposition was followed by the progressive loss of optical density with time, and in what follows the term "decomposition of visual purple" means the loss of the typical colour since our only sure guide to the presence of visual purple is its characteristic wavelength absorption curve.

Theoretical

When visual purple solutions are bleached by light the colour changes can be expressed by assuming that an intermediate substance, "transient orange", is first formed which is rapidly decomposed at room temperatures to "indicator yellow" [Lythgoe, 1937]. Wald [1937] describes these changes in much the same terms. As its name implies the latter substance changes colour with the acidity of the solution, being pale yellow in alkaline and deep yellow in acid solution. Indicator yellow is

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unstable at room temperatures between about *p*H 3·5 and 6·0, but becomes progessively more stable in solutions of greater acidity or alkalinity. Visual purple is bleached by strong acids and alkalis, and the breakdown product has the same indicator properties and is indistinguishable spectroscopically from the product formed when it is bleached by light. The same also is true when visual purple is bleached by heat. This was shown by warming an alkaline solution (*p*H 9·56) to 46° C. for about 2 hr., by which time the original purple colour had been replaced by a very pale yellow. On making the solution acid the colour immediately changed to a deep yellow. The change was reversible.

The formation of indicator yellow complicates the determination of the velocity constant for the decomposition of visual purple, and still further complications are introduced owing to the fact that the indicator yellow is itself thermally unstable under the conditions of some experiments. Transient orange is very rapidly destroyed even at low temperatures. It is not known whether it is an intermediate stage in the thermal bleaching, but if so it must be destroyed so rapidly at the temperatures used in the present experiments that its formation will not be detectable. Since the decomposition of transient orange is very rapid we shall, in effect, be measuring the rate of decomposition of visual purple to the relatively stable indicator yellow.

The optical density D_t of a solution at any time, t, is the sum of the densities of visual purple, indicator yellow and impurities absorbing light. If the densities are expressed in terms of extinction coefficients and concentrations, the relation becomes

$$D_t = \alpha cl + \alpha' c' l + D_i, \qquad \text{.....(i)}$$

where α, α' are the extinction coefficients and c, c' the concentrations of visual purple and indicator yellow respectively, and l is the length of the cell; αcl and $\alpha' c' l$ are therefore the densities of the visual purple and indicator yellow respectively. D_i is the density of light-absorbing impurities.

The concentration of indicator yellow formed up to any time is z times the concentration of visual purple destroyed, where z is the stoichiometric relation between the two substances. If we call the initial concentration of visual purple, c_0, then $c' = z(c_0 - c)$. In this and what follows immediately it is assumed that the indicator yellow formed is stable.

Substituting $z(c_0 - c)$ for c' in (i)

$$D_t = \alpha cl + \alpha' z(c_0 - c)\, l + D_i. \qquad \text{.....(ii)}$$

In the final bleached condition $c = 0$. Let us call this final density D_f, therefore

$$D_f = \alpha' z c_0 l + D_i. \qquad \text{.....(ii}a\text{)}$$

Substituting in (ii) and rearranging

$$D_i - D_f = c(\alpha l - z\alpha' l). \qquad \text{.....(iii)}$$

At the beginning of a period call the density D_0 and since the concentration of visual purple is then c_0,

$$D_0 - D_f = c_0(\alpha l - z\alpha' l). \qquad \text{.....(iv)}$$

If the decomposition of visual purple is a reaction of the first order, then

$$-\frac{dc}{dt} = k_1 c,$$

where k_1 is the velocity constant. Integrating and finding the constants

$$k_1 t = \log_e \frac{c_0}{c}. \qquad \text{.....(iv}a\text{)}$$

Substituting the values of c and c_0 from equations (iii) and (iv), and eliminating $\alpha l - z\alpha' l$,

$$k_1 t = \log_e \frac{D_0 - D_f}{D_i - D_f}. \qquad \text{.....(v)}$$

If, on the other hand, the decomposition of visual purple is a reaction of the second order then

$$-\frac{dc}{dt} = k_2 c^2,$$

where k_2 is the velocity constant. Integrating and finding the constants

$$\frac{1}{c} - \frac{1}{c_0} = k_2 t \quad \text{or} \quad c_0 k_2 t = \frac{c_0 - c}{c}.$$

Substituting the values for c and c_0 from equations (iii) and (iv) and eliminating $\alpha l - z\alpha' l$,

$$k_2 t = \frac{D_0 - D_t}{D_i - D_f} \cdot \frac{1}{c_0}. \qquad \text{.....(vi)}$$

Up to this point it has been assumed that indicator yellow is stable. Under many conditions, however, this is not so and it loses its colour on heating. This is a further complication in determining the rate of thermal decomposition of visual purple, and it is necessary to apply a correction to the observed density readings in order to bring them to the values which would have been observed had the indicator yellow been stable.

The greatest absorption of light by visual purple is at 502 mμ. and the density falls off progressively towards 400 mμ. The absorption of light by indicator yellow, on the other hand, increases towards shorter wave-lengths. For each hydrogen-ion concentration there is a wave-length in the visible spectrum where the density of the indicator yellow

formed on bleaching is exactly the same as that of the parent visual purple. This is known as the cross-over wave-length. A series of absorption curves taken on a solution at different stages of bleaching would intersect at this wave-length provided that all the transient orange but none of the indicator yellow had been destroyed. At the temperatures used in the present experiments transient orange is very rapidly destroyed and any loss of density at the cross-over wave-length is a measure of the thermal destruction of indicator yellow. During an experiment, therefore, readings were taken at this wave-length concurrently with the readings of the loss of density at the maximum absorption of visual purple (502 mμ.). From previous work we know the densities of indicator yellow at different wave-lengths and for different acidities and it follows that we can calculate the loss of density at 502 mμ. due to the decomposition of indicator yellow knowing the decomposition at some other wave-length. For this purpose we first found the ratio of the density of indicator yellow at 502 mμ. to its density at the cross-over wave-length [Lythgoe, 1937]; we then multiplied the loss of density at the cross-over wave-length by this ratio. The resulting figure was then added to the measured densities of the solution at 502 mμ., thereby giving the density which would have been found had the indicator yellow been stable. These corrected values are those required in the equations.

Apparatus and preparation of solutions

The optical densities were measured by a photoelectric spectrophotometer [Bayliss *et al.* 1936]. The instrument was fitted with a new rotating shutter which reduced the time occupied by a single reading to 5 sec. and so made it possible to follow quicker changes.

Arrangements were made to keep the visual purple at a constant temperature during an experiment and to change this temperature rapidly between experiments. The optical cell containing the visual purple was placed in a hollow cell holder through which either heated or cooled water could be pumped. The water was delivered from a 2 gall. reservoir, the temperature of which was controlled by a thermoregulator of the expanding liquid type. It was fitted with a calibrated side arm and tap for the adjustment of the amount of mercury it contained and consequently of the temperature at which it regulated. A platinum wire was placed in the capillary of the regulator. As the temperature rose, the mercury made contact with the platinum and the current flowing in the closed circuit operated a "Sun-vic" relay. The relay switched off a heater in the reservoir and operated an electromagnetic clip which directed the

water returning from the optical-cell holder, through a cooling spiral before passing to the reservoir. When the temperature fell the contact in the thermostat was broken so that the heater was switched on and the water was returned direct to the reservoir. When temperatures below those of the surrounding air were needed, the heater was removed from the reservoir and the cooling spiral was immersed in an ice bath.

The temperature of the circulating water was measured by a thermometer situated near the cell holder. It was found that the thermostat maintained the temperature of the flow water to within about $\pm 0{\cdot}1°$ C. over the range 5–60° C. The time taken for the contents of the cell to reach a steady temperature and the relation of this temperature to that read on the thermometer in the circulating water were determined by means of a calibrated thermocouple immersed in the centre of the cell when filled with water. In the experiments readings were not begun until the visual purple was known to have attained a steady temperature. All figures for temperature have been corrected so as to show the actual temperatures of the visual purple. No special arrangements were made to stir the 0·4 c.c. of solution in the cell since it was thought that the frequent rotation accompanying a set of readings was sufficient to mix the liquid.

The solutions of visual purple were prepared from *Rana esculenta* by the method given in a previous paper [Lythgoe, 1937]. The retinæ were expressed, shaken with 0·6 p.c. NaCl solution and filtered through fine wire gauze. The filtrate containing the rods and pigment was centrifuged and the residue washed several times with 0·6 p.c. NaCl. The residue was then desiccated and extracted with petrol ether. After two washings with an acid buffer (pH 4·4–4·6) and one with 0·6 p.c. NaCl, the rods were shaken for 10 min. at 0° C. with 1 p.c. digitonin solution (1 c.c. for 12 frogs). After centrifuging strongly, the supernatant fluid was buffered by the addition of $M/20$ buffer solution [Clark & Lubs, see Clark, 1928] in equal volume. The final concentration of hydrogen ions in the solutions was measured by a Kerridge [1926] glass electrode used in conjunction with a valve electrometer [Dubois, 1930].

Conduct of experiments

For every preparation of visual purple the absorption curve was measured in the visible spectrum both for the unbleached solution and also after bleaching for a fixed time [Lythgoe, 1937]. At pH 4·2 to 5·57 the measurements were made with the solutions cooled to about 10° C. since above this temperature the destruction of indicator yellow is inconveniently rapid. The cross-over wave-length, the initial density at that

wave-length and the final light-bleached density of the solution (D_f) were given by these readings. The optical cell was then refilled; the heating circulation was turned on and after a suitable interval, readings of the density were taken over a period of at least half an hour. These readings were made alternately at 502 mμ. and at the cross-over wave-length, each reading being paired with a control. At lower temperatures where the destruction of the visual purple was slow, the same solution was used for the experiment at the next higher temperature, but otherwise a fresh sample from the same preparation of visual purple was used for each experiment. The velocity of the reaction was usually determined at four different temperatures for each preparation.

Results

Table I shows a typical set of readings at *p*H 6·83 and temperature 50·6° C. The method of correcting the results for the decomposition of indicator yellow is also shown. Column i gives the times at which the

Table I. Thermal decomposition of a visual purple solution at *p*H 6·83, showing method of correction for loss of indicator yellow. Temperature 50·6° C. Cross-over wave-length, 432 mμ. Initial density at 432 mμ., 0·282 (18·2° C.). Final density at 502 mμ. (D_f), 0·118 (18·2° C.). The figures in brackets are interpolated or extrapolated values.

i Time in sec.	ii Density 502 mμ.	iii Density 432 mμ.	iv Loss of density at 432 mμ.	v Loss of density at 502 mμ., i.e. col. iv × factor 0·37	vi Col. ii + col. v $= D_t$	vii $\frac{1}{t}.\log_{10}\frac{D_0 - D_f}{D_t - D_f}$
0	0·379			[0·022]	0·401 (D_0)	—
94		0·220	0·062	0·023		
188	0·360			[0·024]	0·384	0·00014
280		0·215	0·067	0·025		
373	0·341			[0·025]	0·366	0·00015
467		0·212	0·070	0·026		
560	0·325			[0·026]	0·351	0·00015
653		0·209	0·073	0·027		
746	0·311			[0·027]	0·338	0·00015
847		0·207	0·075	0·028		
933	0·300			[0·028]	0·328	0·00014
1275		0·204	0·078	0·029		
1368	0·272			[0·029]	0·301	0·00014
1461		0·203	0·079	0·029		
1555	0·263			[0·029]	0·292	0·00014

readings were taken. Column ii gives the measured density of the solution at 502 mμ. at those times. Column iii gives the densities of the solution at the cross-over wave-length, whilst column iv shows the loss of density at that wave-length since the beginning of the experiment. From the previous paper we know that the ratio of the density of indicator yellow

at 502 mμ. to its density at the cross-over wave-length is about 0·37 in a solution of *p*H 6·83. The figures in column iv multiplied by 0·37 give the calculated loss of density at 502 mμ. (column v) the figures in brackets being interpolated values. In column vi the latter values have been added to the observed densities at 502 mμ. (column ii) to give the densities (D_t) which would have been observed had the indicator yellow been stable.

Table II shows a typical set of readings for conditions where indicator yellow is stable. The example chosen is *p*H 9·56, temperature 47·2° C.

TABLE II. Thermal decomposition of a visual purple solution in which the loss of indicator yellow is so small that correction is unnecessary. *p*H 9·56. Temperature, 47·2° C. Final density at 502 mμ. (47·2° C.) (D_f), 0·081. (Skeleton values only are shown.)

Time in sec.	Density 502 mμ. $= D_t$	$\frac{1}{t}\cdot\log_{10}\frac{D_0-D_f}{D_t-D_f}$
0	0·316 (D_0)	—
31	0·307	0·00055
124	0·285	0·00049
216	0·265	0·00049
309	0·251	0·00046
402	0·237	0·00044
525	0·217	0·00045
628	0·207	0·00043
788	0·189	0·00043

The values of D_0, D_t and D_f were substituted in equations (v) and (vi) and both $\log_{10}\frac{D_0-D_f}{D_t-D_f}$ and $\frac{D_0-D_t}{D_t-D_f}$ were plotted against time to determine the order of the reaction. In Figs. 1 and 2 $\log_{10}\frac{D_0-D_f}{D_t-D_f}$ has been plotted against time for two experiments. The results at *p*H 6·83, shown in Fig. 1, have been corrected for destruction of indicator yellow. No correction was necessary for the results at *p*H 9·56 shown in Fig. 2, since in solutions more alkaline than *p*H 7–8 the thermal decomposition of indicator yellow is negligible. The points fall very nearly on the straight lines which would have been obtained from a reaction of the first order. Nearly all the results show that the reaction proceeds too slowly towards the end of an experiment. The deviation can be seen in Fig. 2. Although the error in the logarithmic function is grossly disproportionate to the error in the measurement of density towards the end of an experiment when D_t and D_f are nearly equal to one another, the errors are consistently in the same direction. For most experiments, however, the results are in much worse agreement with the equation for a reaction of the second order (equation (vi)). It appears that the thermal decomposition of visual purple is a first-order reaction but that strict experimental verification is impossible owing to complicating factors.

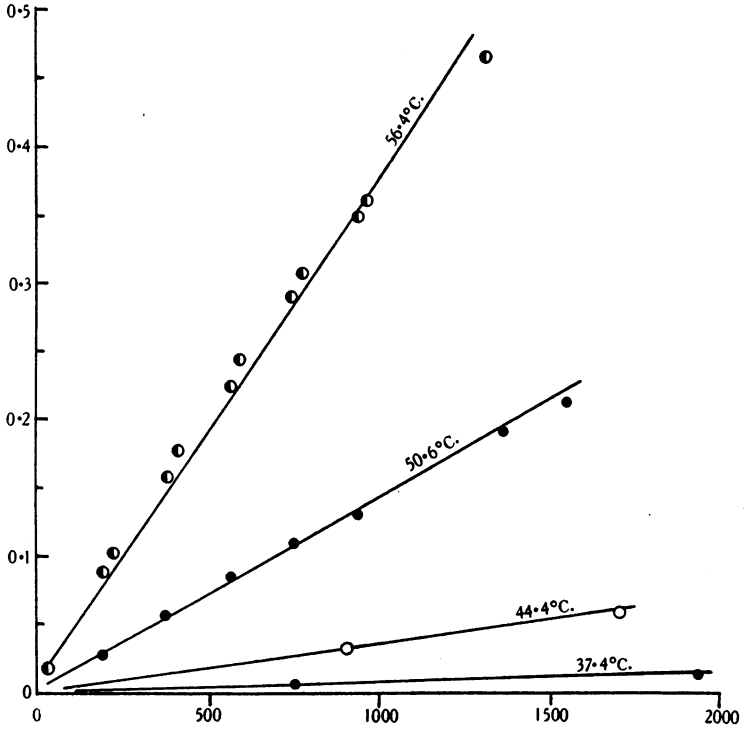

Fig. 1. Abscissæ: time in sec. Ordinate: $\log_{10}\frac{D_0 - D_f}{D_t - D_f}$. The values of D_t have been corrected for the fading of indicator yellow. The temperatures of the visual purple solutions are printed on the straight lines. For the experiment at 56·4° C. the figures of the ordinate must be doubled. *p*H 6·83.

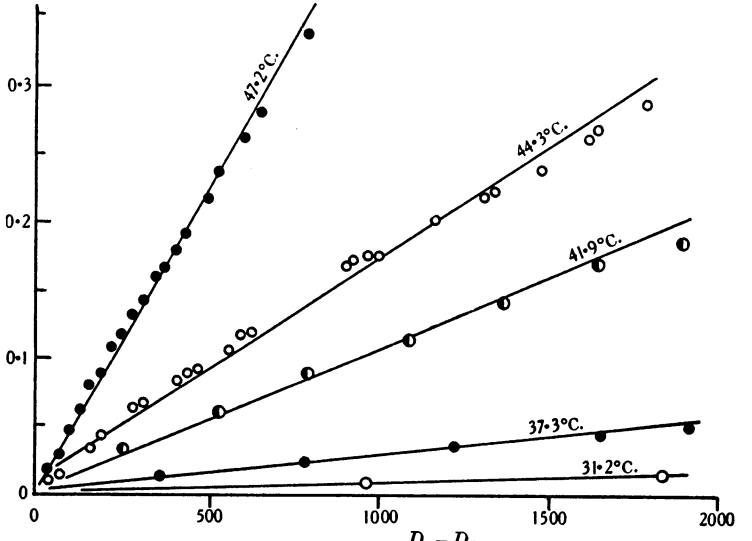

Fig. 2. Abscissæ: time in sec. Ordinate: $\log_{10}\frac{D_0 - D_f}{D_t - D_f}$. The temperatures of the visual purple solutions are printed on the straight lines. *p*H 9·56.

Chick & Martin [1911] have shown that the denaturation of protein does not proceed as a reaction of the first order if the concentration of hydrogen ions changes during an experiment. In the buffered solutions of visual purple used by us bleaching produced no change in hydrogen-ion concentration outside our limits of error in measurement, but a very slight change, sufficient to account for the deviation from the straight line, may have occurred.

It is probable that some irregularities in the results arise from complicated changes in the optical density of the heated solutions other than those described under the theoretical section. Some of these changes were as follows: (1) the higher the temperature at which alkaline solutions of visual purple were bleached, the greater were their final densities. A similar change seemed to occur in slightly acid solutions. (2) During the course of thermal decomposition, solutions of visual purple often showed a sudden increase in density. The increase was not associated with any visible coagulation or precipitation. (3) The density of visual purple solutions at short wave-lengths seemed to change on keeping. The changes in the absorption spectrum were different from those which would have been expected from a breakdown of visual purple to indicator yellow.

Graphs, similar to those in Figs. 1 and 2, were drawn for each experiment, and the slopes of the best-fitting straight lines were found. The derived velocity constants (k) are quoted throughout this paper on the basis of natural logarithms and with seconds as units of time. The logarithms of the velocity constants are plotted against the reciprocal of the absolute temperature in Fig. 3. It will be seen that for any given pH, the points fall on a straight line, and it follows that the Arrhenius equation is obeyed. The heat of activation, E, was calculated from the formula $\log_e k = c - E/RT$, where R is the gas constant and c is also a constant. For all concentrations of hydrogen ions the value of E is about 44,000 cal. per g.-mol., but there is some indication that in acid solutions it is less whilst in alkaline solutions it is more. This value is in the range 20,000–60,000 cal. found for most simple molecules. For hæmoglobin $E = 60{,}000$ cal. and for egg albumen $E = 135{,}000$ cal. [Chick & Martin, 1910]. From Ewald & Kühne's data, Wald [1935] calculated that for visual purple $E = 75{,}000$ cal. Some caution is necessary, however, in the interpretation of heats of activation when there are intermediate links in a chemical change, the links being in equilibrium with one another [Steinhardt, 1937]. Transient orange might possibly be such a link. The ratio of the velocity constants for a 10° rise of temperature (Q_{10}) is about 10 between

30 and 40° but somewhat less at higher temperatures. This value is very high compared with those for ordinary chemical reactions, but of the same order as those for reactions involving proteins.

Equation (v), which is that for a reaction of the first order, can be solved without knowing the concentrations of the solutions. To obtain the value of k_2 from the second order equation (vi), it is necessary to know c_0, a quantity whose absolute value is not yet known with any accuracy. By using D_0 in the equation instead of c_0, it is possible to obtain relative

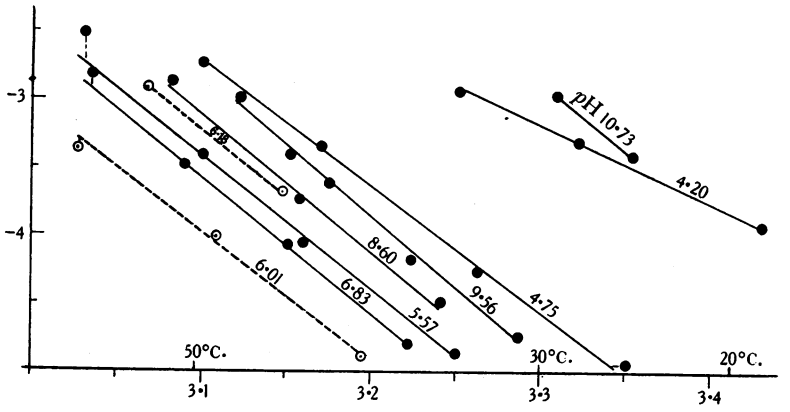

Fig. 3. Influence of temperature on the rate of decomposition of visual purple at various acidities. Scale of abscissæ: $1000/T$, where T = absolute temperature. Ordinate: $\log_{10} k$, where k is the velocity constant (time in seconds: natural logarithms). The upper dotted line is for a dialysed solution of low salt content; the lower dotted line is for a solution 2·0 *M* NaCl. The *p*H values of the solutions are printed on the lines.

values for k_2, and, using these values, E can be calculated. If the thermal decomposition of visual purple is a reaction of the second order, the calculated heat of activation is 53,000 cal.

It is quite possible that we are in reality dealing with a heterogeneous reaction, for it is well known that many reactions which at first sight appear to take place in solution are found on closer investigation to take place almost entirely on the walls of the containing vessel. In order to exclude this possibility the area of solution exposed to quartz was approximately doubled by adding quartz chippings to the quartz optical cells. In one experiment a solution of visual purple at *p*H 6·85 was prepared and the rate of decomposition at 44° C. was measured both with and without the addition of the chippings. The experiments were followed for one hour and in both the velocity constants of the reaction

were almost identical showing that an increase of surface exposed to the solution has no effect on the rate of the reaction.

Although the thermal bleaching of visual purple appears experimentally to be a homogeneous reaction of the first order, it does not follow that the mechanism of the reaction is unimolecular. If the mechanism were bimolecular and involved water, the concentration of the latter would remain constant during an experiment and the observed reaction would be of the first order. Reactions involving water are usually profoundly influenced by hydrogen-ion concentration and it is of importance to investigate the effect of this factor on the rate of bleaching.

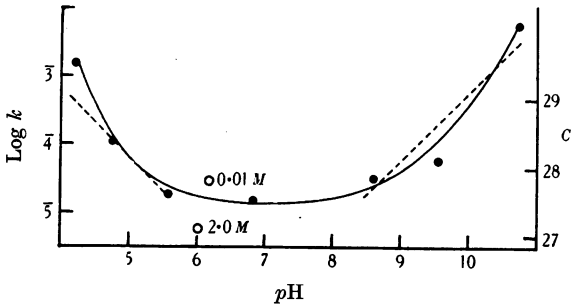

Fig. 4. Influence of *p*H on rate of thermal decomposition. Abscissæ: *p*H of visual purple solutions. Ordinate: the values of $\log_{10} k$ (Fig. 3) at 36° C. are given on the left. On the right are given the values of C in the approximate equation $\log_{10} k = C - 10{,}000/T_{\text{abs.}}$ Results from solutions with high (2·0 *M*) and low (0·01 *M*) salt content are indicated by open circles. For explanation of dotted line see text, p. 34.

The relation between *p*H and velocity constant at 36° C. is shown in Fig. 4. It will be seen that visual purple is thermally most stable at about neutrality, that it is fairly stable between *p*H 5·5 and 8·5, but that its stability falls off rapidly outside this range. It will be seen from Fig. 3 that the ratio between the velocity constants at different concentrations of hydrogen ions is approximately the same whatever the temperature at which comparison is made. It follows, therefore, that the relationship shown in Fig. 4 will also hold for other temperatures provided the velocity constants are multiplied by a factor. When graphs with the same co-ordinates as those used in Fig. 4 are plotted for reactions which are catalysed by water, the shape of the graph should indicate the roles played by the H^+, OH^- and undissociated H_2O in the reaction (see for instance Skrabal, 1927). If equal scales are used for ordinates and abscissæ, a reaction which is catalysed by H^+, OH^- and H_2O gives a graph with a horizontal portion due to catalysis by undissociated water

and with two side arms at 45° corresponding to accelerated catalysis as the number of H^+ or OH^- ions increases. It is possible that our results shown in Fig. 4 do fall on three straight lines but the side arms seem to be steeper than the dotted lines at 45° which would be required by the simple hypothesis. The reaction with H^+ and OH^- seems to be more nearly proportional to the square of the concentration of H^+ and OH^- ions.

The velocity of a reaction can be represented by the formula $k = PZe^{-E/RT}$, where k, E, R and T have the same definitions as have been used already, Z is the total number of collisions and P is the steric factor which is less than unity, $e^{-E/RT}$ is the energy of activation factor. Since E has the same value for all concentration of hydrogen ions it follows that the variation in k with pH at a fixed temperature must be due to changes in PZ, the term concerned with the effective collisions.

The fact that a single straight line is obtained when log k is plotted against the reciprocal of the absolute temperature suggests that the reaction is not composite (see for instance Hinshelwood, 1933), and one can, therefore, extrapolate without introducing serious error. The empirical equation $\log_{10} k = C - 10{,}000/T_{\text{abs.}}$ is approximately true, where the value of the constant C varies with pH. These values of C are given by the ordinates in Fig. 4. On calculation it is found that at 0° C. 1 p.c. of the visual purple would be destroyed in about 2750 hr. at pH 7, and in about 700 hr. at pH 9·6. These are useful figures to bear in mind in the dialysis of the solutions.

The addition of sodium chloride. Most workers since Ewald & Kühne have supported the view that visual purple is a protein. If this is so one would expect the thermal decomposition to be influenced by salt concentration. The experiments so far described were made on solutions having an effective salt concentration of about 0·025 M. Unfortunately the preparation of salt-free solutions of visual purple seems to be impossible since, when dialysed against pure water, the solutions become progressively more acid and the visual purple is bleached. The salt content can, however, be reduced. One solution was prepared by dialysing it in a cellophane sac against a 0·01 M buffer solution, pH 7·0, for 3 days in the dark at 0° C.: the final pH value of the solution was 6·18. The thermal decomposition of this solution was considerably faster than that of one of the usual solutions having the same concentration of hydrogen ions. The results of this experiment are given in Figs. 3 and 4. When, on the other hand, the NaCl content was increased to 2·0 M, the thermal decomposition was considerably retarded (pH 6·01). It was found, on the other hand, that for both the solution of high salt content and for the

solution of low salt content the heats of activation were the same as those of standard solutions of the same acidity.

The changes in thermal decomposition with salt concentration are similar to those found by Chick & Martin [1911] in their work on the denaturation of egg albumen and support the view that visual purple is either a protein or closely allied to one.

The thermal decomposition of indicator yellow. If indicator yellow is kept in solution more acid than about *p*H 3·5 or more alkaline than *p*H 6·5 it is only slowly decomposed at room temperatures (20°) or when warmed gently. An attempt was made to measure its thermal decomposition in the range of acidities where it is unstable but this was only partially successful on account of the variety of unknown reactions which have been described on p. 32. Two moderately satisfactory experiments were made, one at *p*H 4·75 and other at *p*H 6·83. The visual purple was first bleached by light under the standard conditions and the subsequent changes in the density of the indicator yellow were followed in the same way as for visual purple except that the wave-length used was different. For the solution at *p*H 6·83 a wave-length of 410 mμ. was chosen since this permitted both accurate and rapid records of the changes in density. For the solution at *p*H 4·75 readings were taken at

TABLE III. Velocity constants, k (time in seconds: natural logarithms) for the destruction of indicator yellow at various temperatures

*p*H 4·75		*p*H 6·85	
10·4° C.	$k = 1{\cdot}6 \times 10^{-5}$	27·6° C.	$k = 1{\cdot}0 \times 10^{-4}$
18·5	$= 1{\cdot}45 \times 10^{-4}$	44·6	$= 6{\cdot}4 \times 10^{-4}$
25·4	$= 3{\cdot}2 \times 10^{-4}$		
33·5	$= 1{\cdot}0 \times 10^{-3}$		

450 mμ., a wave-length which is near the maximum absorption of the acid form of indicator yellow. The results were calculated on the assumption that the thermal destruction of indicator yellow is a reaction of the first order. The greatest difficulty lay in the determination of the final density. The latter could not be determined by heating the solution until all the indicator yellow had disappeared since one or more of the unknown reactions already mentioned would probably occur. It was felt that the most satisfactory value was one which assumed that the solutions contained impurities similar to those described previously [Lythgoe, 1937]. The velocity constants for the temperatures investigated are given in Table III. At *p*H 4·75 the heat of activation was about 24,000 cal. This value was very little influenced by assuming different values for the

final density. The calculated heat of activation at *p*H 6·83 was 46,000 cal. but this value was obtained from velocity constants at only two temperatures and cannot be regarded as reliable.

Summary

1. The rate of decomposition of visual purple by heat has been measured at a variety of temperatures and concentrations of hydrogen ions. The reaction was followed by the loss of optical density with time.

2. When bleached by heat the product of decomposition, indicator yellow, appears to be identical with that formed by bleaching in light and by acids and alkalis.

3. Equations are developed to express reactions of the first and of the second orders when the product of bleaching (indicator yellow) is itself coloured. A method of correction for the destruction of indicator yellow is described.

4. The rate of thermal bleaching of visual purple solutions can be expressed best as a first order reaction. Some deviation occurs, but a closer approximation cannot be expected owing to complicated changes which occur in the solutions. The reaction is homogeneous and the chemical mechanism is either unimolecular or it is bimolecular with an excess of one reactant, probably water.

5. The Arrhenius equation is obeyed, giving a heat of activation of 44,000 cal. per g.-mol. This figure appears to be independent of the *p*H of the solutions.

6. The thermal decomposition of the solutions is accelerated by a decrease and is retarded by an increase in salt concentration.

7. The behaviour of visual purple solutions to heat is closely similar to that of protein solutions.

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