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ON THE QUINONE PHENOLATE THEORY OF INDICATORS. A SPECTROPHOTOMETRIC STUDY OF THE "END-POINTS" AND "FADING" OF PHENOLSULFOPHTHALEIN INDICATORS.¹

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One of the chief reasons for the suggestion² of the development and study of a sulfophthalein series of indicators covering a wide range of hydrogen-ion concentrations at which the useful color changes take place, was that phenolsulfophthalein showed sharper and more intense color changes than phenolphthalein, and was apparently free from the objectionable "fading" of the intense color which diminishes the usefulness of phenolphthalein. The work of Green³ and especially the fine quantitative work of Kober⁴ and Marshall, showed that over 90% of the intense color of phenolphthalein salts fades in an excess of alkali. The spectrophotometric work by White⁵ in 1915 and by Guy⁶ in 1916, and the work of Lubs,⁵ as well as the data by Birge⁷ in coöperation with us, showed that phenolsulfophthalein and other indicators of this series are *over twice* as deeply colored in alkalies as phenolphthalein and yet show very little, if any, fading in an *excess* of alkali. As this question of fading is a very important one in the use of indicators in analytical work, in the study of the acidity of culture media for wood-destroying fungi and bacteria, and in the measurement of the affinity constants (because of the necessity for determining the "end-point" or maximum value of the specific absorption index which is necessarily lowered by fading), it seems worth while to publish a few of our results to show those interested in the use of these indicators about what

¹ This article is one of a series which we are publishing in coöperation with Dr. Haven Metcalf, in charge Forest Pathology, Bureau of Plant Industry, Department of Agriculture, on quantitative studies of the various chemical and physical factors governing the growth of fungi on culture media and trees.

² See White and Acree, New Orleans address, *Science*, **42**, 101 (1915); Dissertation, Univ. of Wisconsin, 1915; *THIS JOURNAL*, **39**, 648 (1917); **40**, 1092 (1918); Lubs and Acree, *Ibid.*, **38**, 2772 (1916); Lubs and Clark, *J. Bact.*, **2**, 1, 109, 137 (1917); Birge and Acree, *THIS JOURNAL*, a later number.

³ Bistrzycki, *Ber.*, **36**, 2337, 3558, 3565 (1903); Green and Perkin, *J. Chem. Soc.*, **85**, 398 (1904); *Ber.*, **40**, 3724 (1907).

⁴ Kober and Marshall, *THIS JOURNAL*, **33**, 59 (1911); see also Slagle and Acree, *Am. Chem. J.*, **42**, 126, 137-9 (1909); **39**, 533, 542 (1908).

⁵ *Loc. cit.*

⁶ Unpublished results on a number of sulfophthaleins, including affinity constants.

⁷ *THIS JOURNAL*, a later number and succeeding articles giving the general equations and theory covering fully this class of indicators, and describing the application of the spectrophotometric method to the study of tautomerism in this series.

can be expected of the phenolsulphophthalein series under average conditions. The results will be discussed under the questions (a) whether an excess of alkali necessary to secure the "end-point" causes slow or rapid fading; (b) whether different solutions made up from the same sample of indicator give the same results; and (c) whether bacteria, molds, etc., cause chemical changes of the indicator on long standing, especially in an ice box. The indicators reported upon here are phenolsulphophthalein and the tetrabromo- and tetra-nitro derivatives, and corresponding data for the rest of the sulphophthalein series will be presented shortly.

Experimental.

The details of the spectrophotometric method used will be reported in full in a later article.¹ The general plan was to find the wave-lengths at which the indicator solutions give a transmission of 20, 40, 60, 80, etc., %, and to calculate the corresponding absorption indexes, and to draw a curve through these points. In some cases the maximum absorption could not be measured and it was necessary to calculate the absorption index for the peak of the curve. This was done by the usual method of ratios. The values for the absorption index N given in the following paragraphs are therefore arbitrary but are fully as useful as absolute values in showing any changes taking place.

Experiments on the Effect of Time on the Fading of a Solution of 0.00005 M Phenolsulphophthalein and an Excess of Alkali.—In the following table N is the *specific absorption index* in arbitrary units for the middle or *peak* of the green absorption band. The solution of 0.00005 M phenolsulphophthalein was mixed with 5 equivalents of sodium hydroxide and the measurements at different frequencies were made as rapidly as possible. Special experiments were also made in which the temperature of the solutions was the same before and after the mixture was made:

Date.	Time.	N .
July 16.....	10.30 A.M. (5 mols alkali)	425
July 16.....	11.30 A.M.	422
July 16.....	4.15 P.M.	420
July 19.....	10.00 A.M. (7 mols alkali)	420

These results show clearly that the phenolsulphophthalein does not fade perceptibly in long-time periods or with an *excess* of 3 molecules of alkali. It might be thought that a certain amount of fading takes place instantaneously, but this seems to be disproved by the fact that a further quantity of *free* alkali (2 mols) produces no decrease in the absorption index such as that observed by Kober and Marshall for phenolphthalein.

Experiments on the Effect of Time on the Fading of 0.0001 M Solutions of Phenolsulphophthalein in Buffer Solutions.—As we are using buffer solutions in making up culture media for wood-destroying fungi it was in-

¹ Unpublished work by Brightman, Meacham, Hopfield, Birge and Acree.

interesting to determine whether such buffer solutions cause a fading of phenolsulfophthalein. Accordingly, a phosphate buffer solution having a P_H value of 6.5 and a concentration of 0.05 molar and containing 0.0001 M phenolsulfophthalein was made on July 1st and found to have an absorption index of 16.2. On July 11th this same solution was measured again and found to have an absorption index of 16.3. The results showed that neither chemical changes nor bacteria nor molds have changed the absorption index to any extent larger than the experimental errors involved in the work.

Another buffer solution having a P_H of 6.5 ± 0.02 and a concentration of 0.025 molar, which was made on July 26th and measured, was investigated again on July 27th. The following table shows that no changes larger than experimental errors were observed.

Date.	Telescope reading for 70% transmission.	Telescope reading for 60% transmission.
July 26.....	10.290	10.132
July 27.....	10.299	10.142

These results show clearly that any changes which may have taken place in the color intensity are well within the experimental errors involved in the measurements.

Experiments on the Absorption Produced by Adding 5 Mols of Alkali to 0.0005 M Solutions of Phenolsulfophthalein Which Stood Various Time Periods.—It has been observed that in some cases the solutions of the sulfophthaleins become infected with molds and bacteria and the indicator is thereby destroyed and gives a smaller absorption when treated with an excess of alkali. We have made some experiments to determine whether these changes take place when accurately standardized stock solutions of the indicator are kept in an ice box, and have found that under these conditions there is no destruction of the indicator. But in practical use of indicators, it is important to know whether such solutions remain practically unchanged under ordinary experimental conditions. We have found that even if the solution is not kept on ice there will be no appreciable destruction of the indicator if the solutions are given ordinary care to prevent them from becoming contaminated. For example, a 0.0005 M phenolsulfophthalein solution was made on July 16th and found to have an absorption index of 425 when treated with 5 mols of alkali. On July 22nd a portion of the same 0.0005 M indicator solution was treated with 5 mols of alkali and found to have an absorption index of 430, which is identical with that of July 16th within experimental errors.

Experiments to Determine whether Different Portions of the Same Sample of Solid Indicator Give the Same Absorption Index when Treated with an Excess of Alkali.—In accurate work with indicators, especially photometric work, it is necessary to test the purity of the indicator before

extensive measurements are undertaken. This subject has not received the attention in the past which it deserves and we are therefore undertaking careful work on the purification and analysis of all of our indicators. This analysis will involve not only volumetric and gravimetric analyses but also spectrophotometric analyses. Spectrophotometric analysis of our best *commercial* samples of phenolsulfophthalein indicates that there is still possibly 5% of impurity present. But even slightly impure samples of indicator may be used for comparative measurements and we have therefore carried out a number of measurements on the same sample to determine whether such sample is mixed uniformly and will give uniformly consistent results. For example, on July 14th a 0.00005 *M* solution of phenolsulfophthalein and 5 mols of alkali gave an absorption index of 425. On July 25th another solution was made from a portion of the same sample of solid indicator and was found to have an absorption index of 420. On July 31st another fresh solution was made under the same conditions and was found to have an absorption index of 425. As these results do not vary more than 1% it is clear that the same sample of solid indicator should give results agreeing within 1% when the proper experimental conditions are carefully observed.

Experiments on the Fading of the Color Produced by an Excess of Alkali in Solutions of Tetrabromophenolsulfophthalein.—Experiments on the action of an excess of alkali on tetrabromophenolsulfophthalein, and similar experiments by Lubs and one of us on a number of other indicators described in an earlier publication,¹ show that these indicators are apparently fully as satisfactory as phenolsulfophthalein in that they do not fade appreciably in color when treated with an excess of alkali. Even after standing two days a solution of tetrabromophenolsulfophthalein in an excess of alkali showed the same absorption index within the experimental errors.

As we did not get the top of the absorption band for this case we have used the value of the absorption index at telescope reading 11.50 for the sake of comparison. These values were not obtained by measuring the values at exactly 11.50 but were taken from absorption curves drawn in the usual manner.

The *N*/97,160 tetrabromophenolsulfophthalein solution was made at 4:15 P.M., June 21, 1918.

Date.	Time.	Telescope reading.	Absorption index.
June 21, 1918.....	4:30	11.50	64.6
June 22, 1918.....	9:00	11.50	65.2
	2 mols alkali added	11.50	65.2
	Great excess alkali added	11.50	65.2

¹ Lubs and Acree, *THIS JOURNAL*, 38, 2772, 2783 (1916).

Experiments on the Fading of the Color Produced by an Excess of Alkali in Solutions of Tetranitrophenolsulfophthalein.—White¹ has shown that tetranitrophenolsulfophthalein produces an intense color in aqueous and alcoholic solutions which is hardly intensified with alkali up to 2 molecules, but which is caused to fade to a faint yellow by an excess of alkali. We have studied these changes quantitatively by spectrophotometric methods and have been able to verify them in a general way. In deep layers an excess of alkali does not apparently intensify the color. On the other hand, this color fades out fairly rapidly, depending upon the experimental conditions, and in time periods varying from a few minutes to 12 hours becomes a faint yellow. It is evident that tetranitrophenolsulfophthalein shows the same fading exhibited by phenolphthalein and is not as satisfactory an indicator as the halogen and alkyl derivatives of phenolsulfophthalein.

Conclusions.

In the spectrophotometric study of the series of phenolsulfophthaleins which we are using in extensive investigations it has been found that these indicators have a number of properties which make them the best series yet developed.

1. These sulfophthaleins are twice as deeply colored as phenolphthalein in alkalies, and show sharper color changes.

2. The excess of alkali necessary to produce the "end point" of the neutralization of the indicator does not cause any appreciable fading in either short- or long-time periods in the case of phenolsulfophthalein and its tetrabromo derivative.

3. The color of phenolsulfophthalein in phosphate buffer solutions does not fade appreciably, even in considerable time periods.

4. Standardized stock solutions of phenolsulfophthalein can be kept in an ice box without appreciable change, or even at ordinary temperatures if care is given to prevent contaminations, and will then show the same absorption index when treated with an excess of alkali at different time periods.

5. Different samples of the same lot of *solid* well-mixed phenolsulfophthalein will give the same absorption index when treated with an excess of alkali.

6. An excess of alkali in solutions of tetranitrophenolsulfophthalein causes a fading of the intense red color to a light yellow, the time of fading depending upon the amount of alkali and other experimental conditions.

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¹ *Loc. cit.*, see especially Diss. and **THIS JOURNAL**, **40**, 1097 (1918).