band of capri blue was first found it seemed necessary to show that this band could not be due to an impurity. Some of the capri blue was converted into leuco base at *p*H 7.5, was partially shaken out with purified ether, and later partially reoxidized. In spite of the considerable opportunities for fractionation thus afforded the ratio of $\epsilon_{\max.}$ for the two bands was within 3% the same as before. The sample of dinitrodibromofluorescein that we first employed was an old commercial sample that we distrusted. A new sample was therefore prepared by the standard method[16] and fractionated. The absorption spectrum was identical with that given by the old sample, both in fuming sulfuric acid and in dilute alkali, although in the latter case our sample showed a little sharper maximum and therefore presumably was a little purer.

In the experiments on the vacuum bench the stannic chloride was fractionally distilled, the middle portion being employed. The tube containing the dye was exhausted, dry methyl cyanide was distilled in, and then the desired amount of stannic chloride was distilled in and the tube sealed off for the spectrometric measurements. It was impossible to obtain high enough concentrations of stannic chloride to give the second acid addition, since the methyl cyanide is a sufficiently strong base to neutralize stannic chloride to form a white compound, not extremely soluble.

Summary

When in aqueous solution acid is added to dyes of the type of methylene blue the first addition of H^+ is at the central nitrogen, giving a band of lower frequency than that of the original dye. But with capri blue the first addition is to one of the amino nitrogens and a band of higher frequency than the original. These facts are interpreted in terms of a rule of Lewis and Calvin. In the case of capri blue, however, a small amount of the isomeric form, with H^+ on the central nitrogen, is found. In more than 70% sulfuric acid a second H^+ adds. Here there are several isomers possible of which two are found with capri blue, three with methylene blue and the change in the relative amounts of the latter in going from 78% to fuming sulfuric acid is found.

In experiments in which the added acid is not H^+ but boron trichloride or stannic chloride in acetone, and methyl and phenyl cyanides, the extraordinary similarity but not complete identity of the absorption curves of these addition compounds and those with H^+ is exhibited in the cases of methylene blue, capri blue, and Bindschedler's green. In the case of capri blue an interesting steric repulsion between stannic chloride and the dimethylamino group is exhibited.

Another class of dyes, phenolphthalein, eosin and dinitrodibromofluorescein, are indicators in the range from 95% to fuming sulfuric acid and illustrate further the difference between acid addition at central and end positions.

By using dinitrodibromofluorescein as indicator, we have been able to extend the determination of the acidity function of Hammett and Deyrup well into fuming sulfuric acid. It is shown that in the more concentrated acids used by those authors a large part of the color change results from the addition not of H^+ but of another acid, namely, sulfur trioxide. In the range of our titrations and presumably all the way to pure SO_3 the acidity function $(-H_0)$ measures not $\log a_{H^+}$ but $\log a_{SO_3}$.

Berkeley, Calif. Received February 23, 1943

[16] See Colour Index No. 771.

[Contribution from the Chemical Laboratory of the University of California]

Dimeric and Other Forms of Methylene Blue: Absorption and Fluorescence of the Pure Monomer[1]

By G. N. Lewis, O. Goldschmid, T. T. Magel and J. Bigeleisen

In order to understand the absorption spectra of dyes as a class it is desirable to analyze the absorption of a few typical dyes in the fullest detail. Having studied in the preceding paper the spectra of compounds between methylene blue and acids, which compounds are obviously stoichiometrically different from the parent dye, it remains to consider those forms that are, or appear to be, stoichiometrically identical.

This task is simplified by the work of Rabinowitch and Epstein[2] on the dimeric ion, which they conclude exists in considerable amount in a solvent of high dielectric constant, such as water, where the coulombic repulsion is not great enough to overcome the addition forces.

In ethanol or 95% ethanol no appreciable amount of dimer was found, at least in the en-

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[1] This paper is a collection of sporadic work done through a period of four years. The first experiments on absorption and the study of the fluorescence spectrum were carried out by O. G. The construction of the spectrophotometer and the first experiments on the molecular polymer were made by T. T. M. All the remaining experiments were made by J. B.

[2] Rabinowitch and Epstein, This Journal, **63**, 69 (1941).

tirely similar case of thionine. We find, however, that at low temperatures the dimeric band of methylene blue becomes pronounced even in ethanol; for, not only must the amount of dimerization increase with lowering temperature when ΔH is constant, but here the ΔH of dissociation must become considerably greater at low temperatures, owing to the effect of changing dielectric constant upon the coulombic repulsion between the ions.

We show in Fig. 1, curve 1, the molar extinction coefficient of methylene blue in ethanol at room temperature and this may be taken as the curve of the pure monomer. Curves 3 and 4 show relative extinction coefficients at 0.69×10^{-5} M and 2.2×10^{-5}, respectively, in 95% ethanol at 110°K. Taking the band at 16500 cm.$^{-1}$ in curve 4 as due chiefly to the dimer and using the mass law, we may find the amount of dimer left in the more dilute solution of curve 3. Thence by trial and error we obtain curve 2 for the pure monomer at 110°K. The calculation was made a little awkward by the fact, noted by Rabinowitch and Epstein,[2] that the band of the dimeric ion nearly coincides with the one of the subsidiary bands of the pure monomer.

The correctness of curve 2 has been demonstrated in the course of other work in which leuco methylene blue was illuminated[3] in a rigid solvent. Among other interesting bands, that of methylene blue is in some cases very prominent and is identical with the one shown in curve 2.

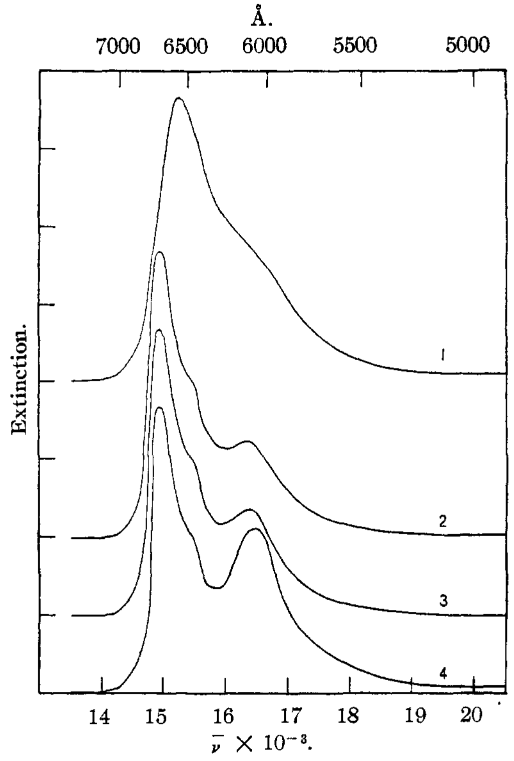

Fig. 1.—Absorption spectrum of methylene blue (1) in ethanol at room temperature—pure monomeric ion; (2) in 95% ethanol at 110°K. and infinite dilution—pure monomeric ion; (3) and (4) in 95% ethanol at 110°K. and at 0.69 and 2.2×10^{-5} M, respectively—showing the band of the dimeric ion, which nearly coincides with a subsidiary band of the monomer.

The Polymerization of Methylene Blue Chloride in Solvents of Low Dielectric Constant

The dimerization of methylene blue ion, which is prominent in water, disappears when we go to solvents of lower dielectric constant. However, if we proceed further in the same direction a new phenomenon appears. If to a solution of methylene blue in alcohol we gradually add hexane, we come to a point where rather abruptly the blue color changes to a pink, and if more hexane is added a pink precipitate appears.

When, in order to obtain the absorption spectrum of methylene blue at low temperature, methylene blue was cooled to liquid air temperatures in EPA (5 ether, 5 isopentane, 2 alcohol by vol.)[3] a queer absorption curve was obtained. This is shown in curve 1 of Fig. 2. At an intermediate temperature, 193°K., we obtained the absorption shown in Fig. 2, curve 2. At this temperature the band at about 20300 cm.$^{-1}$ diminishes with increasing dilution in a way that is roughly consistent with the assumption of a dimeric molecule. But as the temperature is lowered, or the percentage of hydrocarbon in the solvent is increased, the new band not only increases in intensity but the maximum moves far toward the red until we reach the condition seen in curve 1. We assume that the polymerization does not stop with the dimer but increases to higher aggregates all of which presumably are electrically neutral, containing equal numbers of methylene blue ions and chloride ions. These may be called molecular polymers to distinguish them from the ionic dimers of the preceding section. It will be noted that this case is very different from that of crystal violet where the production of ion multiplets caused no new bands to appear, but only slightly shifted the position of the monomeric bands.

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[3] The method was the same as that of Lewis and Lipkin, THIS JOURNAL, 64, 2801 (1942).

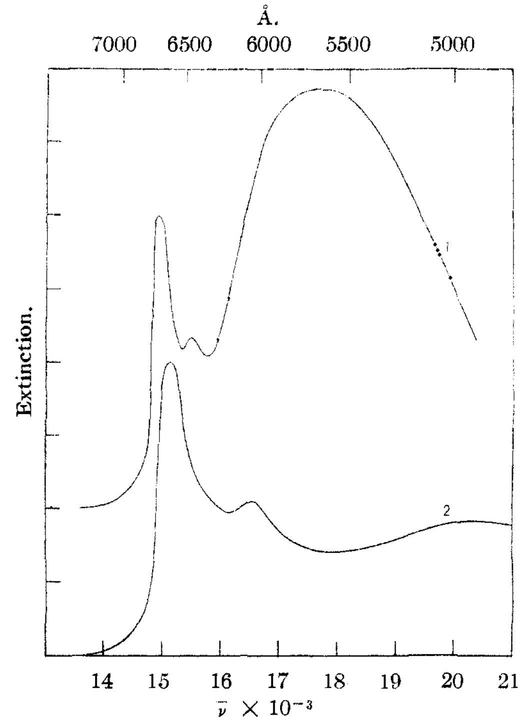

Fig. 2.—Absorption (arbitrary ordinates) of methylene blue in EPA at about 3×10^{-5} *M* (1) at 90°K. and (2) at 193°K. Curve 2 shows at 16500 cm.$^{-1}$ a little of the dimeric ion as well as the broad band at 20300 cm.$^{-1}$ of the dimeric molecule. The greater part of the absorption shown in curve (1) is attributed to higher polymeric molecules.

Amphoteric (Hydrolytic) Addition

In the paper just preceding, on methylene blue as indicator, we have not discussed the behavior of such dyes in basic solution, complicated as it is by formation of colloidal or insoluble substances, and also by demethylation and other chemical changes. However, there is a type of addition, independent of *p*H, to which we must now give attention.

When we determined the molar extinction of methylene blue in water, at so low a concentration that the amount of dimer is negligible, we obtained a curve very similar to the curve in ethanol given in the paper just preceding. The maximum absorption in water was slightly shifted to 15040 cm.$^{-1}$ but $\epsilon_{\text{max.}}$ dropped from 9.2×10^4 in ethanol to about 8.4×10^4 in water, at 6.3×10^{-7} *M*. Rabinowitch and Epstein obtained in their most dilute solution only 3.8×10^4 in a buffered water solution. While we have been unable to explain this great discrepancy, a review of their work together with our own experiments leads to the conclusion that in all aqueous solutions both of methylene blue and thionine a considerable part of the dye is in some colorless form, the amount being essentially independent of *p*H.

The only substance that seems consistent with this behavior is the one that would be obtained by simultaneous addition of OH^- at the central nitrogen and of H^+ at one of the amino nitrogens. Such an addition may be called a hydrolytic, or more generally an amphoteric, addition.[4] Using the same numbering system as in the paper just preceding, we may write the reaction

$$D^+ + H_2O = D^{1,3}OH,H^+ \qquad (1)$$

Inspecting Fig. 4 of Rabinowitch and Epstein,[2] showing the effect of temperature on the absorption of thionine, and considering only the absorption peak of the monomer D^+ (5970 Å.), we see that this peak at their two highest concentrations always increases with the temperature. However, at their lowest concentration of 2.5×10^{-5} *M* the height of the peak changes very little with the temperature and even appears to have a maximum at about 30°. It would appear by extrapolation that at still lower concentration the temperature coefficient of $\log I_0/I$ at room temperature would be negative.

We have verified this conclusion by experiments on methylene blue, which shows an almost identical behavior. In the more concentrated solutions a high positive temperature coefficient of ϵ is found. But as we proceed to lower concentrations we obtain the results shown in Fig. 3. At the highest of the three concentrations and at the lowest temperature, the temperature coefficient is chiefly due to the dissociation of $(D^+)_2$. But in the case represented by curve 1 where the amount

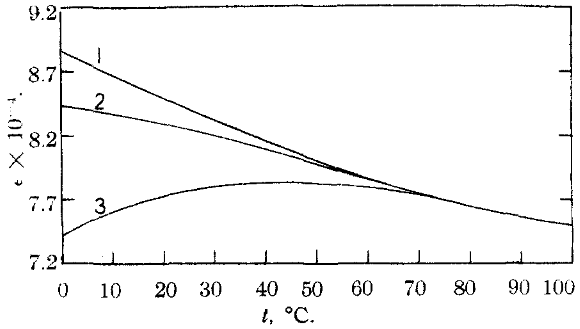

Fig. 3.—Molar extinction coefficient of methylene blue in water at 6600 Å. as a function of the temperature: (1) 6.3×10^{-7} *M*; (2) 1.9×10^{-6} *M*; (3) 9.0×10^{-6} *M*. Correction has been made for thermal expansion.

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[4] Similar addition products in the case of crystal violet were assumed by Adams and Rosenstein, This Journal, **36**, 1452 (1914).

of ionic dimer has become negligible, we are measuring the change in the equilibrium between the monomer and the colorless form. It seems remarkable from equation (1) that the colorless form which is produced by hydrolytic addition should increase with the temperature; but we must remember that a dye such as methylene blue is strongly hydrated. It seems likely that there actually is a net loss of water as the reaction goes from left to right.

It is evident that Rabinowitch and Epstein were measuring the simultaneous effect of temperature on more than one equilibrium and that therefore an appreciable correction must be made to their heat of dimerization.

The realization that we have the three substances D^+, $(D^+)_2$, and $D^{1,2}OH,H^+$ in equilibrium also enables us to interpret the change in the absorption curve of thionine in water, observed by Rabinowitch and Epstein when the solution is made 1 *M* in potassium chloride. There is evidently a very great increase in the amount of dimer but we get very different values for the amount of this change if we (1) measure the relative changes in ϵ at 5970 and 5570 Å. or (2) calculate from the change in the height of the monomer band alone, assuming that as it disappears only dimer is formed. The discrepancy can be explained only on the assumption of a colorless form.

Whenever two unipositive ions unite to form a bipositive ion we expect from our knowledge of activity coefficients that the amount of dimer will greatly increase with increasing ionic strength, but we cannot calculate this effect quantitatively with ions so peculiar as these we are dealing with.

It has been shown in the discussion of crystal violet[5] that the remarkable salt effects which this substance shows, even in extreme dilution, are due to the unusual nature of the dye ion. In small unipositive ions the positive charge is localized. In some large unipositive ions, especially those of high polarizability, the positive charge is less definitely localized, and may be drawn from one part of the ion to another by an electric field. In ions such as those of crystal violet and methylene blue such induction is undoubtedly important. Here, however, a new phenomenon appears. Owing to the great rapidity of the resonance phenomenon, the ion behaves as though a unit charge appears to be distributed between several widely separated spots; so that we may say that a fraction of the charge is localized at an amino group, another fraction at another group. The result is that a negative ion can closely approach only a fraction of the positive charge. Thus these ions, which may be called ions of *distributed charge*, behave as though they had less than unit charge. Their activity coefficients will fall off less rapidly with the ionic strength than in the case of typical unipositive ions, and this essential difference will continue to infinite dilution.

Thus once more considering equation (1) we have on the right a normal unipositive ion, but on the left an ion which will behave as though it had less than unit positive charge. Therefore with increasing ionic strength we should increase the fraction of colorless form. This we have shown experimentally, and we find that with methylene blue at infinite dilution the height of the main monomeric band is decreased 5% when the solution is made 1 *M* in potassium chloride and nearly as much with 1 *M* potassium nitrate.

This significant change, however, does not account for enough colorless substance to explain the large effect of salt that we find in the more concentrated solutions of methylene blue nor the still larger effect found for thionine. Hence there is no doubt that the colorless substance also polymerizes. Rabinowitch and Epstein found a reversal of the temperature effect in 75% ethanol. Here the $(D^+)_2$ is less important and their results can be interpreted as due to hydrolytic or solvolytic addition.

The Fluorescence of Methylene Blue

The absorption curve of the monomeric ion of methylene blue has a shoulder to the right of the main peak closely resembling the one found in crystal violet. In the latter case it has been shown[5] that the subsidiary band is due to an isomer. However, if the molecule of methylene blue is planar, and all the evidence points in this direction, there seems to be no opportunity for such isomerism. That in this case the shoulder is due merely to the vibrational resolution of a single electronic band was shown as follows.

It was pointed out by Lewschin[6] and has been very thoroughly illustrated by Hausser, Kuhn and Kuhn[7] that in the case of a single electronic band with vibrational resolution the fluorescence spectrum should be a sort of mirror image of the absorption spectrum. That this is the case with

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[5] Lewis, Magel and Lipkin, THIS JOURNAL, **64**, 1774 (1942).

[6] Lewschin, *Z. Physik*, **72**, 368 (1931).

[7] Hausser, Kuhn and Kuhn, *Z. physik. Chem.*, **29B**, 417 (1935).

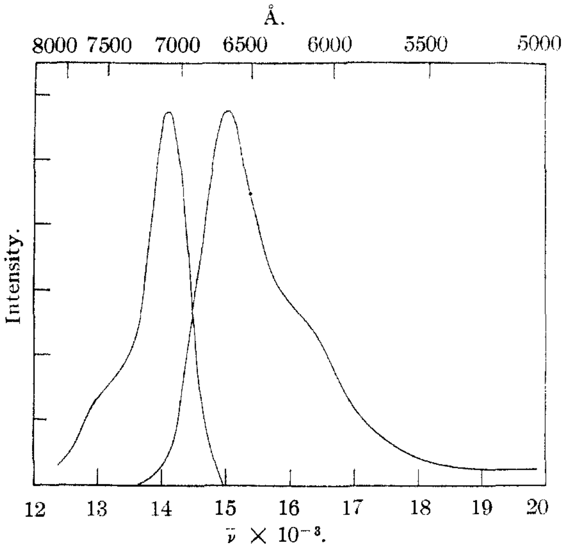

Fig. 4.—The absorption and fluorescence of methylene blue: right, absorption curve in glycerol at room temperature; left, emission curve in glycerol at −25°; the ordinates are direct microphotometer readings, no corrections having been made for plate character; nor has correction been made for self absorption, as shown by the rapid falling off on the high frequency side of the fluorescence curve.

methylene blue is shown in Fig. 4 which gives the absorption and emission curves of methylene blue in glycerol. Finer structure could doubtless be obtained, for example in 95% ethanol at 110°K., but this figure will suffice to show that the subsidiary bands of methylene blue are vibrational.

Summary

The dimeric methylene blue ion which Rabinowitch and Epstein demonstrated in aqueous solution also occurs in 95% ethanol at low temperature and at high concentration. The absorption curve of the pure monomeric ion is obtained in ethanol at room temperature and, by extrapolation, in 95% ethanol at 110°K. The latter curve is identical with one obtained by illuminating leuco methylene blue at low temperature in a rigid solvent.

In addition to the ionic dimers in solvents of high dielectric constant, molecular dimers and polymers are found in solvents of low dielectric constant. Their absorption curves are given.

There is a colorless form of the ion obtained by hydrolytic addition, in which a hydroxide ion goes to the central nitrogen and a hydrogen ion to one of the amino nitrogens. It is shown that in pure water, and especially in a molal salt solution, a considerable part of the dye is in the colorless form. At infinite dilution in water the absorption of methylene blue at its main peak diminishes with increasing temperature, in contrast with the increase found by Rabinowitch and Epstein in concentrated solutions. Except at infinite dilution the colorless ion itself polymerizes. In order to explain the effect of salts, the concept of an ion of a *distributed charge* is introduced.

By comparing the fluorescence and absorption spectra it is shown that the subsidiary absorption bands are due to the vibrational resolution of a single electronic band.

Berkeley, Calif. Received February 23, 1943

[Contribution from the Division of Plant Nutrition, College of Agriculture, University of California]

The Separation and Quantitative Estimation of Amylose and Amylopectin in Potato Starch

By R. M. McCready[1] and W. Z. Hassid

It has long been recognized that starch can be separated into two fractions of widely different physical properties. Maquenne and Roux[2] designated these fractions as amylose and amylopectin. The former is easily soluble in water and forms a slightly viscous solution; the latter is more insoluble and gives relatively opalescent and highly viscous solutions. Various methods have been employed to isolate the two starch fractions, but no quantitative separation has yet been effected. Different workers reported the amylose fraction to vary from 17 to 60%.[3] It now appears that the fractions designated as amylose and amylopectin were by no means identical, and did not represent the same portions of the starch.

When starch grains are swollen in water at 60 to 80°, they are not ruptured, but the crude

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(1) Present address, Western Regional Research Laboratory, Albany, California.

(2) L. Maquenne and E. Roux, *Compt. rend.*, **137**, 88 (1903).

(3) Z. Gatin-Gruszewska, *ibid.*, **146**, 540 (1908); H. C. Sherman and J. C. Baker, This Journal, **38**, 1885 (1916); A. R. Ling and D. R. Nanji, *J. Chem. Soc.*, **123**, 2666 (1923); T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941).