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A QUANTITATIVE STUDY OF THE CHANGES PRODUCED IN THE ABSORPTION BANDS OF CERTAIN ORGANIC FLUORESCENT DYE SOLUTIONS BY ALTERATIONS OF CONCENTRATION AND TEMPERATURE

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Abstract

The forms of the absorption bands have been obtained for solutions of rhodamin-B and eosin at different concentrations and temperatures; also for solutions of fluorescein, uranine, and resorcinol-benzein at various temperatures. It has been found that an increase in concentration produces the same general effect on the absorption bands as a lowering of the temperature of the solutions. In a concentrated aqueous solution of rhodamin-B there are two prominent bands in the visible which overlap. Change in concentration produces unsymmetrical modifications in the forms of the bands, one band growing at the expense of the other. Increase in the temperature of the concentrated solution produces the same effect on the bands as a reduction in concentration. Temperture changes produce only a slight effect on the absorption of a dilute solution. Increase in concentration of an alcoholic solution of rhodamin-B produces an increase in the absorption per molecule and a shift of the bands towards the red. Lowering the temperature of a dilute solution produces an effect similar to that due to an increase in concentration. Decrease in temperature of a concentrated solution produces an increase in absorption which is less than for a dilute solution, and produces no observable shift in the bands. Increase in concentration of an alkaline alcoholic solution of eosin produces a slight increase in absorption, and a shift of the bands towards the violet. Decrease in temperature produces a similar effect which is more marked for a dilute solution than for a concentrated solution. Increase in temperature in neutral alcoholic solutions of fluorescein and eosin causes a marked increase in the absorption, but no shift of the bands. The solutions become colorless at very low temperatures. Increase in temperature of an alcoholic solution of uranine produces an increase in the intensity of the band, and a shift of the band towards the red. Increase in temperature in alcoholic solutions of resorcinol-benzein causes a decrease in absorption with no appreciable shift of the bands.

A theory recently proposed by Merritt to account for the form of the absorption bands of the organic dye solutions on the long wave-length side has been tested with the data herein recorded and has been verified for dilute aqueous solutions of rhodamin but not for the other solutions.

SOLUTIONS of the organic dyes usually possess absorption bands that are sharply defined and relatively simple in form, while the fact that there are prominent bands in the visible spectrum makes it possible to measure the absorption by means of the spectrophotometer rather than by more complicated methods. Such solutions seem therefore specially suited for use in studying the absorption of liquids. The fact that the organic dyes often show fluorescence and anomalous dispersion adds to the significance of such a study.

The work described in the present paper deals chiefly with the influence of temperature upon the absorption of certain fluorescent dyes.

EXPERIMENTAL

Measurements of absorption were made of fluorescein, eosin, uranine, and resorcinol-benzein in alcoholic solution, and of rhodamin-B both in alcoholic and aqueous solutions. A few measurements were also made with solid films of rhodamin and of eosin.

With the exception of uranine, which was the ordinary commercial dye, from Heller and Merz, all of the materials used were of unusual purity. The rhodamin-B, prepared with special care, was furnished by the Du Pont Company. The fluorescein and eosin were prepared by A. J. Hemmer.[1] The resorcinol-benzein, prepared by C. Wang, has been studied for absorption at ordinary temperatures by Orndorff, Gibbs, and Shapiro.[2] In making the alcoholic solutions the best grade of absolute ethyl alcohol was used. The solutions were kept in a dark room in Pyrex glass.

In measuring the absorption of the solutions two similar cells were used, one containing the solution and the other the solvent alone, and the transmission of each was measured throughout the spectrum by a Lummer-Brodhun spectrophotometer. Slit width corrections for diffraction, as determined by Nichols and Merrit, were applied.[3] If T is the transmittance of the layer of the solution, i.e. the ratio of the two corrected slit widths, then the coefficient of absorption is given by

$$\beta = (1/cd) \log_{10} (1/T)$$

where d is the thickness of the layer of the solution and c the concentration in grams per liter. Since in each case the same solution was used for the whole absorption curve the use of Beer's Law, which is often violated, introduces no error. In most cases the cell-thickness was so chosen as to keep the slit-width at least as great as 0.1 mm, for slit-width corrections are uncertain for widths smaller than this.

The cells were made of two circular plates of Pyrex glass separated by a cylinder of Pyrex glass 2.5 cm in diameter, ground to the desired height. The thickness of the cells ranged from 0.008 cm to 4.00 cm and was uniform to within 0.01 mm. The thin cells were made by using glass cylinders of 3 mm height and fitting into them circular pieces of hard glass of uniform thickness so as to reduce the thickness of the liquid layer. The cells were placed in brass tubes with threaded ends, the heights of the brass cylinders being slightly less than the heights of the glass cylinders. The end plates could thus be forced tightly against the ends of the glass cylinders to prevent leakage. No cement was used. The cooling arrangement, which was designed by Professor Gibbs, consisted of a rectangular brass box 10 cm thick with a

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[1] W. R. Orndorff and A. J. Hemmer, J. A. C. S. **49**, 1272 (1927).

[2] W. R. Orndorff, R. C. Gibbs, and C. V. Shapiro, J. A. C. S. **48**, 1327 (1926).

[3] E. L. Nicholas and E. Merritt, Studies in Luminescence, Carnegie Inst. Pub., No. 152, p. 168.

capacity of about two liters. Two cylindrical brass tubes, into which the cells fitted snugly, passed parallel to each other through the box (Fig. 1). The box was insulated by a heavy coating of felt. By means of a trolley arrangement each cell could be brought in turn in front of the slit of one of the collimators of the spectrophotometer.

For temperatures above room-temperature water was placed in the box and heated with an electric heating coil. For low temperatures the cooling mixture that proved to be the most satisfactory consisted of a mixture of equal parts of carbon tetrachloride and chloroform, which was cooled by carbon dioxide snow. This forms a slush at approximately −75°C. For lower temperatures petroleum ether was used, which was first cooled down by adding carbon dioxide snow and then cooled still more by bubbling liquid air through it.

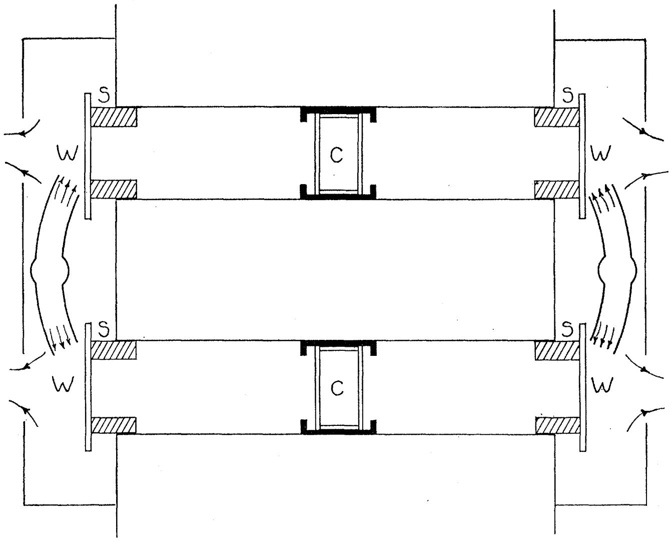

Fig. 1. Illustrating method of preventing moisture from accumulating on the cells.

Considerable difficulty was experienced in preventing moisture and frost from accumulating on the end plates of the cells. The method which proved successful is illustrated in Fig. 1. Cork stoppers, *S*, (shaded in the figure) which fitted into the cylindrical openings of the cooling box, were bored out with holes two centimeters in diameter. Glass plates were cemented on to the large ends of the corks with shellac. A heavy coating of shellac was placed on the corks to stop the pores. Two pasteboard-box covers were pierced with two holes each so spaced that when the covers were tied on against the box they were opposite the windows. Streams of dry air were directed against the windows *W* and it was found that when the air was flowing steadily no moisture collected either on the ends of the cells *C* or on the windows at a temperature as low as −90°C.

The low temperatures were measured with a calibrated pentane thermometer.

The results are shown graphically in Figs. 2 to 6, the observed absorption coefficients being in each case plotted against wave-numbers (i.e. the number of waves per millimeter).

Discussion of Results

Rhodamin-B in aqueous solution. There is a marked difference in the absorption bands of dilute and concentrated solutions (Fig. 2, I). Intermediate forms can be obtained by using intermediate concentrations. If we start with a dilute solution and gradually increase the concentration the band towards the red begins to decrease in intensity while the small band towards the violet grows until it is the more intense of the two.

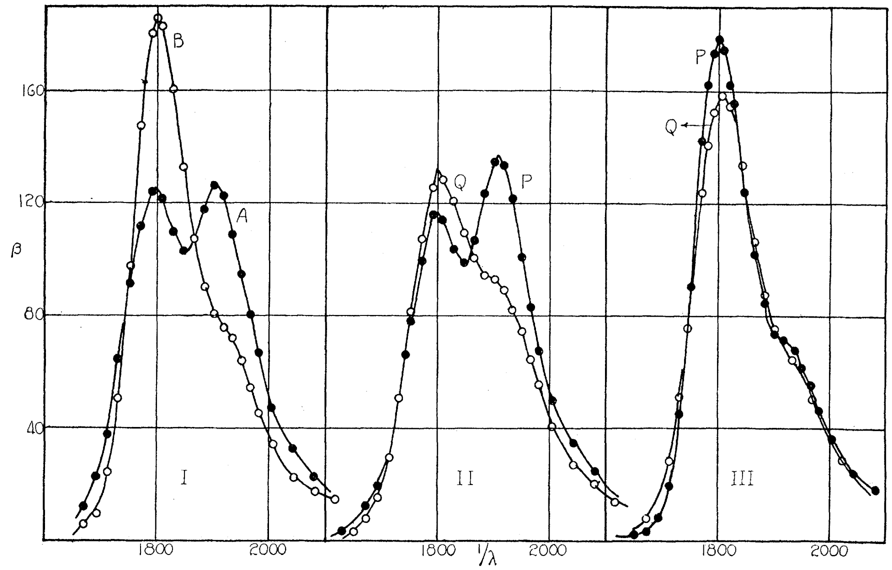

Fig. 2. Rhodamin-B in aqueous solution. I. Concentration effect: *A*, concentration 1000 mg per liter; *B*, concentration 1 mg per liter. Temperature of each 20°C. II. Temperature effect: *P*, temperature 0°C; *Q*, 90°C. Concentration of each 1000 mg per liter. III. Temperature effect: *P*, temperature 0°C; *Q*, 85°C. Concentration of each 1 mg per liter.

Increase in the temperature of a concentrated solution produces the same sort of change as decrease in the concentration (Fig. 2, II). As the temperature is lowered the more refrangible band becomes more pronounced while the intensity of the other is reduced; when the temperature is raised the shorter wave-length band is greatly reduced while the one towards the red is increased.

Changes with concentration similar to those here described have been found by Holmes[4] not only with rhodamin-B but also with a number of

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[4] W. C. Holmes, Ind. and Eng. Chem. **16**, 35 (1924).

other dyes in aqueous solution. Pringsheim[5] observed the same sort of changes in the bands of alkaline aqueous solutions of fluorescein and eosin as the concentrations were varied. The form of the bands for a concentrated aqueous solution of rhodamin-B has also been determined by Uhler and Wood.[6] The effect of temperature changes has not been previously studied.

Holmes concludes that the two bands are probably due to two constitutional forms of the material in dynamic equilibrium, and that equilibrium depends on concentration. The writer is of the opinion that the temperature effect tends to confirm this view. Equilibrium apparently depends on both concentration and temperature.

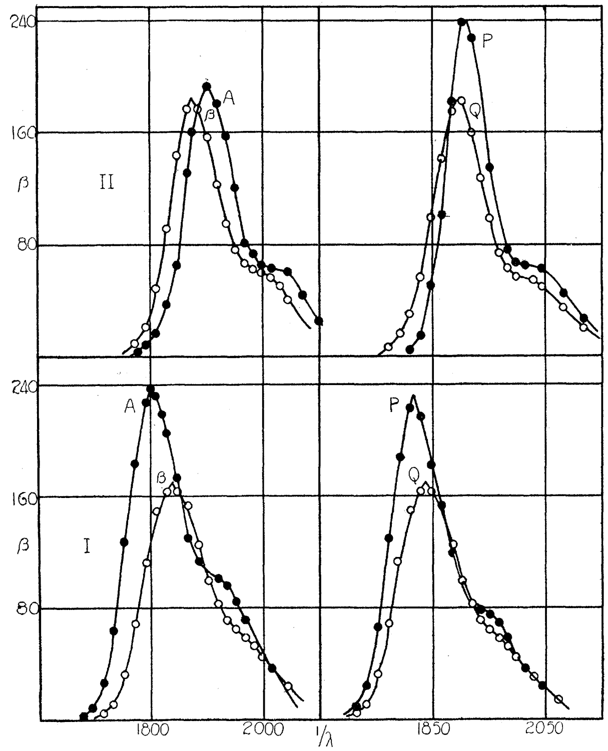

Fig. 3. I. Rhodamin-B in alcoholic solution. Concentration effect (left); *A*, concentration 500 mg per liter; *B*, concentration 1 mg per liter. Temperature of each 20°C. Temperature effect (right): *P*, temperature −70°C; *Q*, 17°C. Concentration of each 1 mg per liter. II. Eosin+12 molecules of KOH in alcoholic solution. Concentration effect (left): *A*, concentration 500 mg per liter; *B*, concentration 1 mg per liter. Temperature of each 20°C. Temperature effect (right): *P*, temperature −75°C; *Q*, 20°C. Concentration of each 2 mg per liter.

It has been found that changes in concentration and temperature affect the form of the bands less with dilute solutions than with concentrated solutions. There is no observable difference in the absorption curves for concen-

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[5] P. Pringsheim, Fluorescenz und Phosphorescenz, p.183 (1923).

[6] Uhler and Wood, Atlas of Absorption Spectra.

trations of 1 mg per liter and 2 mg per liter, whereas there is a marked difference in the curves for concentrations of 500 mg per liter and 1000 mg per liter. The effect of change in temperature for a dilute solution is shown in (Fig. 2, III). It seems reasonable that thermal agitation should destroy the equilibrium, spoken of above, more readily when the molecules of the material are close together then when they are further apart.

The observed changes are not due to any permanent alteration of the material, for the same curves were found at a given temperature no matter how often the solution had been heated and cooled.

Rhodamin-B in alcoholic solution. The effect on the absorption bands of changes in concentration is quite different from the effect on the bands of aqueous solutions (Fig. 3, I). The most striking effect is the shift of the bands towards the longer wave-lengths as the concentration is increased. It will be seen that most of the shift occurs on the long wave-length side of the band. The absorbing power increases with increase in concentration, i.e., the same number of molecules absorb more light in a concentrated solution than in a dilute one. Baly[7] found the reverse effect for pyridine, namely, that as dilution increased the coefficient of absorption increased until a certain value of dilution was reached after which there was no further change.

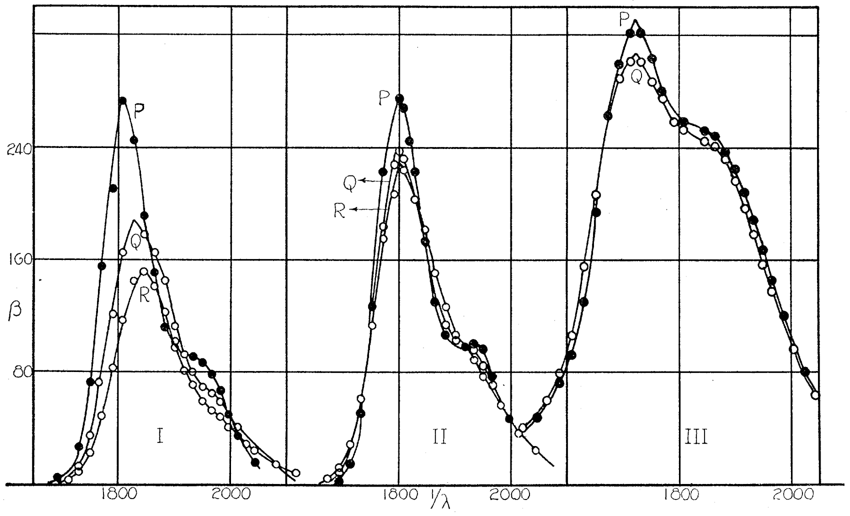

Fig. 4. Rhodamin-B in alcoholic solution. Temperature effect: I. *P*, temperature −90°C; *Q*, 18°C; *R*, 60°C. Concentration of each 4 mg per liter. II. *P*, −70°C; *Q*, 15°C; *R*, 60°C. Concentration of each 500 mg per liter. III. Film on glass plate; *P*, −75°C; *Q*, 20°C.

As in the case of aqueous solutions a lowering of the temperature produces the same effect on the bands as an increase in concentration. The similarity of the curves in Fig. 3, I is striking. The changes produced in the absorption bands by temperature variations are much more marked in dilute solutions (Fig. 4, I, II). In fact for a concentration of 500 mg per liter no shift of posi-

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[7] E. C. C. Baly, Phil. Mag. **31**, 417 (1916).

tion can be detected in the crests of the bands for a temperature change of 138 degrees. A cell, which contained a solution of concentration 500 mg per liter, was suspended in liquid air in a transparent Dewar cup and the position of maximum absorption was determined with the spectrophotometer. The peak of the band was found to remain in the same position, when the solution was frozen, as at room temperature. For a solution having an intermediate concentration of 160 mg per liter the bands shifted slightly in the same direction as for the more dilute solutions as the temperature was varied. The change in the magnitude of the absorption coefficient is not so great in concentrated solutions as in dilute solutions.

As further evidence of the reduction in temperature effect with increase in concentration, consider the curves of Fig. 4, III which were obtained for a film of alcoholic solution, evaporated to dryness on a glass plate. Here the solvent has been removed, and we have the equivalent of an infinite concentration. As will be observed the effect of change in temperature is very small. The position of the crest was determined at liquid air temperature and found to remain in the same position.

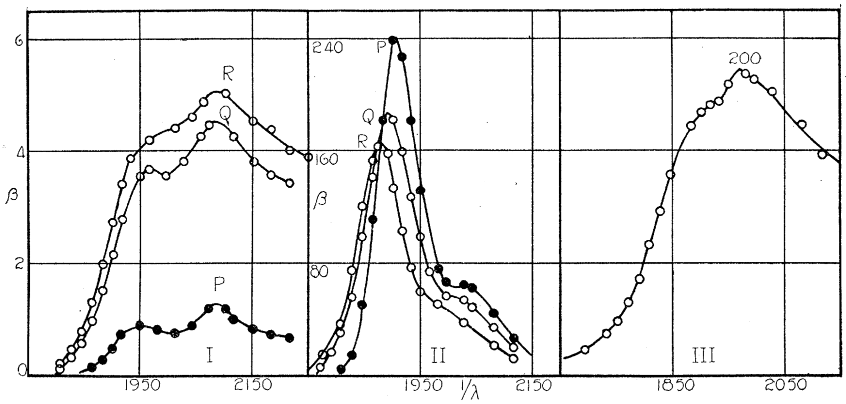

Fig. 5. I. Eosin in neutral alcoholic solution. Temperature effect: *P*, −65°C; *Q*, 20°C; *R*, 60°C. Concentration of each 500 mg per liter. II. Eosin+12 molecules of KOH in alcoholic solution. Temperature effect: *P*, −75°C; *Q*, 20°C; *R*, 60°C. Concentration of each 2 mg per liter. III. Film of eosin+12 molecules of KOH on glass plate. Temperature 20°C.

Concentrated alcoholic solutions behave like dilute aqueous solutions; both give rise to similar absorption bands in the same position in the spectrum; temperature changes produce similar effects.

The probable explanation which has been given for the behavior of rhodamin in aqueous solution will not hold for alcoholic solutions, for there is not the growing of one band at the expense of the other with alcoholic solutions as the concentration and temperature are varied. Moreover, the temperature shift for dilute aqueous solutions is not so marked as for dilute alcoholic solutions. The solvent plays a decided role.

An explanation which is sometimes given for the shift of the bands resulting from change in concentration is the combination, or tendency towards combination, of the molecules of the solute with those of the solvent, which results in a loading of the molecules. It is a well established fact that a loading of the molecules of a substance, which absorbs selectively, causes a shift of the bands towards the long wave-lengths and an increase in absorbing power. For example, neutral fluorescein and neutral eosin have similar absorption bands, but the eosin bands are further towards the red and the absorbing power of eosin is greater than that of fluorescein (Figs. 5, I and 6, I). (Eosin is formed from fluorescein when four bromine atoms replace four hydrogen atoms). But the loading of the molecules of the rhodamin by a tendency to combine with the alcohol will hardly account for the facts, because it seems reasonable that the loading per molecule would be greater in a dilute solution than in a concentrated solution, and that the absorption per molecule would increase with dilution, which is the reverse of what has been found. It may be that there is a tendency towards association of the rhodamin molecules in alcohol which would result in what would be equivalent to a loading of the molecules. It seems reasonable also that a lowering of the temperature of a dilute solution would aid association which would account for the shifts towards the red and increase in absorbing power.

Eosin+12 molecules of KOH in alcohol. The description which has been given of the changes produced in the absorption of alcoholic solutions of rhodamin by alterations of concentration and temperature applies to alkaline alcoholic solutions of eosin except in one particular, namely, the bands shift in the opposite direction from those of rhodamin (Figs. 3, II and 5, II, III). It has been found that shifts of the bands are more marked for a dilute solution than for a concentrated solution, as is true of rhodamin in alcoholic solution. The curve for the film is similar to the curves which Pringsheim[5] found for very concentrated solutions of alkaline eosin.

The behavior of alkaline eosin is more in accord with the behavior of dye solutions in general than that of rhodamin. Investigators have found that in nearly all cases where there is a shift of the bands due to a decrease in concentration the shift is towards the red. Moreover, it is the general rule that an increase in temperature causes the bands to shift towards the red. No satisfactory explanation of these changes has been given.

Eosin and fluorescein in neutral alcoholic solution. (Figs. 5, I and 6, I). The absorption bands of the two solutions are similar except for position, the fluorescein bands being further towards the violet. Temperature effects are similar, an increase in temperature causing the absorption to increase. No shift occurs in the crests of the bands as the temperature is varied. Both solutions become nearly colorless at low temperatures.

Ionization might possibly account for the changes. If the absorption bands were due to ions of definite types there would be no shift of the bands as the number of ions varied, but the amount of the absorption would depend on the number of ions. The ionization would probably increase with a rise in temperature which would account for the observed increase in absorption as the temperature is increased.

Resorcinol-benzein in neutral alcoholic solution. The two absorption bands are similar to those of neutral fluorescein, but the absorbing power of the solution is greater. Since the two substances are similar in chemical constitution it might be expected that temperature changes would produce similar effects on the bands. Such is not the case however, for the absorption of the resorcinol-benzein solution increases with a lowering of the temperature while the reverse effect is found for the fluorescein solution (Fig. 6, I and II). The behavior of these two chemically similar substances shows very strikingly that there is as yet no way of predicting how a change in temperature will affect the absorption of a given substance in solution.

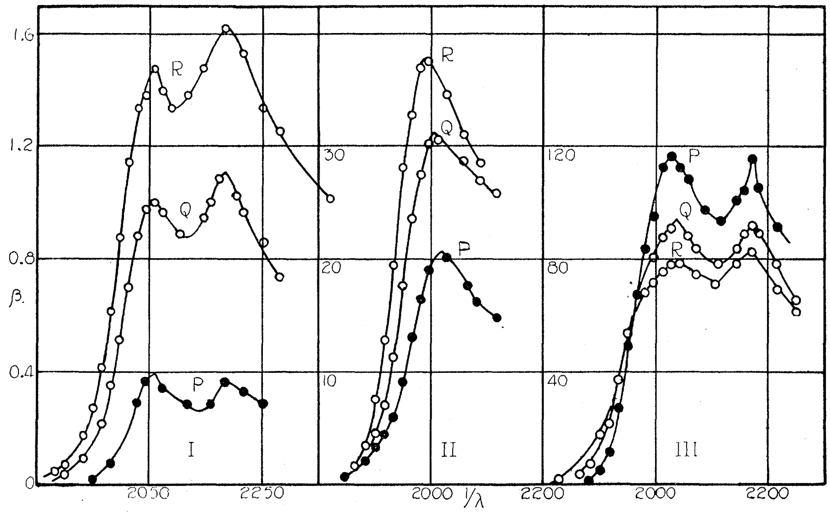

Fig. 6. I. Fluorescein in neutral alcoholic solution. Temperature effect: *P*, −55°C; *Q*, 16°C; *R*, 60°C. Concentration of each 500 mg per liter. II. Uranine in alcoholic solution Temperature effect: *P*, −60°C; *Q*, 20°C; *R*, 60°C. Concentration of each 20 mg per liter. III Resorcinol-benzein in neutral alcoholic solution. Temperature effect: *P*, −60°C; *Q*, 20°C *R*, 60°C. Concentration of each 6 mg per liter.

Uranine in alcoholic solution—(Fig. 6, II). Uranine is a sodium salt of fluorescein about 95 per cent pure, sodium carbonate and salt. It might be expected that uranine would behave like alkaline eosin since both are alkaline forms of fluorescein. It does not, as an inspection of the curves reveals, except in one particular, namely, an increase in temperature causes the bands to shift towards the red.

Test of Theory proposed by Merritt. In developing a theory to account for the great width of the absorption bands in solutions as compared with the narrow bands that are observed in gases, Merritt[8] finds that in certain cases it is to be expected that the relation between the absorption coefficient and the frequency on the red side of the band will be given by an expression of the form

$$\beta = Ke^{sh\nu/kT}$$

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[8] E. Merritt, Phys. Rev. **28**, 684, (1926).

where h is Planck's constant, k is Boltzman's constant, T the absolute temperature, and s a constant determined by the relative values of the electric moment of the absorbing molecule in the normal and the excited states. This relation has been verified by Merritt in the case of dilute aqueous solutions of rhodamin-B and uranine at room temperature and for these substances s was found to be very nearly unity.

To test the relation the values of $\log \beta$ for the long wave-length side of each band were plotted against wave-numbers. If the above relation holds a straight line should result; and if the number of absorbing molecules is not altered by temperature changes—a condition that is most likely to be met in the case of dilute solutions—the theory shows that the slant of this line should be proportional to $1/T$.

In the case of dilute solutions of rhodamin both in water and in alcohol, straight lines were in fact obtained and the slopes of the lines increased with a lowering of the temperature as demanded by the theory. For dilute aqueous solutions the slopes were also nearly in the inverse ratio of the absolute temperatures; the ratio of the slopes was 5 to 10 per cent greater than the inverse ratio of the absolute temperatures. But in the case of dilute alcoholic solutions the ratio of the slopes was about 20 percent less than the inverse ratio of the absolute temperatures. With the concentrated solutions there was scarcely any approach to agreement with the relation that would be expected if K were constant. Since a considerable temperature change in the number of absorbing molecules is to be expected in concentrated solutions as the result of dissociation or temporary chemical changes, this is not surprising.

In conclusion, the writer wishes to express his indebtedness to Professor Merritt, at whose suggestion and under whose guidance the work was done; and to Professor Gibbs and Mr. C. V. Shapiro for helpful suggestions.

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September, 1927.