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Potentiometric Study of β-Naphthoquinone Sulfonate. A Further Contribution to the Semiquinone Problem

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Quinones can, in general, by partial reduction be converted into quinhydrones. It has been very difficult, as yet, to study this process potentiometrically for a quinone proper. This task has been accomplished only for substances that may be termed quinoid compounds only in a wider sense such as the imino substitution products of quinones (the case of Wurster's dyes), the phenazine and the γ,γ'-dipyridyl derivatives, and some others, all of which form cationic quinhydrones. These half reduced forms have been recognized as semiquinone radicals, in contradistinction to bimolecular meriquinones. With respect to those cationic semiquinones the rule has been pointed out that their existence is favored as the acidity of the solution increases. In a recent review on this subject[1] the author advanced the idea that, conversely, in the case of the true quinones, which furnish anionic reduction products, the existence of the intermediate forms should be favored as the alkalinity of the solution increases. The first case where the formation of a semiquinone in a very alkaline solution has been observed is the case of the indigo sulfonates discovered by Shaffer, published by this author only preliminarily as yet, and reported and experimentally confirmed by the writer.[1] While this paper was in preparation, Hill and Shaffer[2] discovered the formation of semiquinones of anthraquinone sulfonates in alkaline solution. The reason why it has been difficult to discover a suitable case of a quinone proper is the fact that true quinones are very sensitive in the presence of alkali and are irreversibly destroyed by it. So, in the very *p*H range in which the formation of a semiquinone on partial reduction might be expected to manifest itself, the system will break down due to the destruction of the quinone. This irreversible reaction frustrates any study of equilibria by potentiometric, optical, or other methods.

It will be shown in this paper that β-naphthoquinone sulfonate is a quinone of the properties desired, in so far as it is relatively resistant at such a *p*H where the formation of the half-reduction product is already distinct.

Fieser, in his studies on the oxidation reduction potentials of quinones,[3] investigated this dye also, but he restricted himself to potentiometric titrations at *p*H not greater than 5. This *p*H limit was sufficient for his problem. He observed irregularities in the titration and unexplainable color changes when titrating in an alkaline solution, but he did not attempt to interpret these irregularities. The color phenomenon which he saw is due at least in part to the formation of a semiquinone.

When this yellow dyestuff is reduced, in an acid solution, an ordinary reduction to the almost colorless leuco dye takes place. The titration curve is precisely that of a two-electron system, the index potential[4] being 14.3 millivolts at 30°. When the reduction is performed at *p*H 9 to 12, an intermediate intense brown color arises, which fades out on complete reduction. The potentials are perfectly stable to *p*H 9 and the drifts are very moderate and not very disturbing even at *p*H 11. In this *p*H range a fairly accurate titration curve can be obtained. At higher *p*H the potentials are no longer stable. The separation of the steps begins at *p*H 9, and is more distinct at greater *p*H's, but nowhere is the separation so great as to produce a jump of the potential at 50% of the whole titration. The two steps always overlap so much that *in maximo* about 50% of the total dye in the form of the semiquinone is in equilibrium with the other forms. The index potential, in the best case throughout the *p*H range with stable or nearly stable potentials, is never greater than about 28 millivolts. It reaches this value at *p*H a little greater than 10, and remains so throughout the accessible *p*H range to *p*H 11.5. From a titration experiment at constant *p*H the normal potentials can be derived. The normal potential of the system: fully oxidized + fully reduced dye, or the "mean normal potential," E_m, is the potential at 50% reduction. The normal potential of the system: half-reduced

(1) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935).

(2) Edgar S. Hill and Philip A. Shaffer, *Proc. Am. Soc. Biol. Chemists*, 1936, p. li, to be reprinted in *J. Biol. Chem.*, 1936.

(3) L. F. Fieser, THIS JOURNAL, **48**, 1097 (1926).

(4) L. Michaelis, *J. Biol. Chem.*, **96**, 103 (1932); *cf.* also ref. 1.

form + fully reduced form, E_1, and that of the system: totally oxidized + half-reduced form, E_2, can be derived from the index potential according to the principle previously developed.[1] When this is done and all normal potentials are plotted against pH, the following diagram is obtained.

As one follows these curves from right to left, one sees that the three normal potentials in the region of pH 12 to 9.4, lie in what we may call their natural order, *i. e.*, $E_2 > E_m > E_1$. At pH 9.4 the curves cross each other, and to the left from the crossing point there appears what one may call the reversed order, $E_1 > E_m > E_2$. The

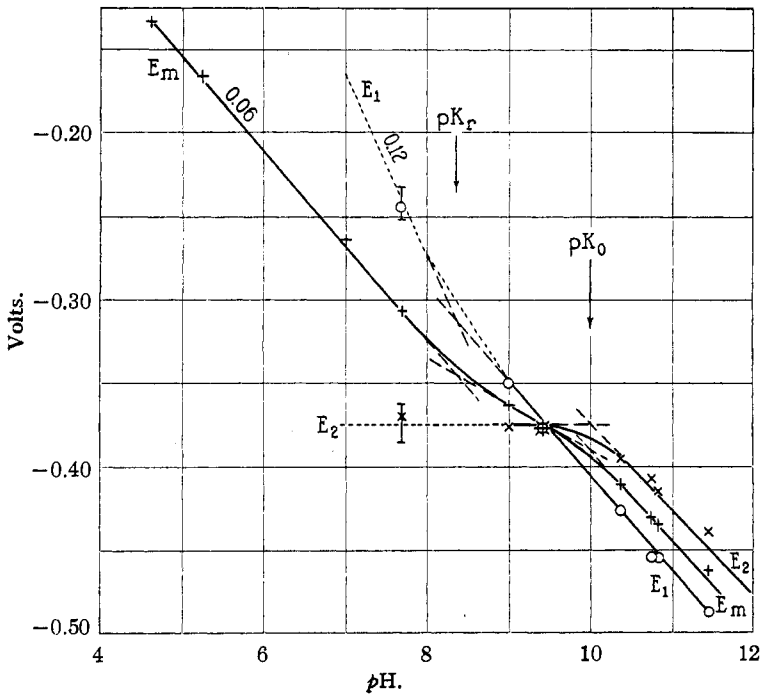


Fig. 1.

three curves diverge rapidly to the left from the crossing point indicating that the existence of a semiquinone rapidly vanishes as pH decreases. Even at pH 7.5 the method just permits, and that with no great accuracy, locating the points for E_1 and E_2, and for pH < 7.5 these two curves cannot be obtained experimentally, but are plotted according to extrapolation on theoretical reasoning.

These curves have been plotted on the assumption that the intermediate form is a semiquinone, not a bimolecular meriquinone. We have now to discuss why this interpretation should be adopted. In order to decide between these two possibilities two criteria have been previously developed. The first of these criteria is of avail only if, at some pH, the separation of the two steps is great enough to bring about a distinct jump of the potential at 50% reduction; which does not occur in this case. But the second criterion can be applied also in the case of overlapping. In the case of a semiquinone, the shape of the titration curve should be independent of the total concentration of the dye. The shape does in fact remain the same within the limits of error when the concentration is varied 1:12. However, the change in the form of the curve in a case like this, where the two steps always greatly overlap, may be small and might under certain conditions be within the limits of error. So another criterion is desirable. This criterion is a colorimetric one and consists in determining whether the amount of the intermediate brown substance is changed when the solution is diluted with the same buffer solvent, containing no dye. This test is very sensitive under just those conditions where the other test becomes insensitive. In the case of a semiquinone there can be no change on diluting; in the case of a meriquinone there will be such a change. In fact, no change was observed, and so the assumption of a semiquinone is corroborated. This test together with a detailed discussion of its theory, will be treated in a special section of this paper.

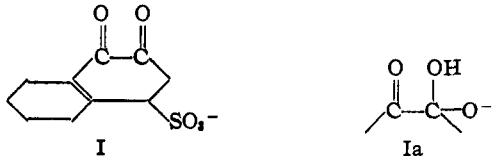
Considering the bends in these curves, the E_m curve shows that there is an acid dissociation constant of the reduced form, pK 8.2, the existence of which is understandable. A second constant of the reduced form may be expected on account of the second OH group. This is $\gg$ 11 and so outside the reach of the experiment. Its existence can be shown by the fact that on reduction with hydrosulfite the leuco-dye is colorless at pH < 11, but yellow in 1 N sodium hydroxide. That is: at pH $\ll$ 11 the color intensity is diminished on complete reduction, at pH $\gg$ 11 it is increased.

Furthermore, there is a dissociation constant of the oxidized form, pK = 10.0. It is confirmed by the fact that the oxidized dye is yellow at

< 10 and almost colorless at higher *p*H, and furthermore by an acidimetric titration of the dye with the glass electrode. Such a dissociation constant can be due only to one of the two CO-groups. Just as carbon dioxide combines with OH^- to form the CO_3H^- ion, so obviously this =CO group combines with OH^- to form the anionic side chain, $=C(OH)O^-$. One might also say, the =CO side chain is to a small extent hydrated to form $=C(OH)_2$, and this can detach a proton. The assumption of a hydrated form of an orthoquinone is in agreement with Fieser and Peters.[5]

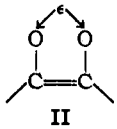
The bends in the E_m curve are accompanied by bends in the E_1 and E_2 curves according to the principles previously developed. The change of slope by 0.03 volt per *p*H unit, in the E_m curve at *p*H 10.0, is accompanied by a change, by 0.06, of slope, in the E_2 curve, but by no change in the E_1 curve, etc. The semiquinone shows no dissociation constant. It cannot, however, be excluded that there is such a one, at *p*H < 8. If this be true, the divergence of the dotted parts of the E_1 and E_2 curve would, say, from *p*H 7 on, stop and all three curves would run parallel to each other to the left of *p*H 7. The maximum amount possible of semiquinone in this *p*H range is extremely small, perhaps only a fictitious magnitude. So no experimental test is available.

From the knowledge of the dissociation constants and the slopes we can derive the following structures of the various forms at various *p*H. At *p*H < 8, formulas I, II and III hold. Ia is the ionized form of the quinone, IIIa that of the hydroquinone. In the semiquinone, II, ϵ is the shared electron.

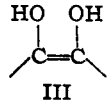


I

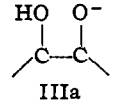
Ia



II



III



IIIa

There remains one obvious question to be answered: Why does a quinone react with two molecules of phenol to form phenoquinone, a molecular addition compound, and why does a quinone not combine with one molecule of a hydroquinone to form a comparable dimolecular meriquinone but instead dismute into two molecules of a semiquinone? The answer is this. Primarily there may always be a chance for a quinone and a phenolic OH group to form a molecular compound due to a hydrogen bond. But a dismutation into radicals may also be possible. What really happens depends on the stability of the compound to be formed. If phenoquinone were to dissociate so as to give rise to radicals, one of the split products would be the radical C_6H_5O. This radical is incapable of existence. If, however, a quinone and a hydroquinone even primarily formed a bimolecular compound, it would immediately split into the two semiquinoid radicals which due to the resonance energy of the electron shared between two atoms of the same kind, *i. e.*, the two oxygen atoms, is a more stable compound than the dimeric molecule with its weak hydrogen bond. One might express the situation by saying: in this case the single-electron bond[6] is a stronger bond than the hydrogen bond. This is an acceptable statement, as the hydrogen bond is weak in general, whereas a single electron bond between two atoms of the same kind may be quite strong due to the resonance energy.

Potentiometric Experiments

Sodium naphthoquinone sulfonate was obtained from a commercial preparation by purification according to Folin's[7] borax method. The tests for purity recommended by Folin were satisfactory. This preparation was once more recrystallized from 80% alcohol. The analysis gave: S, 12.44% (calcd. 12.36); Na, 9.01% (calcd. 8.85). The buffers used were acetate and veronal,[8] both always at ionic strength 0.1; and phosphate, in both of its *p*H ranges, prepared by mixing $M/15$ Na_2HPO_4 either with $M/15$ KH_2PO_4 or with $M/10$ NaOH. Glycine must be avoided because it reacts chemically with the oxidized form of the dye; borate likewise, because it may react with the reduced form. The volume of the buffer was in most experiments 25 cc., the dye 2 to 3 mg. which amount was more widely varied in some special experiments concerned with the effect of concentration. The most satisfactory method of titration was the reductive titration

(5) Louis F. Fieser and Mary A. Peters, THIS JOURNAL, **53**, 793 (1931).

(6) L. Pauling, *ibid.*, **53**, 3225 (1931).

(7) O. Folin, *J. Biol. Chem.*, **51**, 389 (1922).

(8) L. Michaelis, *ibid.*, **87**, 33 (1930).

with the leuco-form of Rosindulin GG,[9] and the technique essentially the one previously described for this reductant.[10] The volume of the reductant for the complete titration was usually 1.8 to 2.8 cc. After finishing the reductive titration, nitrogen was displaced by hydrogen, some

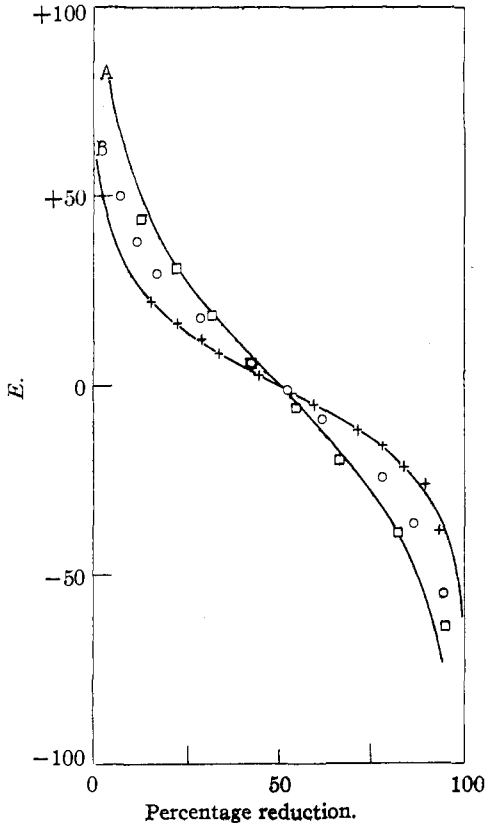


Fig. 2.—Curve A calculated for an ordinary two-electron system without step formation. Curve B calculated for a one-electron system; or for a two-electron system with step formation, if K, the formation constant of the semiquinone, equals 4. +, Reductive titration experiment at *p*H 4.62; ⊙, at *p*H 9.42; □, at *p*H 10.74. The reference point of the potential is the normal potential (E_m) at the particular *p*H. Potentials expressed in millivolts. +, coincide with the calculated Curve A; □, nearly coincides with the calculated Curve B; ⊙, is midway between these two.

drops of 1% solution of colloidal palladium added[11] and the hydrogen potential thus obtained used for calculating *p*H. The potentials were plotted against cc. of reductant, the zero and 100% points of reduction graphically determined. All curves were, within the limits of error to be discussed presently, symmetrical around their mid-point at 50% reduction. The mean normal potential, E_m, was put equal to the potential at 50% reduction. The index potential, E_i, was determined by an easy graphical interpolation; it is the difference of the potential at 50%, and at 25% (or 75%) reduction. From E_m and E_i the two normal potentials E_1 and E_2 were determined by the method previously developed. The errors in E_i, consequently, involve an error in E_1 and E_2. Within the *p*H range with perfect constant potentials, *p*H < 10, the errors in E_i are certainly small, say 2 or 3 tenths of a millivolt. Even so small an error would have a considerable bearing on the evaluation of E_1 and E_2, if the index potential is only very little larger than its minimum value 14.3, say if it is 15 or 16. In Fig. 1, the influence of an error of ±0.2 millivolt in E_i, on E_1 and E_2, is symbolized for the experiment at *p*H 7.6. If E_i is larger, the influence of an error in E_i upon E_1 and E_2 is much smaller. At *p*H > 10, where the potentials are no longer perfectly stable, the error in E_i may be larger. But here, an error even of ±2 millivolts in E_i, upon the evaluation of E_1 and E_2, is so small that it scarcely would show up on the scale used in Fig. 1.

Figure 2 shows three examples of titration experiments described in the legend. Table I shows the results of the whole set of experiments, used for the construction of Figure 1.

Figure 3 shows the acidimetric titration of the oxidized form of the dye with 0.1 *N* sodium hydroxide using the glass electrode with direct galvanometer reading.[12] There is evidently a buffering effect of the dye in the neighborhood of *p*H 10, and this effect is not exhausted after the consumption of one equivalent of sodium hydroxide, indicating further changes of the quinone due to the excess of alkali. It is of no use to apply the corrections otherwise available to calculate more accurately the dissociation constant because the *p*H range is so high that the *p*H measurement by the glass electrode is no longer in full agreement with a true hydrogen electrode. Suffice it to show the buffering effect in confirmation of the reality of the $pk = 10.0$ as shown in Fig. 1.

Theory and Experimental Test of the Dilution Effect

Above this criterion was used: if the equilibrium depends on the volume of the solvent,

(9) L. Michaelis, *J. Biol. Chem.*, **91**, 371 (1931).

(10) L. Michaelis, E. S. Hill and M. P. Schubert, *Biochem. Z.*, **255**, 66 (1932).

(11) Einar Biilmann and Andreas Klit, *Z. physik. Chem.*, **130**, 566 (1927).

(12) L. Michaelis, *Science*, **83**, 213 (1936).

TABLE I

Amount of dye, m.	Vol., cc.	pH	Buffer	E_m (Pot. at 50% redn. referred to normal H_2-electrode), mv.	E_1 ($E_{25\%} - E_{50\%}$)	$E_m - E_1$ or $E_2 - E_m$	K	Maximum ratio semiquinone / Total dye
1.0	25	4.62	Acetate	−132	14.3 ±0.2	Very large	0	0
1.0	25	5.23	Acetate	−166.5	14.2 ±0.2	Very large	0	0
1.0	25	7.00	Phosphate	−267	14.3 ±0.2	Very large	0	0
1.2	25	7.68	Phosphate	−308	15.0 ±0.5	$-65\ ^{+8}_{-15}$	0.01	0.05
1.0	25	9.01	Veronal	−367	19 ±0.5	−12 ±2	.4	.24
0.8	25	9.42	Veronal	−378	21.1 ±0.5	0 ±2	.8	.31
1.2	20	10.36	Phosphate	−413	27.3 ±2	+15.5 ±4	3.1	.47
1.2	25	10.73	Phosphate	−431	32 ±2	+24 ±4		
0.4	25	10.81	Phosphate	−439	29 ±3	+19 ±5	4	.50
1.0	25	11.44	Phosphate	−474	32 ±4	+24 ±8		

1. Potentials are expressed in millivolts and referred to the normal hydrogen electrode. All experiments have been performed at 30 ± 0.1°.

2. The column for K, the effective formation constant of the semiquinone, has been calculated according to equation 8 of the review.[1]

3. The column for maximum amount of the ratio semiquinone: total dye, is calculated as follows. Putting in equation 12 of the review,[1] $x = a$, one obtains

$$(s/a)_{max.} = \frac{K - 2\sqrt{K}}{K - 4}$$

For the case $K = 4$, this expression has no definite value. In this case, two methods are available. Either, one takes advantage of the fact that the denominator and the numerator have the common factor $\sqrt{K} - 2$. If this factor is removed, we obtain

$$(s/a)_{max.} = \sqrt{K}/(2 + \sqrt{K})$$

This formula can be applied also when $K = 4$. Or, one applies equation (15-*b*) instead of (12). Either of these methods gives

$$(s/a)_{max.} = 1/2 \text{ when } K = 4$$

4. The limits of error in the column for E_1 have been estimated on the ground of the perfection of each experiment which mainly depends on whether the potentials are perfectly constant or show a drift in time. The influence of these errors on the evaluation of the column $E_m - E_1$, is, approximately shown. The evaluation of the two last columns, can be considered only as pretty fair approximations.

the intermediate form is a bimolecular compound; if it is independent of the volume, it is a semiquinone. This statement requires a thorough theoretical consideration in order to avoid erroneous conclusions from the experiment. For, the objection may be raised that under certain conditions the dependence, on the volume, of the equilibrium in the case of a bimolecular intermediate form may be so small as to render the criterion useless. It is our task to arrange the conditions of the experiment so as to avoid this ambiguity.

Let us designate the molar amount (not the concentration) of the totally oxidized form as t, that of the reduced as r, that of the intermediate, if it is a bimolecular meriquinone, as m. Starting with all the dye in the t-form, in the amount a, and adding x moles of a univalent reductant, the following equations hold

$$t + 2m + r = a$$
$$2m + 2r = x$$
$$\frac{r \cdot t}{m} = \kappa v$$

where v is the volume in liters and κ is the equilibrium constant of this dissociation: $m \rightleftharpoons r + t$. As the acidic dissociation of r and t are not taken into consideration in this formula, κ will depend on pH, and is what has been called an "effective constant,"[1] in contrast to a true constant. Eliminating t and r, we obtain

$$m = 1/2\,(a + \kappa v \pm \sqrt{(a + \kappa v)^2 - 2ax + x^2})$$

This equation shows in fact that generally m depends on v. In our experiment, v will be of the order of magnitude 10^{-2} to 10^{-3}, a much smaller, around 10^{-5} or less, and x somewhat smaller than a but of the same order. However, κ may possibly vary over a wide range, according to pH. If $\kappa v \gg a$, m will be nearly proportional to v, for the above equation approaches in the limiting case the form[13]

$$m = \pm(2ax - x^2)/4\kappa v \qquad (I)^{14}$$

(13) Using the approximation formula $\sqrt{A^2 + b} = \pm(A + b/2A)$, when $b \ll A^2$.

(14) The one or the other solution has to be chosen according to whether $0 < x < a$, or whether $a < x < 2a$.

If, however, $\kappa v \ll a$, the influence of v will vanish, for (I) becomes in this case

$$m = x/2, \text{ or } m = a - x/2 \qquad \text{(II)}^{14}$$

Here m is independent of v.

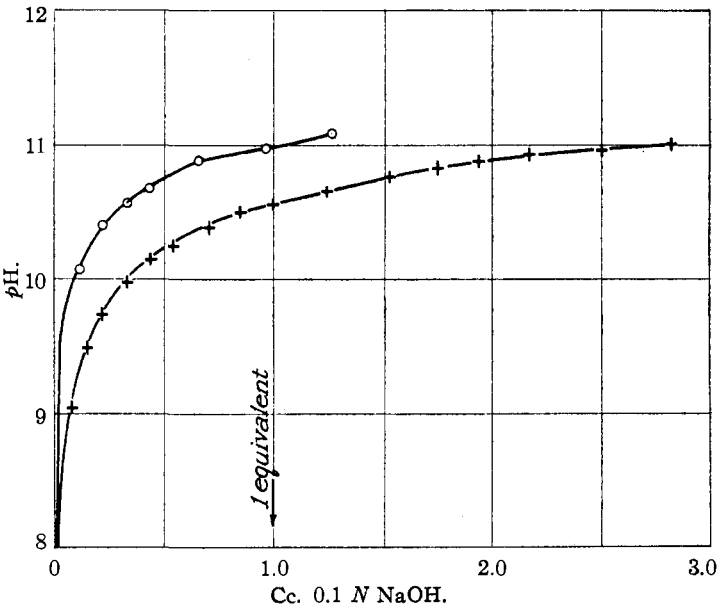


Fig. 3.—+, 20 cc. of the dye solution titrated with 0.1 N sodium hydroxide; ○, 20 cc. of pure water titrated in the same way.

The experiment, therefore, should be performed under such conditions as to make κ as large as possible. Now, we know that even at pH 11, κ cannot be very small. Otherwise there would be a potential jump in the mid-point of titration, whereas in fact the two steps overlap very considerably. If we now operate at pH smaller than 11, so small indeed that the maximum amount of the intermediate form can be just detected by a slight color change during the reduction, we are sure that κ is large enough to approach the condition represented in (I). The pH suitable for this purpose is 9.

Accordingly, the experiment is performed as follows: 2 mg. of the dye is dissolved in 3 cc. of the following freshly prepared buffer: 6.18 g. of sodium veronal and 19.2 cc. of 0.1 N hydrochloric acid, dissolved in carbon dioxide-free water to a volume of 100 cc. (pH = 9.04 at 30°). This solution of the dye is distributed into three test-tubes, each containing 1 cc. One of these is used only as color test for comparison with the others. Now some milligrams of glucose are added to the second tube and the solution is warmed. Looking always through the whole length of the tube one sees the color gradually changing from yellow over brownish-yellow to a pale yellow. The same experiment is performed with the third tube after diluting it with 10 cc. of the buffer. The color changes, on heating, are the same as before. According to the theory, the maximum amount of the brown substance, if it be a meriquinone, should be not appreciably more than one-eleventh of that in the other experiment, at any rate much smaller. If this were true, the brown substance would not have been noticed at all at the higher dilution. Although the amount of the brown substance cannot be determined quantitatively with precision by this method, the estimate on crude observation is sufficient to rule out a bimolecular compound. One may raise the objection that the experiment has been performed at a much higher temperature than the titration experiments. To obviate this objection, the tubes, after completing the reduction, are cooled to room temperature and the process reversed by gradual oxidation by oxygen. The tube with the smaller volume is simply shaken, the other poured alternately from one tube into another. The process is reversed showing the same result as at the higher temperature.

Summary

β-Naphthoquinone sulfonate, on reduction in alkaline solution, forms an intermediate product of brown color which is proved to be a semiquinone radical, in contradistinction to a bimolecular meriquinone. The maximum amount of semiquinone which can exist in equilibrium with the other forms of the dye is about 50% of the total. This is the case at pH 12.5 to 10. At lower pH this amount decreases and becomes vanishingly small at any pH < 7.5. According to the previously developed theory of the semiquinones, the three normal potentials E_1, E_2, E_m are derived from the titration curves, plotted against pH, and discussed. Among other features, the existence of an acidic dissociation constant of the quinone (disregarding the ionization of the sulfonic acid group), is shown.

So the constitution of a true quinhydrone, in the strict sense, has been proven to be that of a semiquinone radical, in complete analogy to the cationic semiquinones described previously.

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