acid. Filter and dry the solid which separates between the ether and acid layers. A quantitative yield results. This substance proved a very difficult one to purify. Although we tried different combinations of hydrochloric acid, alcohol and water, we were unable to remove the small amount of yellowish impurity, and the analyses for chlorine did not give constant values. We found it advisable, therefore, to make the free amidine and then the phenyl isocyanate derivative.

Dibenzyltoluenylamidinemonophenyl Ureide, $(C_6H_5CH_2)_2NC(NCO\text{-}NHC_6H_5)C_6H_4CH_3(p)$.—Moisten 3 g. of the amidine hydrochloride with water and add a slight excess of concentrated potassium hydroxide solution. Extract with ether, and dry the solution obtained with calcium chloride. On evaporation the free amidine, a yellowish crystalline solid melting at about 80°, and very soluble in all organic solvents, results. Dissolve a known weight of this material in absolute alcohol, and add the theoretical amount of phenyl isocyanate. On evaporation of this solution, fine white needles in a quantitative yield deposit, which crystallize very readily from alcohol to a constant melting point of 158°.

Calc. for $C_{29}H_{27}N_3$: N, 10.0%; C, 80.36%; H, 6.23%. Found: N, 9.75; C, 80.38; H, 6.56.

CAMBRIDGE, MASS.

ON THE SULFONPHTHALEIN SERIES OF INDICATORS AND THE QUINONE-PHENOLATE THEORY.[1]

BY H. A. LUBS AND S. F. ACREE.

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In developing a method for the colorimetric determination of the hydrogen-ion concentration of bacteriological cultures and culture media, one of us and Clark[2] found that many of the available indicators were unsatisfactory for reasons previously described. On this account a number of new indicators of the methyl red and sulfonphthalein types were synthesized. The phenolsulfonphthaleins had appeared especially promising from the work of White[3] and one of us on this group, not only because the compounds show brilliant color changes but also because their chemical constitution is of such a nature that the substitution of suitable groups should cause their affinity constants to vary to almost any desired degree and give a series of indicators which can be used to measure a wide range of hydrogen-ion concentrations; furthermore, the new compounds ob-

[1] Published by permission of the Secretary of Agriculture as a joint article from the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C., and the Forest Products Laboratory, Madison, Wisconsin.

[2] Lubs and Clark, *J. Wash. Acad. Sci.*, **5**, 609.

[3] *Science*, **42**, 101; address at New Orleans Meeting of Am. Chem. Soc., and unpublished dissertation, 1915, Univ. of Wisconsin.

tained would give evidence on the quinone-phenolate theory[1] proposed by one of us. The additional evidence obtained by us from a study of these new compounds and the spectrophotometric data recently by Professor J. S. Guy, confirms so beautifully the theory that there can now be hardly any doubt of its correctness. A further study of the phenolsulfonphthalein series from the standpoint of the quinone-phenolate theory will be continued by Acree and his co-workers by the use of (1) colorimetric, conductivity and catalytic methods, and (2) the hydrogen electrode in order to measure the equilibrium and ionization constants. The "normal and abnormal salt catalysis" by organic and inorganic compounds will be especially investigated by the methods outlined by one of us in earlier papers,[2] in order to try to obtain a series of indicators having only "normal" effects, especially in culture media for fungi. The "salt effects" of organic and inorganic compounds of various classes have been extensively investigated by Clark and Lubs in order to obtain a series of indicators as nearly free from these influences as possible. A preliminary account of this phase of their investigations on the biochemical applicability of indicators will be published shortly.

The phenolsulfonphthalein series was chosen for a further study of the quinone-phenolate theory of indicators because they possess properties which are almost ideal for allowing us to obtain certain crucial data which cannot be secured in the phenolphthalein, rosolic acid, alizarin and analogous series. This theory postulates that the change of color produced in solutions of phenolphthalein by the addition of alkalies is not due chiefly to the rearrangement of a colorless lactoid (A) and the formation of a mono-basic carboxylate salt corresponding to (F), containing a simple *colored* quinone group, as formerly assumed by others, but arises because of the formation of a quinone group and a phenolate group (G) or its ion. These two groups can combine and form the intensely colored inter- and intra-molecular quinone-phenolate complex (H) similar to the deeply colored double compounds, such as $O : C_6H_4 : o.2NaOC_6H_3$, which Jackson and Oenslager[3] made by combining separate molecules of quinones and alkali phenolates, and which Slagel[4] showed to be formed in aqueous solutions.

Although in the earlier papers the deep colors of the dibasic (carboxylate, *phenolate*) salts, $(KOOCC_6H_4)C(:C_6H_4:O)(C_6H_4OK)$, of phenolphthalein isolated by Meyer and Spengler,[5] and the deep blue color of the potassium and silver *phenol* salts of the carboxyl esters of phenol-

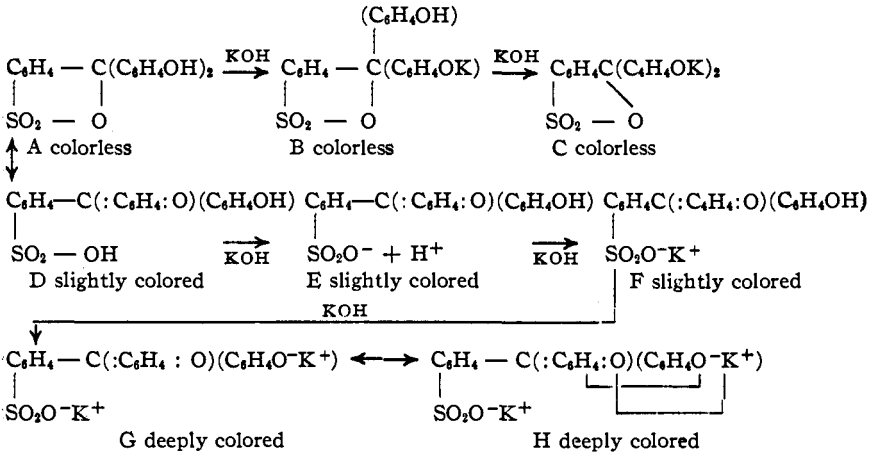
[1] Acree and co-workers, *Am. Chem. J.*, **39**, 528, 789; **42**, 115.

[2] Brunel and Acree, *Ibid.*, **36**, 120; **41**, 474; Slagle and Acree, *Ibid.*, **42**, 130; Loomis and Acree, *Ibid.*, **46**, 586, 632.

[3] *Am. Chem. J.*, **18**, 1; **34**, 441.

[4] *Ibid.*, **39**, 534, 535; **42**, 122.

[5] *Ber.*, **38**, 1318.



phthalein[1] and tetrabromophenolphthalein,[2] $(ROOCC_6H_4)C(:C_6H_4:O)$-(C_6H_4OK), of aurine,[3] $(HOC_6H_4)C(:C_6H_4:O)(C_6H_4OK)$, and of analogous substances were the basis of the quinone-phenolate theory, we knew that strict logic required the present synthetic work and further quantitative verifications. In other words, the *apparent* color of the dibasic phenolphthalein salts might really arise from admixed deeply colored monobasic salts, just as sodium chloride can be made deeply colored by evaporating it with a solution of a small quantity of dye. Likewise it was clear that the *apparent* color of the phenol salts of the carboxyl esters of phenolphthalein might really arise from the saponification of small amounts of the ester group and the formation of traces of a colored *monobasic* carboxylate salt, whose anion containing a quinone and phenol group would be analogous to the anion of monobasic deeply colored aurine salts containing a quinone and phenol group. This work, therefore, did not disprove absolutely the possibility that the color in all of these cases really arises from small amounts of substances containing a quinone and a phenol group.

The disadvantages encountered in testing this theory in the phenolphthalein series can be readily overcome by using the analogous phenolsulfonphthalein compounds, which differ from the phenolphthaleins simply in that the weak carboxyl group —COOH, is replaced by the strong sulfonic acid group $—SO_3H$, of (D). The phenolsulfophthaleins possess the following advantages:

1. They are much more soluble in water and alcohol and exist to a large extent in the colored quinoidal form of the acid, (D) and (E), whereas the phenolphthaleins are practically only colorless lactoids, (A).

[1] Green and King, *Ber.*, **40**, 3724.

[2] Nietzki, Burckhardt and Schraeder, *Ibid.*, **28**, 48; **30**, 177.

[3] Friedländer, *Ibid.*, **26**, 172.

2. Whereas the pure slightly colored monobasic salt[1] (F) has not been isolated in the phenolphthalein series, a number of such salts have been made[2] in the phenolsulfophthalein series and their colors and absorption spectra in solution have been shown to be almost identical with those of the free acid (D) and (E) and entirely different in character and intensity from those of the corresponding deeply colored dibasic salts (G) or (H).

3. Their dibasic salts (G) or (H) have been isolated[3] and their colors and absorption spectra in solution are as different in character and intensity from those of the acids (D) and (E) and monobasic salts (F) as are the intense colors of the phenol salts of quinoidal phenolphthalein esters, $RCOCC(: C_6H_4 : O)(C_6H_4OK)$ or of aurine, $(KCC_6H_4)C(: C_6H_4 : O)(C_6H_4OK)$, from the fainter colors of the corresponding free ester or aurine.

The solubilities of all the phenolsulfophthaleins studied so far are about 100 times those sufficient for a satisfactory study of the color changes. For instance, White[2] has shown that the solubility of phenolsulfophthalein is about 0.03 gram per 100 cc. water, and the other compounds made by him and those since prepared by Lubs[3] and Clark have similar solubilities. The colors of these solutions indicate that considerable quantities of the quinoidal form (D) and (E) are present whose strong sulfonic acid group makes the solution markedly acid. This conclusion has been confirmed by White, who has shown by conductivity measurements that approximately 65% of the phenolsulfophthalein exists in the quinoidal form.

The strongly acid properties of the phenolsulfophthalein series are in marked contrast to those of the weak phenolphthaleins. The carboxyl and phenol groups of phenolphthaleins differ so little in strength and the alkali is partitioned between them to such an extent that visible quantities of the "dibasic quinoidal carboxylate phenolate salt" (G) and (H) are formed by the addition of the smallest quantities of standard alkalies. On the other hand, a consideration of the phenolsulfophthaleins corresponding to (D) and (E) shows that the molecule contains a very weak phenol group which is suppressed still more in ionization by the strong sulfonic acid with an affinity constant close to those of the strong mineral acids. This series was chosen for the present studies because it was predicted that they would act as "self-indicators" and that a large fraction of one molecule of alkali would be neutralized by the strong sulfonic acid group before the useful P_H range would be reached, or, to be more explicit, before any *phenol* salt could be formed and an appreciable

[1] Kober and Marshall, THIS JOURNAL, 34, 1431, 1432, isolated the colorless carbinol monobasic salts.

[2] White, Dissertation, New Orleans address, *Science*.

[3] *Loc. cit.*

change of color occur. The experimental evidence has confirmed this prediction and the quinone-phenolate theory. Small quantities of added alkali are first neutralized practically completely by the strong sulfonic group without any appreciable change of color and a simultaneous tautomeric rearrangement of (A) into (D) ensues. As fast as the sulfonic acid is neutralized the ionization of the remaining portion is suppressed more and more by the increasing amount of sulfonate anions formed and finally becomes so small that the alkali is neutralized very appreciably by the hydrogen ions from the phenol groups and the *intense, different* color of the dibasic quinone-phenolate salt and its anion appears. The amount of alkali necessary to produce this sharp change of color is about 0.85–0.87 molecule in the case of phenolsulfophthalein, and about 0.98 molecule in the case of thymolsulfophthalein, and hence will necessarily depend upon both (*a*) the value of the constant for the equilibrium between the lactoidal and quinoidal structure, and (*b*) the relative magnitudes of the affinity constants of the sulfonic acid and of the phenol. The equilibrium constants under (*a*) are being measured and are therefore not adequately treated in this paper. By substituting negative bromo or nitro groups in the $—C_6H_4SO_3H$ residue, the affinity constant of the $—SO_3H$ group is increased, while that of the phenol group and the P_H ranges are not changed.[1] Phenolsulfophthalein and phenolnitrosulfophthalein[1] have the same P_H range, 6.8–8.4, and thymolsulfophthalein and thymolnitrosulfophthalein[1] have the same P_H range, 8.0–9.6. The ionization of the phenol group is simply suppressed more by the nitrosulfonic acid group and nearly one molecule of alkali can be added before the deeply colored dibasic salt is visible. By the substitution of negative bromo or nitro groups in the phenol residue, however, the affinity constant of the phenol groups is greatly increased. For example, the affinity constant of phenol, about 10^{-10}, is increased about 700 times or to 6.8×10^{-8}, by the introduction of one nitro group in the ortho position, and increased about 2,700,000 times, or to 2.7×10^{-4}, by its conversion into *o,o*-dinitrophenol. It follows then that the introduction of these negative groups into the phenol residues of (D) by the ordinary substitution methods should, and does, make the phenol group much stronger and hence a smaller P_H value will still give enough phenolate anions to begin to give the color; in other words, the addition of smaller amounts of bases will cause the intense color changes in these substituted compounds. On the other hand, the substitution of methyl, isopropyl or amino groups in the phenol residues should, and does, lower the affinity constant of the phenol group and raises the P_H rays from 6.8–8.4 for phenolsulfophthalein to 7.2–8.8 for *o*-cresolsulfophthalein and 8.0–9.6 for thymolsulfophthalein. These alkyl groups, therefore, raise the 0.85

[1] See Lubs and Clark, *J. Wash. Acad. Sci.*, **6**, 483 (1916).

mol alkali required to change the color of the phenolsulfophthalein to about 0.95–0.98 molecule of alkali required to raise the P_H value enough to give the intense color change in *o*-cresolsulfophthalein and thymolsulfophthalein. The introduction of two bromine atoms into the thymol groups then raises the affinity constant of the thymol phenol group and lowers to 0.90–0.92 molecule the amount of alkali necessary to give the P_H value required for the intense color change.

In general, the introduction of negative chloro, bromo, nitro, etc., groups into the phenol residues might be expected to change the P_H range in a fairly regular manner for all these compounds. It is, therefore, interesting to note[1] that the difference between the P_H ranges for thymolsulfophthalein, 8.0–9.6, and for dibromothymolsulfophthalein, 6.0–7.6, is about 2.0, which is the same as the difference between the useful P_H range for *o*-cresolsulfophthalein, 7.2–8.8, and dibromo-cresolsulfophthalein, 5.2–6.8. This value 2.0 is also just half the difference between the P_H ranges for phenolsulfophthalein, 6.8–8.4, and *tetra*bromophenolsulfophthalein, 2.8–4.6. We expect to study these relations for a large number of such compounds. It is only fair to state that it is difficult to purify some of these indicators and that some of the quantitative data given in this paper may be modified later.

There is now evidence[2] at hand to show that the source of intense color in these solutions is *chiefly* the inter- or intra-molecular *combination* of a quinone and a phenolate ion in (G) and (H) and not simply the quinone and *non-ionized* phenolate salt. In solutions of the free tetrabromo- and tetranitro-phenolsulfophthalein and of the dinitro-thymolsulfophthalein the phenol groups are highly ionized and give clearly, *without the addition of any alkali*, the colors characteristic of the nearly completely ionized dibasic salts. The addition of strong mineral acids suppresses the ionization of the phenol group and discharges the intense color of the quinone-phenolate ion. The intense color would not fade if it came from the *nonionized* quinone-phenolate grouping. That the quinone group actually combines with the phenolate ion is shown by the fact that the yellow band characteristic of the spectrum[3] of the free acid and monobasic salt, and hence coming from the quinone group, disappears when the dibasic salt is formed. All phases of this problem will be studied by the use of the phenolsulfophthaleins and their salts, esters, and other derivatives.

Phenolsulfophthalein.—White showed that when eleven parts of pure crystalline anhydride of sulfobenzoic acid are fused with 12 parts of phenol at 140° for 6 hours, as described, by Remsen,[4] Sohon, and Holmes,

[1] Lubs and Clark, *J. Wash. Acad. Sci.*, **6**, 483 (1916).

[2] White, New Orleans address, *Science*, **42**, 101.

[3] White, Dissertation, New Orleans address, *Science*.

[4] *Am. Chem. J.*, **20**, 257; **25**, 201.

the mass becomes pasty. It is allowed to cool and is boiled with water and filtered. The same compound has been made by Lubs[1] and Clark by heating the acid chloride and phenol. The red powder is dissolved in dilute alkali and reprecipitated by addition to hot dilute hydrochloric acid. When washed repeatedly with hot water the powder is practically pure. It can be recrystallized from phenol by the addition of ether. The acid is soluble in water to the extent of about 0.3 g. per liter. It is yellow in slightly acid solution, pink in stronger acids, and purple in weak alkalies. Stronger alkaline solutions discharge the color, probably through hydration, and the colorless solution becomes colored again on the addition of acids. When an excess of the solid is agitated with alkaline solutions, or with carbonates or with oxides of the heavy metals, yellow or orange solutions of the monobasic[2] salts are formed, and these salts can be isolated by evaporation of the solutions *in vacuo*. The addition of two molecules of an alkali to the acid produces the deep red dibasic salt and the addition of soluble salts of heavy metals does not produce deep red precipitates of the dibasic heavy metal salts. Solutions containing about 0.03 g. phenolsulfophthalein in 100 cc. water are yellow or orange in color. The addition of 0.01 *N* alkali produces *locally* the deep red color of the dibasic salt where the drop of alkali falls, but this color disappears immediately when the solution is stirred and the dibasic salt is changed to the yellow monobasic salt as follows:

$$\underset{\text{yellow}}{C_{19}O_5SH_{14}} + 2NaOH \rightleftarrows \underset{\text{deep red}}{C_{19}O_5SH_{12}Na_2} + 2H_2O$$

$$\underset{\text{deep red}}{C_{19}H_5SH_{12}Na_2} + \underset{\text{yellow}}{C_{19}O_5SH_{14}} \rightleftarrows \underset{\text{yellow}}{2C_{19}O_5SH_{13}Na}$$

This set of reactions can be continued until about 0.85–0.87 molecule of alkali is added and the red color becomes faint and permanent. It then requires about 2% more 0.01 *N* alkali to change the color into a decided red, which increases in intensity by the addition of more alkali. This set of color changes is characteristic of all the analogous sulfophthaleins, only the quantitative relations and colors varying in different cases.

The following data[3] show the amount of alkali required to produce the permanent color change. Carbon dioxide must be rigidly excluded in the titration of all these sulfophthaleins. Approximately 0.02 *N* alkali was found to be suitable for the titrations. In the column headed "Cc NaOH, theory" are given the number of cubic centimeters of alkali calculated for the sulfophthalein as a monobasic acid, and Columns 5 and 6 give the amount and per cent. of one molecule actually required.

[1] *Loc. cit.*

[2] White, Dissertation, etc.

[3] A new standard alkali solution was used in this and succeeding titrations, which accounts for the fact that 8 required a larger *volume* of alkali than did 6, although Sample 8 was smaller than Sample 6.

No.	Wt. of phthalein.	Vol. of solution. Cc.	Cc. NaOH theory.	Cc. NaOH found.	Per cent. of 1 molecule. Eq.
1	0.0314	300	4.28	3.75	87.6
2	0.0310	150	4.28	3.73	87.1
3	0.0288	125	3.99	3.43	87.2
4	0.0322	200	4.40	3.77	85.6
5	0.0176	200	2.40	2.10	87.5
6	0.0420	150	5.73	4.97	86.6
7	0.0131	100	1.73	1.51	87.2
8	0.0389	300	5.95[1]	4.76	80.0
9	0.0329	300	5.03	3.96	78.7
10	0.0297	300	4.54	4.11	90.5
11	0.0252	300	3.86	3.49	90.4
12	0.0329	300	5.04	4.41	87.5
				Average,	86.3

Titrations 1 to 6, inclusive, were made with material obtained from Hynson, Westcott & Company, Baltimore. The others were made with material of our own preparation,[1] 10, 11 and 12 having been crystallized from phenol by the method already described.

The color changes of phenolsulfophthalein are produced in solutions varying in hydrogen-ion concentration from P_H 6.50 to P_H 8.50, and are very sharp between P_H 7 and P_H 7.5. Six drops of a 0.012% yellow solution of the monosodium[2] salt make an excellent indicator when diluted with 50 cc. of water and give a distinct purple color when only 0.01 cc. 0.1 *N* alkali is added.

Tetrabromophenolsulfophthalein.—This substance was prepared by White by the action of bromine on phenolsulfophthalein in glacial acetic acid. It melts at 270–1°. A concentrated aqueous solution is orange in color with a tinge of blue. The addition of hydrochloric acid decreases the ionization of the phenol group, discharges the blue color, and makes the solution yellow or orange in color. When this solution is diluted and the phenol group ionizes more the blue color comes out strongly again and the addition of alkali makes the solution intensely blue. The addition of hydrochloric acid again discharges the blue color. For example, 12 drops of 0.04% alcoholic solution of the tetrabromo compound added to 50 cc. water gave a solution whose blue color was distinctly discharged by the addition of 0.5 cc. 0.01 *N* hydrochloric acid. The larger affinity constant of the bromophenol group causes the color changes to take place sharply between P_H 3.2 and P_H 4.5. Three to six drops of a 0.04% alcoholic solution of the acid make an excellent indicator.

Tetranitrophenolsulfophthalein.—This compound was made by White

[1] White, Dissertation.

[2] Aqueous solutions of the monosodium salts of all of the sulfophthaleins can be used as indicator solutions.

by treating phenolsulfophthalein with a mixture of concentrated sulfuric and nitric acids. The compound is yellow in strongly acid solutions, but purple in water because of the large ionization of the nitro-phenol group. The addition of alkali does not appreciably increase the depth of the purple color but the addition of strong hydrochloric acid causes this to fade to yellow, which is changed to purple again when the solution is diluted and the ionization of the nitro-phenol group is increased. The large affinity constant of the nitro-phenol group is shown by the fact that White found a molecular conductivity of about 430 for this substance in $N/1000$ solution, of which value about 370–80 as a maximum comes from the sulfonic acid group. The nitro-phenol group is too strong an acid to allow this substance to be a valuable indicator for most work. An excess of alkali causes the purple color to fade to yellow, which immediately changes to purple again when the excess of alkali is neutralized.

o-Cresolsulfophthalein.—This indicator can be prepared either by the method of Remsen and Sohon or that of Lubs and Clark. The fusion of *o*-cresol with either the anhydride or chloride of sulfobenzoic acid gives about 50% yield. The *o*-cresolsulfophthalein can be recrystallized from glacial acetic acid and is soluble in alcohol and water. It is yellow in very dilute acid solution and purple in dilute alkalies. Two to four drops of a 0.4% alcoholic solution make an excellent indicator. Six drops of a 0.2% alcoholic solution added to 50 cc. water gave a distinct color change when 0.01 cc. 0.1 *N* alkali was added. The color changes are produced in solutions having P_H 7.2 to P_H 8.8.[1] The titration of *o*-cresolsulfophthalein with dilute alkalies shows clearly that the cresol group is so weak that practically one molecule of alkali is neutralized by the sulfonic acid group before sufficient phenolate salt is formed to change the color from orange to purple. For example, 0.0567 gram required 14.5 cc. 0.01 *N* alkali, or 98% of the theory, instead of the calculated 14.83 cc. alkali. The alkaline solution shows color phenomena characteristic of nearly all of these sulfophthaleins. A deep blue color is observed in reflected light. In transmitted light the solution appears deep red and the spectrum shows only a red band.

Thymolsulfophthalein.—This substance was prepared by Lubs and Clark by fusing the acid chloride with thymol in the presence of dehydrated zinc chloride 4 hours at 140° with frequent stirring. The melt was boiled with water and the hardened mass was extracted with alcohol to remove the remaining thymol. The cold alcoholic solution gave a precipitate of the thymolsulfophthalein as greenish crystals. The substance is yellow in very dilute acids, pink in concentrated acids, and deep blue in dilute alkalies when viewed by reflected light but deep red in transmitted light. The color changes take place between P_H 8 and P_H 9.75,

[1] Lubs and Clark, *J. Wash. Acad. Sci.*, **6**, 483.

and the useful range lies between P_H 8 and P_H 9.5, which is about the same as that of phenolphthalein. Three to six drops of a 0.04% alcoholic solution makes an excellent indicator. For example, 12 drops of a 0.04% alcoholic soution added to 50 cc. water give a distinct green color change when 0.01 cc. 0.1 *N* alkali is added; 0.02 cc. makes the solution blue.

The isopropyl and methyl groups lower considerably the ionization of the phenol group and make it necessary to add practically one molecule of alkali before the blue color of the dibasic salt becomes visible. Titration of samples kindly furnished by Mr. H. A. B. Dunning showed that 0.0548 g. required 11.50 cc. 0.01 *N* alkali, or about 0.98 molecule instead of the theoretical 11.75 cc. Material prepared by Lubs gave the following data: 0.0564 g. required 11.70 cc., or 0.967 molecule, instead of the theoretical 12.10 cc.; 0.0560 g. required 11.60 cc. or 0.967 molecule instead of the theoretical 12.01 cc. As it requires only about 0.02 molecule of alkali to change the color decidedly from that of the orange-free acid and monobasic salt to the deep blue of the dibasic salt, it is clear that the thymolsulfophthalein gives excellent substantiation of the quinone-phenolate theory that the intense color change is not due to a monobasic salt and simply to the quinone group, but arises from the formation of a compound formed by the union of a quinone and a phenolate ion.

Dibromothymolsulfophthalein.—This indicator was prepared by Lubs and Clark by the action of bromine in glacial acetic acid on the thymolsulfophthalein and is obtained in yellow crystals. It is yellow in acid solution and deep blue in dilute alkalies when viewed by reflected light but red in transmitted light. The best color changes occur between the ranges of P_H 6 and P_H 7.25. About 2 drops of a 0.04% alcoholic solution make an excellent indicator, and become distinctly green in 50 cc. water when 0.005 cc. 0.1 *N* alkali is added; 0.01 cc. makes the solution blue.

The influence of the negative bromo groups in increasing the ionization of the phenol group is made clearly evident by the fact that less alkali is required to give the blue color than was found to be necessary with thymolsulfophthalein. For example, 0.0628 g. of a sample prepared by Dunning required 9.2 cc. 0.01 *N* alkali, or 0.91 molecule, instead of the theoretical 10.06 cc. Of material prepared by Lubs and Clark 0.0555 g. required 7.55 cc. 0.01 *N* alkali, or about 0.85 molecule, instead of the theoretical 8.89 cc. and 0.0643 g. required 9.50 cc. 0.01 *N* alkali, or about 0.92 molecule, instead of the theoretical 10.30 cc.

Thymolnitrosulfophthalein.—This compound was made by Lubs and Clark by heating thymol with the acid chloride of nitrosulfobenzoic acid and dehydrated zinc chloride. The subsequent treatment was like that for thymolsulfophthalein. It forms a powder which appears red or green-

ish blue, depending upon its fineness of subdivision and the way in which it is illuminated. It is yellow in dilute acids and deep blue in dilute alkalies in reflected light and shows a red and a violet band in transmitted light. This compound was made because it was predicted that the nitro group in the benzene sulfonic acid residue would increase the affinity constant of the sulfonic acid group more than that of the phenol group and that practically one molecule of alkali must be added before the intense color of the dibasic salt is observed. The experiments verify the theory. For example, 0.0415 g. requires theoretically 8.12 cc. 0.01 *N* alkali to neutralize the sulfonic acid group. The concentrated aqueous solution of this amount of the indicator is orange in color and the addition of 7.92 cc. or 0.98 molecule produces no change of color; when 8.02 cc., or 0.99 molecule of alkali, are added the blue color of the dibasic salt just began to appear. When 8.22 cc. or about 1.01 molecule of alkali were added there was a distinct change of color. When 0.67 cc. more 0.01 *N* alkali was added the color of the solution was dark green, when 1.2 cc. were added the solution was very dark green, and when 2.15 cc. were added the solution was bluish green. A total of two or more molecules of alkali made the solution dark blue in color. In another experiment 0.0435 g. was dissolved in about 200 cc. of hot water and titrated with 0.01 *N* alkali. One molecule of alkali corresponds to about 8.6 cc. alkali, and it required 8.2 to 8.3 cc., or about 0.96 molecule to produce any noticeable change in color. The addition of 8.5 cc. 0.1 *N* alkali made the solution dark red, 8.7 cc. made the solution very dark, 9.63 cc. made the solution olive-green, and 10.0 cc. made the solution a decided green in color. The addition of an excess of alkali made the solution deep blue and boiling the solution did not cause the color to fade perceptibly through the well-known hydration phenomenon. The excellence of this indicator is shown by the fact that 4 drops of a 0.04% alcoholic solution added to 50 cc. water required only 0.01 cc. 0.1 *N* alkali to give a distinct green color; 0.02 cc. makes the solution blue. The color changes take place between P_H 8.2 and P_H 9.6.

Conclusions.

1. Solutions of sulfophthaleins having no negative bromo or nitro groups in the phenol residues are yellow or orange in color; the colors and conductivities show that the indicators exist largely in the quinoidal form. They are "self-indicators" and can be titrated with from 0.85 to 0.98 molecule of alkali before the intense color change due to the dibasic salt is observed. The monobasic sulfonic acid salt (F) has practically the same orange or faint red color as the free, nearly completely ionized acid. All these facts make untenable theories formerly proposed by others that the intense color changes in the phenolphthalein series arise simply from the quinone group. The data give further evidence for the quinone-

phenolate theory that the intense purple or blue color is produced only when appreciable quantities of the dibasic quinone-phenolate salts are formed, which then give rise to the combination of the quinone group and the phenolate anion. The introduction of negative groups into the benzenesulfonic acid residue increases the affinity constant of this group but does not alter appreciably the affinity constant of the phenol group or the P_H range.

2. Solutions of sulfophthaleins having negative bromo or nitro groups in the phenol residues have high molecular conductivities and low P_H ranges, which fact indicates considerable ionization of the phenol residue. In harmony with this idea is the fact that these solutions exhibit more or less the colors of the dibasic alkaline salts. In conformity with these conclusions is the fact that the addition of mineral acids to solutions of these sulfophthaleins suppresses the ionization of the phenol group and changes the intense color characteristic of the dibasic salts into the fainter yellow or orange characteristic of the quinone group. In the *solid state* these bromo- and nitrophenol derivatives are yellow, while the sulfophthaleins with no negative groups in the phenol residues, or having negative groups in the benzenesulfonic acid group, are generally dark brick-red.

3. The fact that 0.98 molecule of alkali can be added to *o*-cresolsulfophthalein before the deep color appears shows that practically all of the monobasic salt exists as (F) and almost none as the colorless form (B) and that practically all of the dibasic salt is in the forms (G) and (H) and practically none in the colorless form (C). This monobasic salt is, therefore, nearly an ideal indicator because practically none of the alkali and indicator is wasted in forming colorless hydrated or lactoidal salts (C) but all is consumed in the formation of the deeply colored dibasic salts (G) and (H). These relations may vary for the different members of this group. This point is very important in the proposed equilibrium studies of these compounds and no such evidence can be obtained in the phenolphthalein series.

4. The free sulfophthaleins and the monobasic salts give absorption spectra containing a yellow band characteristic of the quinones. When the dibasic salt is formed this yellow band disappears and a deep red band appears. This indicates that the quinone group as such disappears because it combines with the phenolate anion and forms a complex quinone-phenolate group.

5. Solutions of the dibasic salts of some of these sulfophthaleins have different colors when viewed in reflected and then in transmitted light. This phenomenon has been noted by one of us in solutions of crystal violet and other substances and is being investigated to learn the charac-

teristics of the "reflection and transmission spectra" for each of these substances.

6. Solutions of some sulfophthaleins which are yellow in neutral or faintly acid solutions become red in stronger acids and, hence, have been found by Mr. Homer Cloukey to be colored red by such salts as zinc chloride and stannous chloride. But even some neutral salts, such as sodium chloride, change the colors of these solutions and this "salt effect" of acids, bases and salts will be extensively investigated.

7. On account of all of these characteristics of the sulfophthaleins it has been found possible to prepare a series of indicators having a wide range of sensibility of hydrogen ions. By substituting bromo, nitro, methyl, isopropyl, amino and other groups in the benzenesulfonic acid group, and especially in the phenol residue, one is enabled to change the ionization constants of the sulfonic acid and phenol groups greatly and hence prepare indicators covering a wide range of usefulness. The phenol, thymol, and cresolsulfophthaleins, and their bromo derivatives, are highly satisfactory indicators.

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EFFECTS OF LARGE APPLICATIONS OF COMMERCIAL FERTILIZERS ON CARNATIONS.*

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In the investigation of the use of commercial fertilizers in growing carnations by the Illinois Agricultural Experiment Station, it has been found that the lack of appreciation by florists of the relatively high plant food concentrations and often high solubilities of commercial fertilizers, as compared with manure, has often led to a complete loss of a crop of flowers in an effort to produce an extraordinarily large one. On this account, it was considered desirable to study the causes and effects of overfeeding with the more ordinarily used commercial fertilizers.

The fertilizers chosen for the experiment were dried blood, sodium nitrate and ammonium sulfate, acid phosphate and disodium phosphate, and potassium sulfate. For comparison, sodium chloride and sodium sulfate also were used on some sections. Experimental work upon the subject was carried out during the years 1912–15.

Carnations are propagated by means of cuttings, and from these it was

* This paper presents the data contained in the thesis presented by Mr. Muncie in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois.