case examined was a value found comparable with the antiseptic strength of phenol.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE COLOR LABORATORY, BUREAU OF CHEMISTRY, JOINTLY WITH SOIL FERTILITY INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE, NO. 100]

THE SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATIONS AND OF THE APPARENT DISSOCIATION CONSTANTS OF INDICATORS. II. THYMOL BLUE[1]

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In the Sörensen (*P*H) range between 4.5 and 6.0, thymol blue (thymolsulfonephthalein) exists in what will be termed its "mid-form," in which it is yellow, with an absorption band in the violet portion of the spectrum. As the alkalinity of the solution is increased over the *P*H range 6.0 to 12.0, the indicator is progressively and completely transformed into its alkaline form in which it is violet-blue, with an absorption band in the yellow and

TABLE I

THYMOL BLUE: ALKALINE RANGE

*P*H Corr.	Temp. °C.	Indicator per liter of solution Mg.	*E* at 460 μμ	*E* at 595 μμ	R_2	R_1	*K*
5.2	24	40	0.70	...	∞	0.00	..
7.25	24.5	80	1.38	0.07	19.7	.017	(9.01)
7.73	23.5	80	1.35	.23	5.87	.056	(8.96)
7.94	24	80	1.30	.37	3.51	.090	(8.94)
8.31	26.5	40	0.59	.42	1.40	.204	(8.90)
8.50	24.5	40	.56	.57	.98	.279	8.92
8.71	23.5	40	.50	.79	.63	.388	8.91
8.91	22	40	.42	1.03	.40	.507	8.90
9.09	24.5	40	.36	1.25	.29	.610	8.89
9.13	25.5	32	.28	1.035	.27	.631	8.90
9.31	25	32	.25	1.165	.215	.710	8.92
9.53	23.5	32	.205	1.325	.155	.808	8.91
9.75	23.5	32	.18	1.425	.126	.869	8.92
9.98	23	32	.155	1.51	.10	.922	(8.93)
10.74	24	32	.125	1.595	.08	.972	(9.00)
11.37	24.5	32	.125	1.625	.077	.991	(9.37)
0.05 *M* NaOH	24	32	.12	1.64	.075	1.00	..

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[1] Presented before the Dye Section of the American Chemical Society at its Washington meeting, April 21–26, 1924.

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red portions of the spectrum, the maximum of which lies at approximately 596 $\mu\mu$.

Within this range of transformation the degree of transition at any point, which is dependent upon, and may be employed as a measure of, the hydrogen-ion concentration of the solution, may be determined conveniently by spectrophotometric measurements, and given expression in ratios of two types, of which the first (R_1) records merely the degree of development of the band of the alkaline form of the indicator, whereas the second (R_2) records the relative intensities of the respective bands of the mid-form and alkaline form.

The buffer solutions employed were the standard 0.05 M solutions of Clark evaluated by electrometric means. The determinations were made with a well shielded system, comprising a Type K potentiometer in connection with a high-grade galvanometer and a Bureau of Standards certified Weston cell, the entire system having been frequently checked against Clark and Lubs' 0.05 M potassium acid phthalate solution. The recorded Sörensen values have been corrected for minor variations between the temperatures of the electrometric and spectrophotometric measurements on the basis of the data supplied by Walbaum.[4] The recorded temperatures are those of the spectrophotometric measurements.

The absorption measurements were carried out in 1cm. cells with a Hilger wave length spectrometer provided with a Nutting photometer. The recorded extinction coefficients E are averages of numerous duplicate determinations. The R_2 values are ratios of E at 460 $\mu\mu$ to E at 595 $\mu\mu$, whereas the R_1 values are ratios of E at 595 $\mu\mu$ to the corresponding value for an equal concentration of indicator in 0.05 M sodium hydroxide solution.

The apparent dissociation constant K of the indicator may be calculated from the values of either type of ratio. The percentage dissociation D (or the percentage of indicator present in alkaline form) is obtained directly from R_1 values ($D = 100R_1$). Less accurate data may be obtained from R_2 values by means of the formula $D = \frac{100\,c}{R_2\,a + (c - b)}$, where a $= E$ at 595 $\mu\mu$ in 0.05 M sodium hydroxide solution, $b = E$ at 460 $\mu\mu$ in the same solution and $c = E$ at 460 $\mu\mu$ in a solution of P_{H} 5.2. (In the application of the formula it is essential to reduce all values for E to a common basis of indicator concentration.) The calculation of K is made by means of the formula $K = P_{\text{H}} - \log\,[D/(100-D)]$, where D is greater than 50, and by the formula $K = P_{\text{H}} + \log[(100-D)/D]$, where D is less than 50. The tabulated data are based on R_1 values.

The average of eleven values of K, obtained within the portion of the range wherein the dissociation is normal, is 8.91. The value obtained by

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[4] Walbaum, *Biochem. Z.*, **107**, 219 (1920).

Brode[5] was 8.90. Clark and Lubs[6] obtained 8.9 by visual methods and Gillespie,[7] 8.82.

The experimental values of R_1 are plotted against Sörensen values in Fig. 1. Within the limits of experimental accuracy, the resulting curve conforms to the theoretical dissociation curve over the range between less than 10% dissociation and more than 90% dissociation, corresponding to a range of approximately one P_H unit on either side of the mid-point. In the application of the method the employment of the theoretical dissociation curve for the dissociation constant of 8.91 is advocated over this range. Since the diagnostic value of measurements decreases rapidly as these limits are exceeded, the employment of the spectrophotometric method outside this range is of questionable advisability. If such application is made, it should be upon the basis of experimental data rather than upon that of theory.

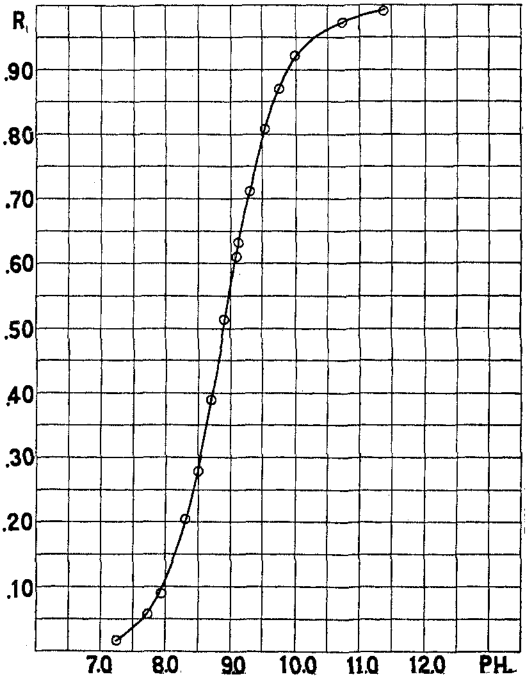

Fig. 1.—Thymol blue: alkaline range.

The values of R_2 obtained are somewhat less accurate, and it appears probable, as was anticipated, that this type of ratio will find but limited application with the sulfonephthaleins, owing to the difficulty encountered in carrying out precise measurements in the violet. With indicators of characteristics similar to those of 1-naphthol-2-sodium sulfonate indophenol, with which both bands occur in regions wherein the eye is relatively sensitive, it affords a decided gain in accuracy.[8]

Since values of R_2 are constant under varying conditions of indicator concentration, however, that type of ratio may be employed to advantage in determining the hydrogen-ion concentration of solutions of such small

[5] Brode, This Journal, **46**, 581 (1924).

[6] Clark and Lubs, *J. Bact.*, **2**, [1] 109, 191 (1917).

[7] Gillespie, *Soil Science*, **9**, 115 (1920).

[8] With respect to this indicator a typographical error was made in the previous paper of this series [This Journal, **46**, 627 (1924)]. The suitable wave lengths for employment are 500 μμ and 625 μμ instead of 550 μμ and 625 μμ as stated.

volume that it may be difficult or impossible to insure a precise and known concentration of indicator. The data of the present investigation are not recommended for general employment in that connection. In view of probable variation in sensitivity of observation in the violet, it will doubtless be advisable for the individual analyst to obtain data on the basis of his own measurements.

Thymol Blue: Acid Range

As the acidity of solutions of thymol blue is increased over the *P*H range 4.5 to about —2.0, there is a second progressive and complete transformation of the indicator into its acid form in which it is red, with an absorption band in the yellow, the maximum of which lies at approximately 544 $\mu\mu$.

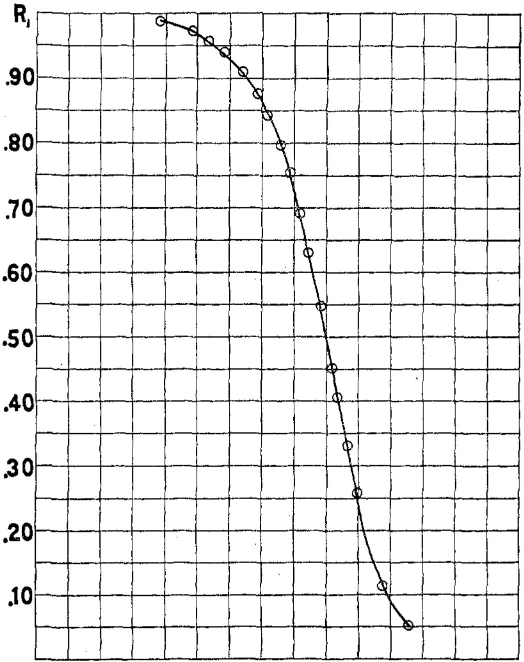

Fig. 2.—Thymol blue: acid range.

The same principles of spectrophotometric evaluation are applicable in both the acid and alkaline ranges of the indicators. Whereas the absorption of alkaline solutions of thymol blue remains unaltered over a period of days or even weeks, that of acid solutions decreases in an appreciable degree within 24 hours. This effect, which may probably be attributed to a process of increasing molecular aggregation, does not impair the accuracy of measurements which are carried out within a reasonably brief period after the addition of the indicator to the solution to be evaluated.

The buffer solutions of Clark were employed to the *P*H value of 1.2 (at which point the spectrophotometric measurements of Brode were discontinued). The *P*H range 1.2 to —1.1 was covered with hydrochloric acid solutions of suitable concentrations, while a portion of the same range (*P*H 1.2 to 0.4) was also covered with mixtures of hydrochloric acid and potassium chloride. Within the *P*H range 0.2 to —1.1 the electrometric measurements were found to be reproducible to within only about 2 mv., but above *P*H 0.2 the agreement in determinations was excellent.

Within the range 1.2 to 0.4 the spectrophotometric measurements on the two types of solutions were in satisfactory agreement. In view of this result and of the data obtained on solutions of phenolsulfonephthalein by Brightman, Meecham and Acree,[9] it may be concluded that the effect of such quantities of salts as are contained in 0.05 *M* buffer solutions upon the absorption of the sulfonephthaleins is practically negligible.

The results obtained in the acid range of thymol blue are summarized in Table II. No data were available for the correction of *P*H values in compensation for differences between the temperatures of the electrometric and spectrophotometric measurements, but such differences were relatively small.

TABLE II

THYMOL BLUE: ACID RANGE

*P*H	2.79	2.39	1.98	1.83	1.68	1.59	1.41	1.23	1.10
R_1	0.053	0.114	0.257	0.325	0.405	0.450	0.548	0.630	0.690
K	(1.54)	1.50	1.52	1.51	1.51	1.50	1.49	(1.46)	(1.45)
*P*H	0.94	0.78	0.59	0.44	0.20	−0.10	−0.33	−0.60	−1.10
R_1	0.753	0.796	0.844	0.875	0.910	0.941	0.947	0.972	0.99
K	(1.42)	(1.37)	(1.32)	(1.29)	(1.20)	(1.10)	(1.02)	(0.97)	(0.90)

The dissociation of the indicator remains normal until the mid-point is reached, but it undergoes an appreciable retardation in the more strongly acid portion of the range. A value of 1.5 is obtained for *K*.

Previous values for *K* obtained by Clark[6] and by Brode[5] are, respectively, 1.7 and 1.75. The accurate determination of the dissociation constant is dependent upon obtaining complete dissociation of the indicator. Neither Clark nor Brode has recorded the conditions under which complete dissociation of the indicator was assumed, but it appears probable that their provisions were inadequate. In the present investigation concd. hydrochloric acid (36%) was employed for the purpose. The evidence obtained indicated that the dissociation of the indicator is incomplete in solutions containing less than 20% of hydrochloric acid.

In the application of the spectrophotometric method in the acid range of thymol blue the evaluation of solutions should be based upon the dissociation curve of the indicator as experimentally determined rather than upon theoretical values.

The Accuracy of the Method

The accuracy obtainable in the spectrophotometric determination of hydrogen-ion concentrations is dependent in a considerable measure upon the technique employed. The most reliable results are not obtained on the obvious basis of the comparative magnitude of extinction coefficients as determined with a constant concentration of indicator. It is much pref-

[9] Brightman, Meecham and Acree, *J. Bact.*, **5**, 169 (1920).

erable to maintain an approximate uniformity in respect to the intensity of the absorption in comparative measurements by means of a suitable variation in indicator concentrations. If such provisions are made as will confine the measurements within the intensity range wherein the eye of the observer is most sensitive, and the employment of the indicator confined within the *P*H zone in which dissociation is greater than 10% and less than 90%, the data of the present investigation indicate that the error by the spectrophotometric method should not exceed 0.02 *P*H.

Summary

Data are supplied for a relatively convenient and accurate spectrophotometric method for determining the hydrogen-ion concentrations of solutions within the effective dissociation ranges of thymol blue.

The apparent dissociation constants of the indicator in its alkaline and acid ranges are determined to be 8.91 and 1.5, respectively.

The dissociation of the indicator is normal over its useful alkaline range, but is appreciably retarded after the mid-point in its acid range has been passed.

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THE SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATIONS AND OF THE APPARENT DISSOCIATION CONSTANTS OF INDICATORS. III BROMOCRESOL GREEN

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The synthesis of bromocresol green (tetrabromo-*m*-cresol-sulfonephthalein) was undertaken by Barnett Cohen in 1922 to supply a sulfonephthalein derivative which would be suitable for substitution in place of methyl red in the Clark and Lubs series of indicators. In the preliminary note on the indicator[3] its apparent dissociation constant was reported as 5.0.

In its alkaline form the indicator is blue, with an absorption band in the red with the maximum at approximately 614 $\mu\mu$.

Through the kindness of Mr. Cohen, the authors were supplied with a sample of his original material and with one of subsequent preparation shown by analysis to be of superior purity. The dissociations of these

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[3] *U. S. Pub. Health Repts.*, **38,** [5] 199 (1923).