rapidly precipitating a black insoluble substance. Attempts to prepare the salts of the common inorganic acids have thus far failed due to their solubility and to rapid condensations. Even in the above picrate a small residue insoluble in dilute acids was found.

VI. Oxidation of 2,4,6-Triaminophenol. — The diaminoquinonimine hydrochloride[1] prepared by oxidation of this phenol was titrated with titanium trichloride, the values found agreeing with the assigned holoquinoid formula.

Summary.

In the aminoquinonimine salts the auxochromic effect of one phenylamino group is, unlike the cases of other holoquinoid salts, the same as the auxochromic effect of one dimethylamino group.

CHICAGO, ILLINOIS.

ON THE QUINONE-PHENOLATE THEORY OF INDICATORS.

THE ABSORPTION SPECTRA OF SOLUTIONS OF PHENOLSULFONPHTHALEIN AND ITS TETRABROMO AND TETRANITRO DERIVATIVES AND THEIR SALTS, AND OF ANALOGOUS SUBSTANCES.

BY E. C. WHITE AND S. F. ACREE.

Received March 28, 1918.

It was shown by White[2] in 1913–15 and later, in 1915–16, by Lubs[3] and one of us that phenolsulfonphthalein and its derivatives and their salts give us the best series of indicators yet found for acidimetry and for the study of the quinone-phenolate theory. The yellow color characteristic of the solutions of phenolsulfonphthalein (I) is not altered perceptibly by the addition of small quantities of alkali up to 75% of a molecular equivalent (III), but the addition of 0.95 molecule[4] and more of alkali produces a deep red color[5] (IV). This indicates that the phenolsulfon-

[1] Carl Heintzel, *Z. Chem.*, 1867, 342.

[2] *Science*, **42**, 101 (1915). Address at New Orleans Meeting of American Chemical Society. Dissertation, 1915, University of Wisconsin.

[3] THIS JOURNAL, **38**, 2772 (1916); Lubs and Clark, *J. Wash. Acad. Sci.*, **5**, 609 (1915); 6, 483 (1916).

[4] In White's earlier titrations on various preparations having more or less impurities the red color appeared on the addition of from 0.80 to 0.90 molecule of alkali. Later preparations required 0.93 to 0.95 molecule of alkali. The phenolsulfonphthalein acts as a "self indicator" and this titration gives us a very accurate criterion of its purity.

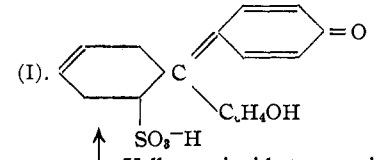
[5] It has been generally overlooked that unsymmetrical dibasic acids have two sets of primary and secondary ionization constants caused by the independent ionization of the non-ionized acid in two directions. We have developed the general equations covering these cases by starting with the relations embodied in the Mass law,

$$\frac{(HAn + HAn') \times H}{H^2An} = K + K_1,$$

$$\frac{An'' \times H}{HAn + HAn'} = \frac{K_2K_2'}{K_2 + K_2'}, \text{ and } K_1K_1' = K_2K_2'.$$

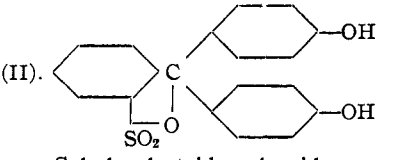
The values of these constants can be ascertained by developing chemical or phys-

phthalein exists largely in the quinoidal state (I), as follows:

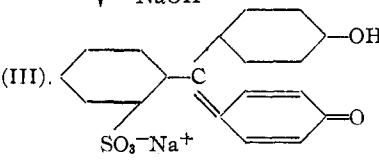


Yellow quinoid strong acid.

NaOH

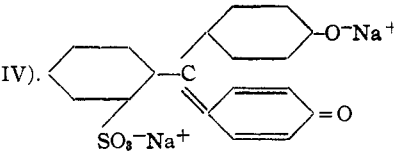


Colorless lactoid weak acid.



Yellow monobasic salt.

NaOH



Deeply colored dibasic salt.

This conclusion was then substantiated by conductivity measurements by White[1] in 1915 which showed that phenolsulfonphthalein and its tetrabromo- and tetranitro-derivatives exist to at least 65%, and perhaps more, in the quinoidal form (I) containing a strong sulfonic acid group. This evidence was later confirmed by Lubs[2] and one of us in measurements of the P_H values of these and other indicators and by Lubs[3] and Clark in a friendly continuation of our problem of making and using a series of phenolsulfonphthalein indicators, especially in their excellent work in bacteriology. For all practical purposes, then, phenolsulfonphthalein and its sodium salt can be treated as a mono-acid in-

ical methods for measuring the concentration of each ion. In the case of the phenolphthalein and phenolsulfonphthalein indicators the non-ionized quinoidal form is in equilibrium with both the quinone phenolate anion $(^-OC_6H_4)(O:C_6H_4:)C(C_6H_4COOH)$, and the quinone carboxylate anion $(HOC_6H_4)(O:C_6H_4:)C(C_6H_4COO^-)$. These two ions give entirely different absorption spectra. The secondary ionization of the quinone phenolate anion produces no great change in color but the secondary ionization of the quinone carboxylate anion gives the intense color common to the bivalent ion $(^-OC_6H_4)(O:C_6H_4:)C(C_6H_4COO^-)$ and monovalent quinone phenolate anion $(^-OC_6H_4)(O:C_6H_4:)C(C_6H_4COOH)$, both of which have the quinone phenolate anion causing the intense color. The constants of a phenolsulfonphthalein having the quinodal form can be expressed by the equations

$$\frac{(HAn' + An'')(red)}{(H^2An + HAn)(yellow)} = \frac{\alpha}{1-\alpha} = \frac{K_1'K_2' + K_1'H}{H^2 + K_1H} = \frac{K_1K_2 + K_1H}{H^2 + K_1H},$$

and these equations can be solved simultaneously by the use of data from spectrophotometric measurements of the indicators in buffer solutions whose hydrogen ion-concentrations can be ascertained by the hydrogen electrode, or by the use of weak bases whose hydrogen-ion concentrations can be calculated or measured. The importance of HAn' and K_1 can be magnified by substituting negative groups in the phenol radicles. We desire to reserve this field of research in connection with our quinone phenolate theory. (B. and A.)

[1] THIS JOURNAL, **39**, 648 (1917).

[2] *Ibid.*, **38**, 2772 (1916).

[3] *J. Wash. Acad. Sci.*, **6**, 481 (1916).

dicator, $^{-}O_3SC_6H_4C(:C_6H_4:O)(C_6H_4OH^{-4})$. These facts in connection with the brilliant color changes, the absence of greatly disturbing "salt effects," the absence of measurable fading in the presence of an excess of alkali characteristic of phenolphthalein and its negative substitution products, and the solubility in water make the phenolsulfonphthaleins the best series of indicators yet developed. We, therefore, intend to make the other derivatives necessary to fill the present gaps and give us a series of phenolsulfonphthaleins covering a range of hydrogen-ion concentrations from 10^{-1} to 10^{-13} or more.

If this theory is correct and the deep color is produced chiefly from the quinone-phenolate group in (IV) it seemed probable that a study of the entire absorption spectrum in the infra-red, visible, ultraviolet and X-ray regions, instead of the total color affecting the eye, would show very clearly the absorption bands due to the quinone-phenol group in (I), to the monobasic salt (III), and to the dibasic salt (IV), and hence allow us to test the theory more thoroughly. The present work with this object in view was completed in May, 1915. It was suspected from the previous evidence, and is now confirmed that the free acid and monobasic salt have practically the same absorption spectra because both contain the non-ionized quinone-phenol group $-C(:C_6H_4:O)(C_6H_4O^-)$. When the phenol group is neutralized partially by the alkali and the quinone-phenolate anion $-C(:C_6H_4:O)(C_6H_4O^-)$ is formed the outer edge of the yellow band begins to shift toward the red and becomes fixed in position when the phenol group is entirely neutralized. This change in intensity is being measured very accurately to calculate affinity constants by Professor J. S. Guy by means of an excellent Zeiss spectrophotometer kindly placed at our disposal by Professor Ingersol.[1] The "salt effect"

[1] Prof. R. T. Birge of the Physics Department of Syracuse University and one of us are coöperating in this work with the aim of securing more extensive quantitative and qualitative spectrophotometric, physical chemical and structural organic relations in dyes and indicators. Prof. Birge's mathematical studies in band series and the organic and physical chemical relations which have led to the quinone phenolate and quinaminone theory of color changes in dyes and indicators may enable us to correlate the absorption spectra in the infra-red, visible, ultra-violet and X-ray regions with chemical and physical properties and with present or new conceptions of their internal structure, expressed to-day in terms of radicals, unsaturated groups such as quinone complexes, combinations of quinones and other complexes, bonds, complex and simple ions, and electrons. We hope that this work will enable us to calculate the real and apparent equilibrium and ionization constants of the individual and total acid, basic and salt groups, and apply the affinity constants very accurately in alkalimetry and acidimetry, especially in studying changes in culture media by wood-destroying fungi in a coöperation with Dr. Haven Metcalf, Chief of the Division of Forest Pathology in the Bureau of Plant Industry, Washington.

Our first object is to complete the systematic spectrophotometric study of the phenolsulfonphthalein group begun by White and one of us and continued by Lubs and Clark and Acree in coöperation. We hope to complete the development of a

due to acids, bases and salts and organic compounds will also be investigated by the same methods.

Experimental.

The instrument used was a Steinheil grating spectrometer with camera attachment.[1] The grating can be rotated so as to bring different parts of the spectrum into coincidence with the intersection of the cross hairs in the telescope. The zero position of the grating is that in which the image of the slit in the spectroscope coincides with this intersection. The angle through which the grating is rotated is read off on a scale on the instrument, and the wave length λ in coincidence with the cross hairs for any given deflection θ of the grating from its zero position is given by the relation

$$\lambda = k \sin \theta$$

in which k is a constant ascertained by the maker of the instrument.

It has been mentioned before that the limit of the solubility of phenolsulfonphthalein is reached when about 0.03 g. is contained in 100 cc. of solution. We therefore used solutions of this concentration, corresponding to 0.0008 molar, and for purposes of comparison made up solutions of other substances in equivalent concentrations. As we had no spectrophotometer available then and could not study the absorption spectrum of each substance accurately we had to content ourselves with mapping out the edges of the absorption bands to discover the general relations between absorption and constitution. It was found that a column of this solution of phenolsulfonphthalein 30 cm. long gave an absorption band of practically maximum width, a column 40 cm. long showing the same absorption. Phenolsulfonphthalein in such a solution and in such length of column absorbs all of the violet and blue, and most of the green. The transmission area extends to the end of the visible red. Salt formation shifts the edge of the absorption band towards the red.

The absorption bands of the sulfonphthaleins and their salts were compared with the bands of other indicators. Phthaleins other than sulfonphthaleins could not be investigated in the free condition, owing to their insolubility in water. The use of alcoholic solutions suggested earlier is difficult, owing to the well-known decolorization of phthalein

sulfonphthalein series of indicators which will, (*a*) show the brilliant color changes observed by White, which will (*b*) cover the most useful ranges of hydrogen-ion concentrations, and which will (*c*) be as free as possible from disturbing influences such as "salt effects" and fading because of hydration. We hope to develop the spectrophotometric method so that it will become a handy and accurate instrument in the hands of chemists and biologists for making extremely accurate measurements involving such colorimetric determinations as oxidations with permanganates, iodimetry, and acidimetry and alkalimetry, especially in turbid and colored solutions.

[1] We are indebted to Dr. J. H. Mathews of the Department of Chemistry for the loan of this instrument.

salts by alcohol, but will be studied. It was found that all of the phthalein salts tested showed approximately the same absorption bands, the transmission for the depth used being entirely in the red. Only in the case of tetrabromophenolphthalein did 2 transmission areas appear, one in the violet and one in the red. It is worthy of note that some solutions, such as that of the salt of tetrabromophenolsulfonphthalein, which as ordinarily viewed appear *blue* to the eye, *transmit* only *red* light. The blue color of such a solution is doubtless due to reflected or fluorescent light; it seems likely that any detailed quantitative study of the color of such substances in solution would have to take into consideration the *reflection* phenomena as well as the *absorption* spectra.

In the following tables we give a few characteristic measurements of spectra of solutions which were photographed on panchromatic plates. Many others were examined but are not recorded here. We are indebted to Dr. Diemer for making the photographs and for many valuable suggestions. The position of the absorption band is given in $\mu\mu$ and the reproducibility is probably not better than 5 to 10 $\mu\mu$.

TABLE 1.—PHENOLSULFONPHTHALEIN.

Substance.	Absorption band in $\mu\mu$.
Phenolsulfonphthalein	569 to end of visible violet
The free acid plus 60% of one equivalent of alkali	569 to end of visible violet
The free acid plus 75% of one equivalent of alkali	568 to end of visible violet
Monobasic salt of the same by titrating the solution to color (85% of one equivalent of alkali)	577 to end of visible violet
The free acid plus 2 equivalents of alkali	639 to end of visible violet
The free acid plus conc. HCl (5 cc. to 30 cc. of solution)	586 to end of visible violet

It will be noted that the solution of phenolsulfonphthalein to which 60% of one molecular equivalent of alkali has been added (III) shows *exactly the same absorption band as does the solution of the free phthalein* (I) and only a very slight shift occurs when 75% of one equivalent has been added. This shifting is not sufficient to change the color of the solution, as it appears to the unaided eye, in any way. It seems likely[1] that the dibasic quinone-phenolate salt (IV) begins to form appreciably at this point, but that the amount of this salt present between the limits 75 to 85% of one equivalent of alkali is not sufficient to become apparent to the eye. From the latter point the addition of more alkali up to 2 equivalents[2] causes a shifting of the edge of the band from 577 $\mu\mu$ to 639 $\mu\mu$, where it remains even upon the addition of more alkali.

The addition of very small amounts of hydrochloric acid produces no visible change. The addition of *concentrated* hydrochloric acid to 0.001 N

[1] This idea has been verified in the recent spectrophotometric work by Professor Guy, and Professor Birge and Mr. Hopfield.

[2] The very accurate spectrophotometric work in coöperation with Prof. Birge and Mr. Hopfield shows *small* changes upon the addition of more than 2 equivalents of alkali, which accords with theory.

phenolsulfonphthalein shifts the edge of the absorption band towards the red. We think it likely that this is due partially to the formation of an oxonium salt, as illustrated by the following formula and will study this problem in detail quantitatively as we have observed the same phenomena in a number of cases:

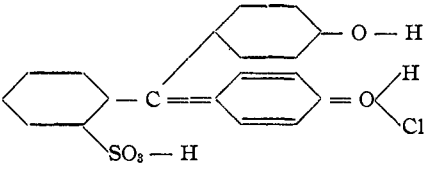


TABLE II.—TETRABROMOPHENOLSULFONPHTHALEIN.

Substance.	Absorption band in $\mu\mu$.
Tetrabromophenolsulfonphthalein	651 to end of visible violet
The same plus a large excess of HCl	604 to end of visible violet
The same plus 2 equivalents of alkali	692 to end of visible violet

Our conductivity work[1] and the P_H values by Lubs[1] and Acree and Lubs and Clark show clearly that the faint blue color of a dilute solution of tetrabromophenolsulfonphthalein, which is greatly intensified by alkalies, arises from the small ionization of the phenol group. If such is the case the addition of more and more hydrochloric acid should, and does, cause a shift of the edge of the band into the shorter wave lengths, namely, from 651 $\mu\mu$ to 604 $\mu\mu$. Consequently, the bluish red solution becomes yellow, but becomes blue again when the solution is diluted. On the other hand, this ionization of the bromophenol group is by no means complete and the addition of more and more alkali up to 2 molecules or more should, and does, shift the edge of the band into the larger wave lengths, namely, from 651 $\mu\mu$ to 692 $\mu\mu$.

TABLE III.—TETRANITROPHENOLSULFONPHTHALEIN.

Substance.	Absorption band in $\mu\mu$.
Tetranitrophenolsulfonphthalein	626 to end of visible violet
The same plus 2 equivalents of alkali	626 to end of visible violet
The same plus conc. HCl (5 cc. to 30 cc.) 7.25 P.M.	611 to end of visible violet
The same—7.40 P.M.	599 to end of visible violet
The same—8.30 P.M.	577 to end of visible violet
The same—Next morning	577 to end of visible violet

Our conductivity work[1] and the study of the P_H values by Lubs[1] and Acree and by Lubs[1] and Clark show that the intense purple-red color of solutions of tetranitrophenolsulfonphthalein arises because of the high ionization of the quinone-nitrophenol group. This is so large that the addition of more alkali to the deep layer used hardly intensifies the color of the solution or changes the position of the bands[2] given in Table III.

[1] *Loc. cit.*

[2] In our present quantitative spectrophotometric work we are using cells of different depths. The intensely colored solutions are studied in thin layers. (B. & A.)

The addition of an excess of alkali causes the deep purple-red color to fade to yellow which changes to purple-red again on the addition of sufficient acid. The addition of more and more hydrochloric acid, however, suppresses the ionization of the nitrophenol group and causes a quick shifting of the edge of the band into the shorter wave lengths and a change of the purple-red color into yellow. The solution then shows a gradual fading, which does not require more than 30 minutes. It is possible that we are dealing here with an oxonium salt effect, hydration phenomena, and with the suppression of the ionization of the phenol group. On diluting the solution and allowing re-ionization the purple-red color returns instantly.

The absorption bands of these 3 sulfonphthalein are so close together that it was decided to photograph the absorption spectra of other members of the aurine and phenolphthalein group to see if there is a general similarity. The spectra of alkaline solutions of aurine, rosolic acid, fluorescein, eosin, iodeosin, phenolphthalein, phenoltetrachlorophthalein, and tetrabromophenolphthalein are shown in Table IV to be close together and close to those of the above sulfonphthalein. This, doubtless, arises from the fact that the deep color in all these cases arises from the quinone phenolate group $-C(:C_6H_4O)(C_6H_4O^-)$, modified in each case by the particular groups characteristic of each of these colored substances. All of this cumulative evidence speaks strongly for the quinone-phenolate theory of indicators.[1]

TABLE IV.—ROSOLIC ACID, FLUORESCEIN AND PHENOLPHTHALEIN SERIES.

Substance.	Absorption band in $\mu\mu$.
Aurine	621 to end of visible violet
Rosolic acid	618 to end of visible violet
Fluorescein	612 to end of visible violet
Eosin	584 to end of visible violet
Iodeosin	606 to end of visible violet
Phenolphthalein	591 to end of visible violet
Phenoltetrachlorophthalein[2]	679 to end of visible violet
Tetrabromophenolphthalein	621–466 and 425 to end of spectrum

Conclusions.

1. It has been shown that the yellow color and the absorption spectrum of phenolsulfonphthalein solutions are not altered by the addition of alkali up to 0.75 molecule. When more alkali is added the yellow color changes to deep red and there is a corresponding shift in the absorption

[1] Accurate spectrophotometric data are now being obtained for the entire spectrum of each member of this sulfonphthalein series in solutions varying from 0.001 *N* to 0.00002 *N*, in the presence of acids, in the presence of bases up to several molecules, and at various temperatures. Salt effects are under investigation. (B. & A.)

[2] This material was kindly furnished us by Dr. L. G. Rowntree of Johns Hopkins Medical School.

band. This is interpreted as evidence that the *intense red color* of the alkali salts of indicators of this series does not come from the *nonionized quinone-phenol group* but arises from the *quinone-phenolate anion.*

2. In accordance with this conception we find that the introduction of negative bromo- and nitro-groups into the phenol group increases the ionization of the phenol group, increases the conductivity, lowers the P_H value and gives to the solution a greater concentration of quinone-phenolate anions and hence increases the *deep red color* and changes the position of the absorption band so as to cut out part of the yellow. The addition of hydrochloric acid suppresses the ionization of the phenol group and changes the *deep red color* into the yellow of the quinone and hence shifts the absorption band so as to include less of the yellow.

3. The similarity of the absorption spectra of the alkaline solutions of sulfonphthaleins, phenolphthaleins, aurine, fluorescein and related substances gives evidence that the deep red color in all these cases arises from the presence of a *quinone-phenolate anion.*

MADISON, WISCONSIN.

[CONTRIBUTION FROM THE ORGANIC LABORATORY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY.]

THE ACTION OF HYDROGEN PEROXIDE UPON URIC ACID.

SECOND PAPER ON HYDROGEN PEROXIDE AS A REAGENT IN THE PURIN GROUP.

BY C. S. VENABLE.

Received April 17, 1918.

It has recently been shown by Venable and Moore[1] that when uric acid is treated with hydrogen peroxide at room temperature in an approximately half-normal alkaline solution, cyanuric acid is produced, with a yield about 50% that of the theory. Evidence was also produced to show that "tetracarbonimide" which other investigators[2] had described as a product of the reaction was itself only cyanuric acid.

Since that paper was published these views have received welcome and efficient confirmation by the independent work of Walters and Wise[3] who have also added valuable experimental evidence of their own. The identity of cyanuric acid with tetracarbonimide can, therefore, now be regarded as definitely established. In consequence the present paper will discuss the action of hydrogen peroxide upon uric acid from a more general point of view—dealing, first, with the influence of temperature and alkalinity, and second, with the mechanism of the reaction.

[1] THIS JOURNAL, **39**, 1750 (1917).

[2] Scholtz, *Ber.*, **34**, 4130 (1901); Schittenhelm and Weiner, *Z. physiol. Chem.*, **62**, 100 (1909).

[3] THIS JOURNAL, **39**, 2472 (1917).