[FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Oxidation–Reduction Equilibrium, over the Whole *p*H Range, of Oxonine and Some Related Dyestuffs

BY L. MICHAELIS AND S. GRANICK

It has been one of the major objectives of research in this Laboratory during the past years to prove that the reversibility of any bivalent oxidation–reduction depends on the fact that an intermediate univalent reaction product, a free radical, is capable of existence in sufficient concentration so that this concentration is not a limiting factor for the rate of the over-all process of the bivalent oxidation or reduction. The evidence for such a statement ought to consist in showing that at any point of oxidative or reductive titration at any one *p*H, the equilibrium concentration of the radical in a reversible system is not a fictitious, unreasonably small but rather a finite, well measurable quantity. A large equilibrium concentration of the radical has been clearly demonstrated for very acid solutions of cationic dyes, and for very alkaline solutions of anionic dyes. In the intermediate range the only evidence so far is the fact that the potentiometric titration curve is just a little bit steeper than that expected for a bivalent oxidation without intermediate step. Here the deviation, as expressed in terms of the index potential, usually exceeds the limits of error but little. In this paper we shall deal with cationic dyestuffs which are able to form semiquinones to an easily detectable amount not only in very acid, but also in alkaline, solutions. Hereby the interpolation of the behavior in the intermediate *p*H range around neutrality can be accomplished with fair accuracy. It will be shown that the index potentials obtained from this interpolation agree satisfactorily with those obtained directly by titration. Our experience with potentiometric titrations has led us to the conclusion that the index potentials experimentally derived are reliable to within ±0.2–0.3 mv. The agreement between interpolation and experiment in the *p*H region around neutrality supports the conclusion that the values of 0.5 to 1.0 mv. above the theoretical minimum value of 14.3 mv. are certainly well beyond our experimental errors.

Those cationic dyestuffs for which free radicals can easily be detected not only in very acid but also in alkaline solution, are not new ones. They are oxazines and thiazines. The reason why no reliable titration curves in alkaline solutions could be obtained so far is the extremely low solubility of the uncharged free bases of some of the dyestuffs, such as thionine or oxonine, or the insolubility of the leuco dye together with the lability of the dye itself in alkaline solution, such as is the case for methylene blue. We shall begin with the first of these two cases. Oxonine (3,9-diaminophenoxazin[1]) was found to be the most suitable dyestuff to start the investigation, because its semiquinone formation constant in alkaline solution was found to be greater than for other comparable dyes. The artifice by which the difficulty arising from insolubility was overcome, consisted in using a buffer containing some organic solvent, such as alcohol or pyridine. Aqueous buffers containing 20% of pyridine were found very useful. To be sure, Geake and Lemon[2] have shown that the semiquinone formation constant may depend on the nature of the solvent. In the present case, however, and under our working conditions the influence of pyridine in the concentration used was found to be negligible. Comparative measurements of the normal potential and the index potential of oxonine, at *p*H around 6 in aqueous buffers and buffers containing 20% pyridine, or at *p*H around 11.5 with 10% or with 20% pyridine, showed no differences that might have been considered decidedly beyond the limits of error. By *p*H in a pyridine-containing buffer we understand the value calculated from the potential at the hydrogen electrode as though it were a purely aqueous solution. The following experiments accordingly fulfill a double purpose: first, to demonstrate the existence of semiquinones in alkaline solution, supplementing the former experiments in very acid solutions; and, second to utililize the data now available for acid and alkaline solutions to interpolate the behavior in neutral solution. One may thus compare the results obtained for approximately neutral solutions with those obtained previously by utilizing directly such index potentials as exceeded the minimum

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(1) S. Granick, L. Michaelis and M. P. Schubert, (a) THIS JOURNAL, **62**, 1802 (1940); (b) **62**, 204 (1940).

(2) A. Geake and J. T. Lemon, *Shirley Inst. Mem.*, **16**, 11 (1938).

value very little, say, by 0.5 to 1 millivolt, so little indeed that their direct utilization for further calculations based upon them, may have been not quite convincing for some critics.

I. Oxonine

(a) **The Optical Properties of Oxonine and its Semiquinone.**—The free base of oxonine, as existing in solutions of $pH > 11$, is red and shows a very diffuse, broad absorption band around 500 mμ. The univalent cation which exists from *p*H 10 to 0, is blue-violet, exhibiting a gorgeous red fluorescence. It has a sharp and intense absorption band with peak at 575 mμ. In acetate buffer containing 20% pyridine, this band is displaced by about 15 mμ toward the red. The fluorescence band lies in the red and orange and has its center at about 600 to 610 mμ. The bivalent cation, as established in strongly acid solution has a sharp absorption band at 650. In almost concentrated sulfuric acid, a trivalent cation is formed, of dirty bordeaux red color, with a very diffuse and weak band around 550 and another region of diffuse absorption at the violet end of the visible spectrum.

The univalent cation shows, with increasing concentration of the dye, a second band around 540, which, on comparison with analogous cases investigated by Rabinowitch and Epstein,[3] may be interpreted as due to a dimerization ensuing from intermolecular interaction of the electric oscillators established by the quinone–benzene resonance. On comparing the intensities of this second band for various concentrations (see Fig. 9 of the previous paper[1a]) for oxonine with those of thionine, one sees that the polymerization is smaller for oxonine than for thionine (hence even very much smaller than for methylene blue). In the presence of pyridine, the polymerization is still smaller than in a purely aqueous solution. A quantitative estimate of the effect of this polymerization for the conditions of the following potentiometric titrations of oxonine has shown that it is entirely negligible for our working conditions, with respect to the evaluation of all constants within the scope of our concern.

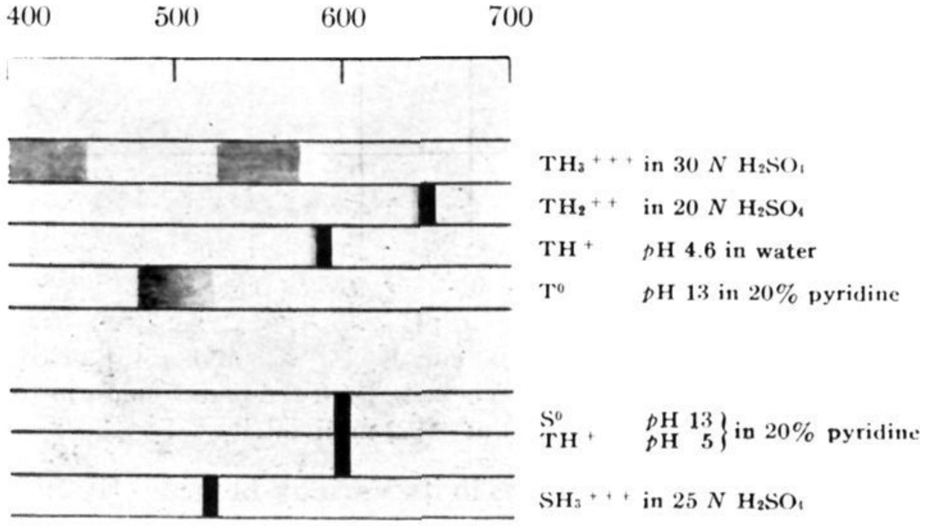

Fig. 1.—The absorption bands of the various quinonoid (T) forms and semiquinonoid (S) forms of oxonine, schematically, as observed in the hand spectroscope.

When in a strongly alkaline solution (0.1 to 1 *N* sodium hydroxide) in 20% pyridine or alcohol, in a concentration of the dye sufficiently high to absorb almost all visible light, the color is diminished by gradually diluting with the solvent, the region of absorption shrinks more and more to the very diffuse band of the uncharged base, in the green and blue, and vanishes almost entirely in such concentrations as still appear pink for the unaided eye. When, however, the color of the concentrated solution is diminished, not by dilution, but by a reducing agent, a new and sharp band arises before fading is complete. Palladium–hydrogen, or sodium hydrosulfite or, more conveniently, because of the slowness of its action, glucose may be used as reducing agent. As the diffuse band of the free base recedes, a new sharp band around 590 mμ arises. It happens to coincide with the band of the univalent quinonoid cation in 20% pyridine solution. Yet, this cation does not exist in such an alkaline solution, nor is there any fluorescence characteristic of it. The color, immediately prior to the complete fading of the red base, is blue-violet, without fluorescence. This band is that of the semiquinone. On oxidizing with air, this band vanishes entirely. The unusually high value of the semiquinone formation constant in alkaline solution for oxonine, together with its favorable optical properties, makes this dyestuff an especially favorable material for the desired studies.

It may be added as a preliminary note that the oxonine radical in alkaline solution also has been identified as a radical by its paramagnetism, as will be described in a further publication.

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[3] E. Rabinowitch and L. F. Epstein, THIS JOURNAL, **63**, 69 (1941).

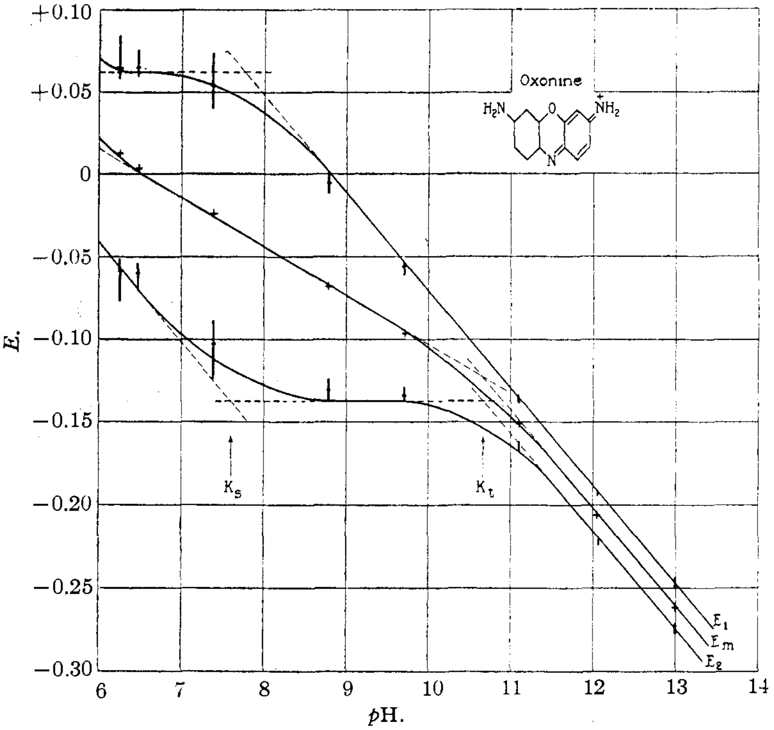

Fig. 2.—The three normal potentials E_1, E_m and E_2, plotted against pH from 6 to 13, for oxonine. The lengths of the marks conform to a probable error in the index potential of ±0.2 millivolt.

(b) Structure of the Dye in its Various Levels of Oxidation and Ionization.—There is no difficulty in writing down the four levels of ionization for the R form. With no proton attached, it is as in formula I. The first proton is attached at position 1, the second at position 2, the third at position 3. We may designate the four ionization levels of R as R^0, RH^+, RH_2^{++}, RH_3^{+++}. The first and second constant of ionization will differ from each other not very much more than postulated according to the statistical effect, the third must be very different. In fact, we have $pk_1 = 6.5$, $pk_2 = 5.5$, $pk_3 = -1.2$, approximately. The T-form, free of detachable protons, T^0, can be written as in formula II. In acid solution TH^+ is formed. The proton is attached not to the $—\dot{N}H_2$ group, but to the $=\dot{N}H$ group (position 1) because of the resonance established hereby. This is the "quinone–benzene resonance," characteristic of almost all dyestuffs with a sharp band spectrum in the range of visible wave lengths.[4] In order to establish the lowest possible level of energy, the second proton in TH_2^{++} is attached to the bridge-N (position 2), whereby the resonance is maintained, as is the sharpness of the absorption band. In fact, the band of TH_2^{++} is of the same type as that of TH^+, only displaced by 80 mμ toward the red. When TH_3^{+++} is formed by forcing upon the molecule a third proton, in strong sulfuric acid, at position 3, any quinone–benzene resonance is abolished, and the absorption spectrum is not only displaced toward the blue but is also very much more diffuse. The attachment of this third proton requires such an extremely acid solution because the NH_2 group to which it is attached is not a regular amino group, but one in resonance with a $=NH_2^+$ group.

The structures of the S-forms may be discussed as follows. Since there are three N-atoms capable of combining with a proton, we have to distinguish the ionization levels S^0, SH^+, SH_2^{++} and SH_3^{+++}. Judging from the parallelity of the E_1, E_2 and E_m curves (Fig. 2) in strongly alkaline solution, the ionization level of S is easily obtained for $pH > 11$. It is S^0. Two tautomeric forms of S^0 are imaginable, S^0a and b (III and V). In both of them, the electrons may be distributed in various ways; so several resonating structures can be written for both. Of these, two are especially worth mentioning, S^0a, α and β (IV) which represent an equivalent resonance system. Although the distribution of the electric charge here is less favorable than in S^0a (III) the resonance energy may be large enough to compensate for the separation of the charges. Since this type of resonance (IV) is the same as in TH^+, one might expect the same type of absorption spectrum. In fact, the radical in the ionization level S^0 has the same absorption spectrum in the visible region as TH^+, which exists in acid solution. A similar resonance for S^0b (V) is not imaginable. So it is suggestive to admit at least in part the resonance structure (IV) for the S^0 form.

The SH^+ form is imaginable in several tautomeric forms by attaching a proton to any one of the available N atoms in S^0. As Fig. 3 shows, it can exist only in a very low equilibrium concen-

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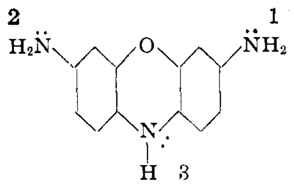
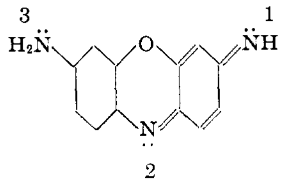
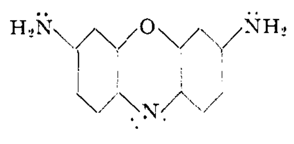
(4) As to the correlation of resonance and color in dyestuffs, see L. Pauling, *Proc. Natl. Acad. Sci.*, **25**, 577 (1939); G. Schwarzenbach, *et al.*, *Helv. Chim. Acta*, **20**, 490 and 654 (1937); THIS JOURNAL, **60**, 1667 (1938).



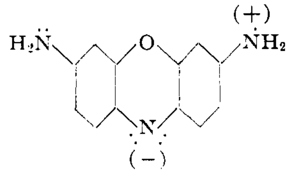
I (R°)



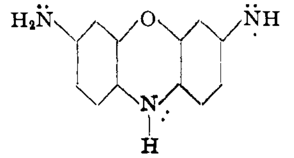
II (T°)



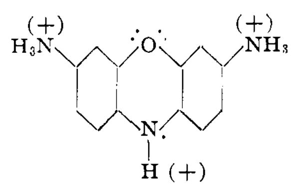
III (S° a)



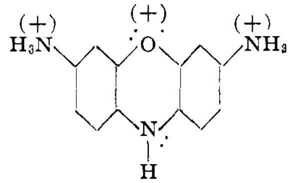
IV (S°-a,α; shifting the charge from left to right, it is S°-a,β)



V (S b)



VI (SH_3^{+++}, α)



VII (SH_3^{+++}, β)

tration. Since there is no spectroscopic evidence for SH^+ either, it will not be worth while to discuss the various possible resonance structures of the various tautomers of SH^+.

On proceeding to SH_2^{++} and SH_3^{+++}, we encounter a new situation, which is similar for both. As can be seen from Fig. 3, SH_2^{++} is capable of existence in equilibrium condition only to a minute extent. It is understandable therefore that spectroscopic evidence is missing. However, SH_3^{+++} can easily be detected spectroscopically. It is that form of the radical described in the previous paper (1, a and b) as existing in very acid solution. We restrict the discussion to this SH_3^{+++} form. It is formulated in (VI), in resonance with (VII). The profound change of type of resonance from that in the lower levels of ionization may seem a mere speculation at first glance. However, the following consideration lends support to such a suggestion, and even seems to make it quite unavoidable.

It has been shown that the spectrum of s-oxazine, the free radical derived from the unsubstituted skeleton of the oxazine dyes, such as arising from partial oxidation of oxazine in an acid solution, differs from the analogous s-thiazine (and also s-selenazine): s-oxazine has one sharp, very intense band, s-thiazine has a complicated series of less intense bands. Furthermore, as has been shown, all dyestuffs derived from oxazine by attachment either of one or of two amino groups, form in strongly acid solution a radical with the same absorption spectrum as does the unsubstituted s-oxazine, and all radicals of dyestuffs derived from thiazine, or selenazine, by attachment of one or two amino groups or even methylated or phenylated amino groups, have the very characteristic, unmistakable complicated spectrum of unsubstituted s-thiazine (or selenazine). It is evident that the side chains, if there be any, are not involved in the electric oscillator responsible for this spectrum. This is solely determined by the nature of the bridge atoms. So, the resonance of the SH_3^{+++} form of oxonine, may with good reason be assumed to be essentially of the same nature as that of the unsubstituted oxazine, and not influenced by the amino side-chains. In SH_3^{+++} both side chains are quaternary amino (not imino) groups and thus can supply no electrons for resonance across the molecule. This may be taken as sufficient evidence for the resonance structure VI $\rightleftarrows$ VII.

(c) Potentiometric Titrations of Oxonine.—The experiments are tabulated in Table I.

(d) The Three Normal Potential Curves for Oxonine.—Sufficient data are now available to construct a diagram of the three normal potentials for oxonine over a pH range from almost concentrated sulfuric acid to pH about 14 (Fig. 3). For the most acid range, the plot may be considered as a schematic one, the values of pH and of the ionization constants being only approximately known. To evaluate pH in this region, Hammett's acidity functions were taken[5] as pH values. The data obtained by Hammett were used by Hall and Spengeman[6] for plotting the acidity function against molarity of sulfuric acid with the remarkable result that this plot beginning with 1 *M* acid, is a straight line over a wide range, with slope 0.5. This holds not only over the range as drawn by these authors, but up to 18 *M* sulfuric acid. This plot has been used for translating the various concentrations of sulfuric acid as used in terms of normality in the previous paper, into pH values.

The ionization constants K_{t_2} and K_{t_3} were

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(5) Hammett and Paul, THIS JOURNAL, **56**, 827 and 830 (1934); **58**, 2182 (1936).

(6) N. F. Hall and W. F. Spengeman, *ibid.*, **62**, 2487 (1940).

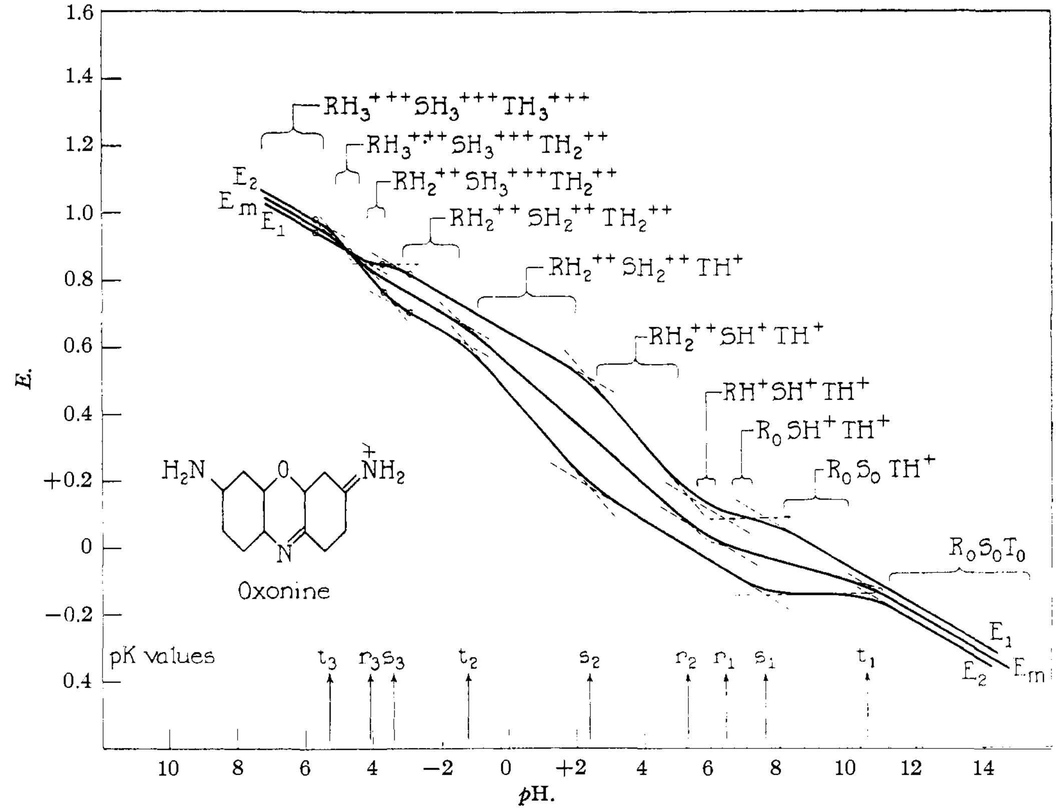


Fig. 3.—The three normal potentials of oxonine plotted against the pH range from −8 to +14. The ionization constants of the R, S and T forms are marked by arrows. The experimental values used for plotting the region of high acidity are marked by circles.

determined optically as follows. A solution of the dye at pH 4.6, containing only TH^+, and another solution in 15 N sulfuric acid, containing only TH_2^{++} were prepared. Then a solution of the dye in such a concentration of sulfuric acid was established, so that its color matched that of the optical mixture of TH^+ and TH_2^{++} in a color comparator, provided the total concentration of the dye was double that of either the pure TH^+ or the TH_2^{++} solution. The acidity function of this sulfuric acid concentration, was considered as its pH, and thus also as pk_{t_2}. In an analogous manner, pk_{t_3} was determined. No great accuracy is claimed for these values.

In this acid region the plot shows schematically in principle what one wants to know. On the other hand, the measurements presented in this paper yield rather accurate data for the plot in its alkaline region (Fig. 2). The alkaline end and the acid end of this plot can now be easily interpolated so as to fit to the number and most probable positions of the bends in the intermediate range. So one arrives at diagram Fig. 3. Those points in the E_1 and E_2 curve fixed by potentiometric and spectroscopic data, in the extreme acid region, are marked by circles.

Now we may compare the interpolated middle section with the results obtained directly from such index potentials which exceed the possible minimum value (14.3 mv.) by not more than 0.5 to 1 millivolt. It can be seen that there is a satisfactory agreement. We may add that in some experiments in the neighborhood of pH 0, the index potential was within the limits of error equal to its theoretical minimum value 14.3, which also agrees with Fig. 3. The bends corresponding to the ionization constants of the S-forms are herewith unambiguously fixed, if the whole picture is to become a coherent one. The accuracy of the ionization constants of S, of course, is not very great. However, the most interesting values, namely, those of the maximum ratio of semiquinone to total dye, $(s/a)_{max}$, are very little affected by such uncertainties in the ionization constants of the S forms. It may be added that the value of pk_{r_3} is rather arbitrary, but some incorrectness

TABLE I

OXONINE, 1 × 10^{-4} MOLAR

Buffer, 100 cc. of buffer consisting of 20 cc. pyridine and the aqueous buffers:	*p*H of the final buffer	E_m potential in volts, at 50% oxidation	E_i Index potential in millivolts 25–50% oxidation	E_i Index potential in millivolts 50–75% oxidation	Method
(1) Acetate buffer, ionic strength 0.1 (*p*H without pyridine 4.45)	6.26	+0.0125	14.8	14.8	Red. with Pd + H_2, titr. with $K_3Fe(CN)_6$
(2) Acetate buffer, ionic strength 0.1 (*p*H without pyridine 4.62)	6.49	+ .0035	15.0	15.0	Same
(3) 45 cc. *M*/15 KH_2PO_4 + 25 cc. *M*/15 Na_2HPO_4; final volume filled up with water	7.42	− .0241	14.9	14.6	Same
			Av. 14.75		
(4) 2.07 g. sodium veronal; 2.5 cc. 1 *N* HCl, filled up with water	8.79	− .0685	15.0	15.0	Same
(5) 1.50 g. dimethyl glycine + 5.4 cc. *N* HCl; filled up with water	9.72	− .0965	16.1	16.1	Red. titrn.[a] with leuco-rosindulin GG
(6) 60 cc. *M*/15 Na_2HPO_4 + 10 cc. *N*/10 NaOH; the rest water	11.11	− .1511	18.9	18.6	Red. with Pd + H_2,[b] titr. with $K_3Fe(CN)_6$
(7) 45 cc. *M*/15 Na_2HPO_4 + 30 cc. *N*/10 NaOH; the rest water	12.05	− .2065	19.0	19.0	Same[b]
(8) 10 cc. 1 *N* NaOH; the rest water	13.05	− .2617	17.5	19.2	Same[b]
			Average 18.35**		

[a] This dyestuff, almost indispensable for reductive titrations because of its very negative potential range and its stability over a wide *p*H range, is no longer commercially available. It can be prepared from Rosinduline 2B (National Aniline and Dye Company) as follows. A solution of 5 g. of this dye in 60 cc. of water is heated in a sealed tube at 180° for twenty-four hours. Orange rosets of the free Rosindon-monosulfonic acid appear on cooling. Acidify the solution with 10 cc. of 2 *N* HCl. Filter off the precipitate. If the starting material is good, the filtrate is practically colorless; yield 1.9 g. or 70%. Convert this acid into its well-crystallized sodium salt (Rosindulin GG) by suspending in 200 cc. of water and adding sodium carbonate just to neutralization. Add 2 g. of sodium chloride, heat to boiling, cool twenty-four hours at room temperature, filter, wash with very little 1% sodium chloride solution. Calculated for the dry sodium salt: N, 6.60; S, 7.55. Found: N, 6.65; S, 7.6. Potentiometric titration of its leuco dye with $K_3Fe(CN)_6$ in acetate buffer *p*H 4.62 shows normal potential −0.1298 volt, with index potential 15.1; 15.2 millivolts. [b] In these alkaline solutions the potential at the end-point drifts back due to a further slow irreversible oxidation. This causes a slight indefiniteness of the end-point only at *p*H 13, hence the slight apparent asymmetry of the index potential in experiment No. 8.

of its value has not much influence on the shape of the curve in the more accessible *p*H range.

It may seem striking that the attachment of the third proton in SH_3^{+++}, according to diagram Fig. 3, is easier than that of the third proton in TH_3^{+++}, the ionization exponents being −3 and −5.7, respectively. The acceptance of this fact is unavoidable in order to bring about the convergence of the three potential curves ensuing in the crossing over at *p*H −5.1. Hereby the almost abrupt increase of the semiquinone formation constant in strongly acid solution is accounted for. The explanation of this fact may be this. The third proton of the T form is attached to a group which is partly a —NH_2 group, partly a =NH_2^+ group, because of the resonance. This makes this group a very weak basic one. In contrast, the third proton of the S form is added to a true —NH_2 group, which is, though weakly basic, yet not weaker than any third basic group of a trivalent base of similar structure.

B. Thionine and Methylene Blue.—Thionine is analogous to oxonine. The difference is essentially that the maximum value of the semiquinone formation constant, holding for strongly alkaline solution, is decidedly smaller than for oxonine. The highest index potential is 15.6 mv. (instead of 19 for oxonine). The excess above the the-

TABLE II

CORRELATION OF *p*H; E_i (INDEX POTENTIAL); $E_2 - E_1$ (DIFFERENCE OF THE NORMAL POTENTIAL OF THE MORE POSITIVE AND THE MORE NEGATIVE UNIVALENT PARTIAL OXIDATION–REDUCTION SYSTEM); LOG *k* (*k* = SEMIQUINONE FORMATION CONSTANT AND $(s/a)_{max}$ (MAXIMUM RATIO OF SEMIQUINONE TO TOTAL DYE)

Approximately interpolated values according to Fig. 3

*p*H	12	10	8	6	4	2	0	−2	−4	−6
$E_2 - E_1$	−24	−46	−182	−110	−230	−315	−180	−120	−40	+35
E_i	19	17	14.6	14.9	14.4	14.3	14.6	15.1	17.7	28
log *k*	− 0.40	− 0.76	− 3	− 1.8	− 3.8	− 5.2	− 3	− 2	− 0.67	+ 0.58
$(s/a)_{max}$ × 100	23	17	1.6	5.7	0.6	0.12	1.5	4.7	19	50

TABLE III

THIONINE, 1 × 10^{-4} MOLAR

Buffer: Na_2HPO_4, $M/15$, cc.	Buffer: NaOH, cc.	Buffer: Pyridine, cc.	Buffer: Water to cc.	pH	E_m	E_i: 25%–50% Oxidation	E_i: 50%–75% Oxidation	Method
60	7 0.1 N	20	100	10.84	−0.0687	15.6	15.6	Reduced with PdH_2, titrated with $K_3Fe(CN)_6$
60	10 0.1 N	20	100	11.16	− .0867	16.0	16.0	
60	13 0.1 N	20	100	11.34	− .0931	15.7	15.5	
..	10 1 N	20	100	12.93	− .1852	16.1	16.3	

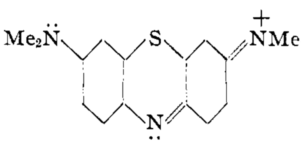
TABLE IV

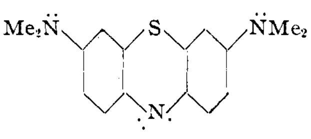
METHYLENE BLUE, 1 × 10^{-4} MOLAR

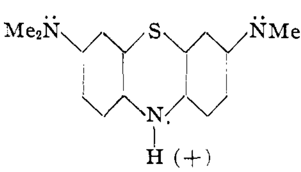
	Buffer	pH	E_m	E_i: 25%–50% Oxidation	E_i: 50%–75% Oxidation	Method
1	35 cc. $M/15$ KH_2PO 35 cc. $M/15$ Na_2HPO_4 20 cc. pyridine Water to 100 cc.	7.56	+0.0020	14.8	15.0	Reduced with Pd + H_2 titrated with $K_3Fe(CN)_6$
2	25 cc. $M/15$ KH_2PO_4 25 cc. $M/15$ Na_2HPO_4 50 cc. absol. ethanol Water to 100 cc.	7.70	+ .0110	14.8	14.7	Reduced with Pd + H_2 titrated with $K_3Fe(CN)_6$
3	Same as no. 1		+ .0022	15.1	15.2	Red. titrn. with Leuco-Rosindulin GG.
4	1.017 N H_2SO_4	0.36	(+ .5025)	14.0	13.9	Red. with Pd + H_2, titr. with $K_2Cr_2O_7$

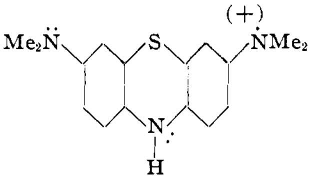
oretical minimum value, 14.3, is distinctly beyond the limits of error. The difference from the behavior of oxonine might tentatively be accounted for by assuming pK_s to be about 8.0 (instead of 7.5). Such a slight difference would be sufficient to turn the whole situation, so easy to decipher for oxonine, into one much more difficult to recognize. No convincing spectroscopic evidence could be obtained for the thionine radical in alkaline solution. In part it may be due to the fact that the maximum ratio of semiquinone is only 8% for thionine, whereas it is 23% for oxonine. It is possible, in addition, that the band of the radical overlaps more with that of the uncharged base of the quinonoid dye.

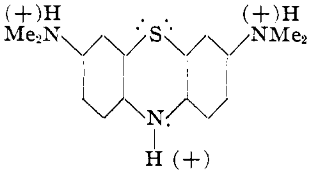
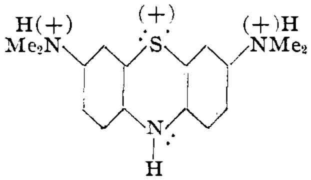
In methylene blue, the situation is somewhat different. For a quaternary base such as methylene blue, no ionization level corresponding to S^0 exists. The various ionization levels of the T forms may be distinguished as T^+ (formula VIII), TH^{++}, TH^{+++}. No form corresponding to S^0 (IX) can be observed. The forms occurring are SH^+ (X and XI), SH_2^{++} (no formula drawn), and SH_3^{+++} (XII and XIII). The essential difference in the plots of oxonine and methylene blue (Fig. 4) ensues from the non-existence, at least within the reachable pH range, of the form S^0. This may be accounted for by the fact that methylene blue in alkaline solution is so unstable that no reliable index potentials can be obtained. The half-schematic plot (Fig. 4) has been, for this reason, extended only over the pH range up to 7. The interpolation method, so instructive in the case of oxonine, cannot be applied for methylene blue, and we have to rely on the index potentials even in the pH region around neutrality. The large concentration effect for


VIII (T^+)


IX (S^0) (there is no evidence for its existence)


X (SH^+, α)


XI (SH^+, β and (reversing right and left) γ)



XII ($SH_3^{+++}\alpha$) ←——→ XIII ($SH_3^{+++}\beta$)

methylene blue indicating dimerization of the T-form makes the accurate determination of the limiting value of the index potential for infinitely low concentration more difficult than for the other dyes. According to the many experiments performed during the past years the value E_i = 14.9 ± 0.2 for *p*H 4.6 does not seem too high. Titration experiments in solutions of higher concentration than 1×10^{-4} *M* distinctly show the effect of polymerization. In the first place, the normal potentials are displaced toward the negative side as shown already by Clark, Gibbs and Cohen.[7] Secondly, the index potentials are changed with increasing concentration. This change is two-fold: they become smaller (values down to 9 mv. could be reached), and the two index potentials (that from 50 to 25%, and that from 50 to 75% titration) become unequal. Without going into the details for the time being, the authors are convinced that 14.9 ± 0.2 is not too high as a limiting value for the index potential. Utilizing this value and those obtained for very acid solutions in the previous paper,[1b] one arrives at the diagram Fig. 4, which does not claim to be very precise, yet at any rate may contribute a good deal to the notion of this frequently yet never exhaustively studied dyestuff.

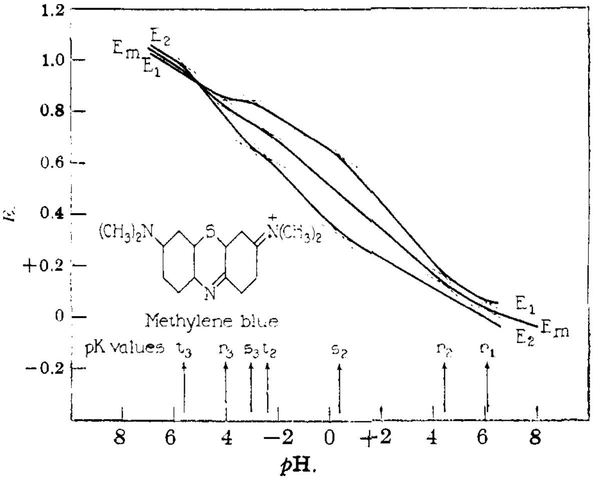


Fig. 4.—The three normal potentials of methylene blue plotted against *p*H, holding for very dilute solutions of the dyestuff.

Summary

Oxazine forms visually detectable semiquinone radicals not only in very acid solutions, as has been shown recently, but also in alkaline solution. Oxonine is the most suitable dyestuff for accurate measurements of the semiquinone formation constant over the whole *p*H range from −8 to +14. From the reliable measurements in alkaline solution, and the relatively fairly accurate measurements in strongly acid solutions, the values for the intermediate *p*H range can be interpolated. The values thus obtained for the intermediate *p*H range agree within the limits of error with those directly obtainable from the slopes of the titration curves. We need not now consider the latter as less reliable within this intermediate *p*H range. The plot Fig. 3 for oxonine may be said to be the most complete set of data for any dyestuff yet investigated, since it includes the three normal potentials, the ionization constants of all the three levels of oxidation, and the semiquinone formation constants over the whole *p*H range. The analogous behavior of thiazine dyestuffs is also discussed.

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(7) W. M. Clark, H. D. Gibbs and B. Cohen, *U. S. Publ. Health Repts.*, **40**, 1131 (1925).