SEMIQUINONES, THE INTERMEDIATE STEPS OF REVERSIBLE ORGANIC OXIDATION–REDUCTION

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Reversible oxidation–reduction systems may be classified in two groups, according to what we call the electron number, that is to say, whether the oxidized form differs from the reduced by one electron (or hydrogen atom) or by two. We also speak of univalent and bivalent oxidation. Cases of univalent oxidation are rather common in inorganic compounds, such as in ferric–ferrous or cupric–cuprous systems, either in the form of simple metal ions or complex compounds. But they are rare, and in fact, have not been known at all until recently, in organic chemistry. Authors to whom we owe very outstanding contributions to our knowledge of reversible oxidation–reduction processes (6) are so much impressed by the lack of univalent oxidations in organic compounds that they correlate the simultaneous loss of two electrons with the trend of electrons towards arrangement in pairs, which is the main principle of Langmuir's and G. N. Lewis' theory of the chemical bond. Such a view, however, can be maintained only in a restricted sense. The situation is rather as follows.

In the metals of the transition series of the periodic systems, as iron and cobalt, a univalent oxidation occurs whenever two levels of oxidation, differing by one electron, are capable of existence. Thus ferrous ions contain one more electron than ferric ions. However, disregarding some exceptional cases, organic compounds possess only atoms with fixed valence numbers. These exceptional cases, such as triarylmethyl with tervalent carbon, or diarylnitride with bivalent nitrogen, are considered as unsaturated compounds—radicals capable of existence only under special conditions depending on the chemical nature of the particular aryl—and they exhibit a strong tendency toward abolishing the unsaturated state by dimerization, as follows

$$2 \text{ triarylmethyl} \rightleftharpoons \text{hexaarylethane}$$

although in some particular cases this latter tendency may be weak. The customary way of studying reversible organic oxidation–reduction systems in an aqueous solvent did not seem to offer any chance of encountering a radical of this kind. As a rule, the oxidized form has a quinoid structure, and the reduced form has the corresponding benzenoid structure as encountered in the leuco dyes. The univalent oxidation of the benzenoid form would lead to a radical with a structure intermediate between the quinoid and the benzenoid form.

As a matter of fact, however, such intermediate forms of oxidation had been known for a very long time. Their simplest representative is quinhydrone. But so great was the aversion to thinking of them as unsaturated radicals, that this substance was taken as a molecular compound of quinone and hydroquinone. It does not seem appropriate to dwell on the many tentative formulas ascribed to quinhydrone. We may just picture Willstätter's (62) formula. He assumes that a residual valence of oxygen holds together one molecule of quinone with one of hydroquinone. In addition, he assumes that no static formula truly depicts the constitution of quinhydrone, but that an oscillation of the bonds takes place so that the reduced half and the oxidized half of the molecule take turns in rapid succession. For this reason he termed these compounds meriquinones, which means that, in a time average, the state of oxidation is evenly distributed (μέρος = part) among the two halves. As regards the even distribution, we shall share his point of view. As regards the molecular size, we shall be compelled to disagree.[1] Willstätter's point of view appeared to be even more justified by the fact that such molecular compounds, between two different molecules, one of a higher oxidation level than the other, do exist, even in ratios other than 1:1, e.g., a compound of one molecule of quinone and two molecules of phenol, or five of quinone and two of *p*-phenylenediamine (Schlenk (46)). In fact, it is very suggestive, that by analogy with a compound consisting of 1 quinone + 2 phenol, a compound of 1 quinone + 1 hydroquinone should also be capable of existence, and that quinhydrone is this compound. Such an assumption would be corroborated by a determination of the molecular weight. No method was available to decide as to the molecular size of quinhydrone. So Willstätter's interpretation of the meriquinoid substances as encountered in many groups of organic dyestuffs was in general accepted. To prove this statement let it suffice to quote, as examples, Schlenk's (46) formula of the meriquinone obtained by partial oxidation of dichlorotolidine or Dimroth's formula for the meriquinone obtained by partial reduction of γ,γ'-dipyridyl.

The first suspicion as to the correctness of the bimolecular structure was uttered by Hantzsch (23), although he had no cogent evidence. He observed that the green compounds obtained by partial reduction either of

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[1] Recently Willstätter, in a paper published with Haber (22), has abandoned his original bimolecular formula.

N-methylphenazonium iodide, or of phenazine, showed two bands in the ultra-violet, a phenomenon which is observed neither in the fully reduced nor in the fully oxidized form, and is absent also in compounds of a doubtlessly bimolecular meriquinoid structure. He failed, however, to find any representative of this group suitable for a determination of the molecular weight by the methods available. Later, E. Weitz (59) succeeded in determining the molecular weight of some compounds of this group by the boiling point method in organic solvents. He worked on the meriquinoid compounds obtained by mixing equivalent amounts of dibenzyl-γ,γ'-dipyridylium dichloride with dibenzyl-γ,γ'-dipyridylium. The latter substance can be considered, as he showed (55), as a free ammonium radical, or better, a double radical, because it contains the ammonium group twice. In mixing these two compounds a deep violet dyestuff is developed, which can be prepared in crystalline form and may be considered as a meriquinoid compound. The problem was whether the molecular weight of this substance is that of a compound or condensation product of the two parent substances, or whether it has the same molecular size as either of the parent substances. The analysis showed that it contains one chlorine atom to two nitrogen atoms; this would agree with either assumption. He determined the boiling point of this substance in water-free methanol in an atmosphere of carbon dioxide free from oxygen. For this purpose he elaborated a special technique of the boiling point method for oxygen-sensitive substances and obtained a molecular weight, corroborated by the good agreement of a very large number of single experiments, of 197 as an average from experiments with solutions more dilute than $M/16$ and 215 from experiments with concentrations greater than $M/16$. The molecular weight of the substance if it be monomolecular would be 373. Assuming 100 per cent dissociation of the chloride ion, the apparent molecular weight should be 187, which agrees very well with the experiment. This result was taken as evidence that the monomolecular radical formula was proven. Assuming the dimolecular formula and complete dissociation of the two chloride ions, one should obtain an apparent molecular weight of 235. Yet it is known how uncertain the interpretation of this method is when applied to an electrolyte. In the same paper Weitz carries out a determination of the molecular weight of dibenzyldipyridinium dichloride (the colorless oxidation product of the above radical) having a molecular weight of 409. Again, assuming total dissociation of the chlorine one should expect an apparent molecular weight of 136. However, he finds in three experiments 253, 243, and 283, which necessitates the assumption that one of the two chlorine atoms is not ionized at all and the other only in part. Such an observation is liable to arouse some doubt as to the general applicability of the method to organic electrolytes. One may recall the fact that later

Wrede (64, 65) was also misled by the freezing point method of another dye, pyocyanine, to which he ascribed a molecular weight double what we think is the real one. Although Weitz may have been fortunate in the above case of the dye radical, a confirmation by another method is desirable. Such a method is the potentiometric oxidative or reductive titration.

THE APPLICATION OF THE POTENTIOMETRIC METHOD TO THE PROBLEM

This method was first applied to the study of meriquinoid compounds by W. Mansfield Clark, B. Cohen, and W. Gibbs (4). They happened to work with a group of meriquinoid compounds of a very labile nature, principally with those of the benzidine compounds. The potentials obtained during the titration showed rapid drifts, so that a real insight into the mechanism of the process could not be obtained. Guided by the current ideas it was quite natural that they did not doubt Willstätter's bimolecular formula. They developed a complete theory for the potential curve obtained on the basis of their assumptions, but had no opportunity to test the theory with their experimental material on account of the drifts of potential. The first case in which a compound of this type, perfectly stable even in aqueous solution, was encountered, is that of pyocyanine, first described by Friedheim and Michaelis (21), and only a little later and independently by Elema (10). Neither the first paper on this subject by Friedheim and Michaelis, nor that by Elema, led to a complete understanding, as the theory presently to be developed had not yet been worked out. It is of no use to dwell on a detailed comment on the preliminary results then obtained. We had better start with the complete theory which very soon after that was developed by these authors (39, 11, 12).

The case of two-step oxidation has many analogies to the two-step dissociation of dibasic acids. So the question may arise as to why the complete theory of such a two-step process has not been previously developed, at least for the case of acidic dissociation. Now, in the case of a two-step dissociation, the titration curve plotted against pH will, in general, show two successive levels separated from each other by a jump. The height of this jump will depend on the ratio of the two dissociation constants of the dibasic acid. The smaller this ratio, the smaller the jump. The smallest ratio which theoretically can occur is 4:1. This is often called the "statistical ratio"; a smaller ratio has been shown to be impossible for statistical reasons. In practice the ratio is always much greater, and there is scarcely a case known where this ratio is smaller than 20:1. For this reason the jump between the two halves of the curve is always so large that any overlapping of the two curves is negligible. The two steps of dissociation can be treated as two simple dissociation curves in succession. In the case of two-step oxidation–reduction we have to deal also with two characteristic constants, to be defined later on, which are analogous to the two dissociation constants, but for these constants any ratio may occur, even smaller than 4:1, and even a ratio smaller than 1:1. In such a case the two halves of the curve are no longer separated by a jump, but overlap. For this reason the theory has to be developed in a more general way, including also the case of overlapping. The theory will be developed here, first for the simpler case with distinct steps, then for the general case including the overlapping.

A. The case of distinctly separate steps

Suppose we have a substance R which can be reversibly oxidized in two successive steps, each involving the loss of one electron. The completely oxidized form may be called T, so that its relation to R is expressed by

$$R \rightleftharpoons T + 2\epsilon \tag{1}$$

where ϵ is the electron. If the intermediate form differs from R and from T only by an electron without change of molecular size, the intermediate form may be called a semiquinone, S, and the following relations hold.[2]

$$R \rightleftharpoons S + \epsilon \tag{2a}$$

$$S \rightleftharpoons T + \epsilon \tag{2b}$$

If, however, the intermediate form is a molecular compound, it may be called a meriquinone, M. We restrict ourselves for the time being to the simplest case, that M is a compound of one molecule R and one molecule T. It is easy to see that the assumption of other stoichiometrical proportions, say R:T = 1:2, will not alter anything essential in the corollaries to follow. Then the following relations hold:

$$2R = M + 2\epsilon \tag{3a}$$

$$M = 2T + 2\epsilon \tag{3b}$$

As all reactions are supposed to be reversible, the intermediary form must be in equilibrium with the two others, and it will be able to undergo a self-oxidation–reduction, or a dismutation or disproportionation, or a kind of Cannizzaro reaction. On applying small letters for concentrations, this equilibrium will be determined in the case of a semiquinone by

$$k \cdot r \cdot t = s^2 \tag{4a}$$

where k may be termed the formation constant of the semiquinone, or

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[2] The symbols used are in alphabetical order: R (reduced), S (semiquinone), T (totally oxidized).

$1/k$ the dismutation constant. If the intermediate form is a meriquinone, the equation for equilibrium will read

$$k' \cdot r \cdot t = m \tag{4b}$$

When a solution containing only R is titrated with an oxidant, the magnitude of k or k' will determine how much of S or M can be formed *in maximo* during the titration. If k is very small the two steps will greatly overlap. If this constant is very large, at the beginning of the oxidative titration only S or M will be formed, and T will arise only after practically all R has been converted into S, or M. In such a case the two steps of oxidation are said to be distinctly separated.

In this case each of the two steps of oxidation can be treated separately, as there is practically no overlapping. In the first half of the titration the chemical process is, for the case of a semiquinone:

$$\mathrm{R} \rightarrow \mathrm{S} + \epsilon$$

and the oxidation-reduction potential is

$$E = E_1 + \frac{RT}{F} \ln \frac{s}{r} \tag{5a}$$

where the constant E_1 may be called the normal potential of the R–S system. For the case of a meriquinone, the potential is

$$E = E_1 + \frac{RT}{2F} \ln \frac{m}{r^2} \tag{5b}$$

Analogous equations may be written for the second step of oxidation, in which the normal potential E_2 necessarily is much more positive than E_1. We shall develop the details only for the first step, as the second step is a repetition of the first; a complete repetition for the case of a semiquinone, and a kind of antisymmetrical repetition for the case of a meriquinone. On comparing equations 5a and 5b, it can be seen that for a semiquinone the potential depends only on the ratio $r:s$, but for a meriquinone also on the absolute amount of the substances. We may rewrite equation 5b in a more suitable form. Let v be the volume of the solution, (R) the absolute amount of R in moles, then we obtain

$$E = E_1' + \frac{RT}{2F} \ln \frac{m}{r} - \frac{RT}{2F} \ln \frac{(R)}{v}$$

This equation shows that the titration curve, i.e., potentials plotted against the amounts of added oxidant, is distorted as compared with equation 5a. The differentiation with respect to v gives

$$\frac{dE}{dv} = \frac{RT}{2F} \cdot \frac{1}{v}$$

or, all variables besides v being kept constant, $\Delta E = 0.03 \log \frac{v_1}{v_2}$. So E depends on v, whereas in the case of a semiquinone the potential is independent of the volume. Thus, in the case of a meriquinone, the potential depends on the volume in which a given amount of substance R is dissolved. On varying this initial volume, one obtains a family of titration curves parallel to each other, the distance amounting to 0.03 volt when the volumes are varied in a ratio 1:10. So the following criteria can be established:

For a semiquinone:	For a meriquinone:
1. The titration curve is symmetrically arranged around its middle ordinate.[3]	1. The titration curve is not symmetrical about its middle ordinate, but steeper in the first half than in the second.[4]
2. The shape of the curve is that of an ordinary one-electron system.	2. The shape of the curve is somewhat between that of a one-electron system and a two-electron system; one half (the steeper one) approaching somewhat that of a one-electron system, the other being more similar to that of a two-electron system.
3. The curve of the potentials as plotted against per cent of oxidation is independent of the volume.	3. The curve of the potentials as plotted against per cent of oxidation depends on the volume in which the initial amount of the substance was dissolved. It is shifted toward the positive side by 0.03 volt when the volume is varied in the ratio 1:10.

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[3] More precisely: Draw an x-axis and a y-axis intersecting in the midpoint of the titration curve. When the second half of the curve is now mirrored in the x-axis (dotted curve), this is a mirror-image of the first half in the y-axis.

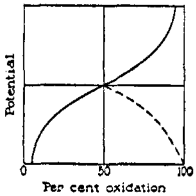

FIG. 1

[4] If the titration concerns the second step of oxidation, the first half is flatter than the second.

When these criteria are applied to an experiment as plotted in figure 2 and described in the legend of this figure, all criteria for a semiquinone and none for a meriquinone are fulfilled. As yet, no case has been found where the result was different from this one.

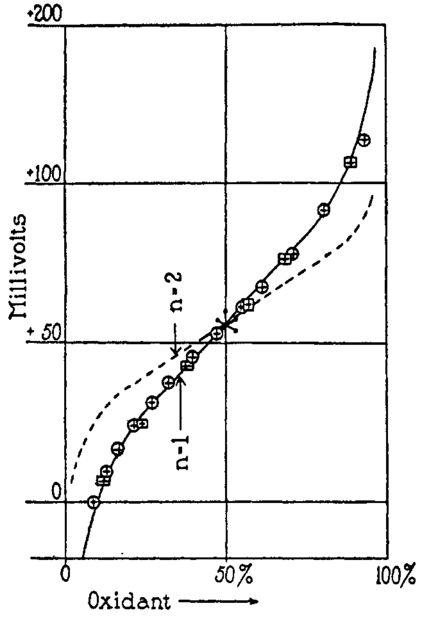

Fig. 2. Showing the independence of the potential of the concentration of the dye. α-Oxyphenazine at pH 1.382 (solution 0.05 M for HCl and 0.1 M for KCl). Only the first step of the oxidation of the leuco dye is plotted. The abscissa indicates the amount of quinone, expressed in per cents of that amount which completes the first step of oxidation; the ordinates are potentials. The first experiment is represented by the circles. After finishing this experiment, the whole solution was diluted with the above acid mixture (containing no dye) to a threefold volume, the dye re-reduced by hydrogen and palladium, the hydrogen expelled, and the titration with quinone was repeated. It furnished the points marked with squares. The drawn out line is the one calculated for an oxidation with the electron number 1; the dash line the one calculated for the electron number 2.

B. The general case, including that with overlapping potentials

In most cases the separation of the two steps becomes less distinct as the pH increases, until finally the overlapping of the two steps becomes complete. The explanation of this fact will be dealt with later on. When, now, for a particular dye the decision for a semiquinone at a sufficiently low pH has been made, we assume that this decision can be maintained also for higher pH, where the above criteria no longer can be applied without modification which could only very laboriously be obtained. This assumption is justified by the fact that no recognizable change, especially with regard to the color, of the intermediate form appears on changing pH. To be sure when the overlapping is considerable, the pure color of the semiquinone will never appear, but only mixtures of it with colors of the other forms. Yet the absorption of the semiquinone can be recognized even in the mixture, owing to the color intensity of most of the semiquinones. So there is no reason to assume that the intermediate form has a different structure according to whether the separation of the steps is more or less complete. The problem now arises as to the analysis of complete titration curves including the cases of overlapping. This problem has been solved in two ways, by Elema and by Michaelis. The formulas arrived at, at the first glance, look so different that their agreement was not recognized at first. Elema, however, succeeded in proving, very ingeniously, that the one formulation is the somewhat disguised equivalent of the other. The advantage of Elema's formulation will be shown later. The other seems the easier one from the standpoint of presentation and is given in what follows.

When overlapping takes place at any point of the titration there will be a mixture of R, S, and T. The whole system being in equilibrium, the oxidation–reduction potential can be expressed in three different ways, according to the three partial oxidation–reduction systems in solution, viz. the system R–T, the system R–S, and the system S–T. The potential, then, can be formulated in any of these three ways:

$$(1)\quad E = E_m + \frac{RT}{2F} \ln \frac{t}{r} \tag{6a}$$

$$(2)\quad E = E_1 + \frac{RT}{F} \ln \frac{s}{r} \tag{6b}$$

$$(3)\quad E = E_2 + \frac{RT}{F} \ln \frac{t}{s} \tag{6c}$$

Notice that the factor 2 appears only in the first equation, as only the R–T system is a two-electron system. E_1 may be called the normal potential of the first step; E_2 that of the second step; and E_m the "mean normal potential." These three normal potentials are not independent of each other. It can easily be inferred from these three equations that E_m is the arithmetic mean of E_1 and E_2, or

$$E_m = \frac{E_1 + E_2}{2} \tag{7}$$

The relation between k and the normal potentials can be recognized by dividing equation 6c by equation 6b and referring to equation 4a. One obtains

$$\frac{RT}{F} \ln k = E_2 - E_1$$

or, at 30°C.,

$$\log k = \frac{E_2 - E_1}{0.0601} \tag{8}$$

In order to evaluate these three equations (6a, 6b, 6c) we have to express r, s, and t in terms of total concentration, a, of the dye, and of the amount x of added oxidant. We have always

$$r + s + t = a \tag{9}$$

Further, after adding x univalent equivalents of oxidant, we have:

$$s + 2t = x \tag{10}$$

x is measured then in terms of equivalents of oxidation for one step, so that at the end point of the titration $x = 2a$. The solution for the three unknowns, r, s, t, requires a third independent equation which is the above mentioned (4a)

$$k \cdot r \cdot t = s^2 \tag{4a}$$

The solution of these three equations (9, 10, 4a) for r, s, and t, is

$$r = a\left(1 + \frac{p}{2}\right) - \frac{x}{2} \mp \frac{1}{2}\sqrt{Y} \tag{11}$$

$$s = -\, a \cdot p \pm \sqrt{Y} \tag{12}$$

$$t = a \cdot \frac{p}{2} + \frac{x}{2} \mp \sqrt{Y} \tag{13}$$

where

$$p = \frac{k}{4 - k} \tag{14}$$

and

$$Y = a^2 p^2 + 2apx - px^2 \tag{14a}$$

So the formation constant k appears only in the form $\frac{k}{4 - k}$, which term changes in sign according to whether $k \gtrless 4$. As to the signs before the square roots, the upper sign should be used when $k < 4$, and vice versa.

For the particular case $k = 4$, the general solution becomes indefinite, but a singular solution can be obtained either by interpolation, or by directly putting $k = 4$ in equation 4a. In this case the solution is

$$r = \frac{(2a - x)^2}{4a} \tag{15a}$$

$$s = \frac{x(2a - x)}{2a} \tag{15b}$$

$$t = \frac{x^2}{4a} \tag{15c}$$

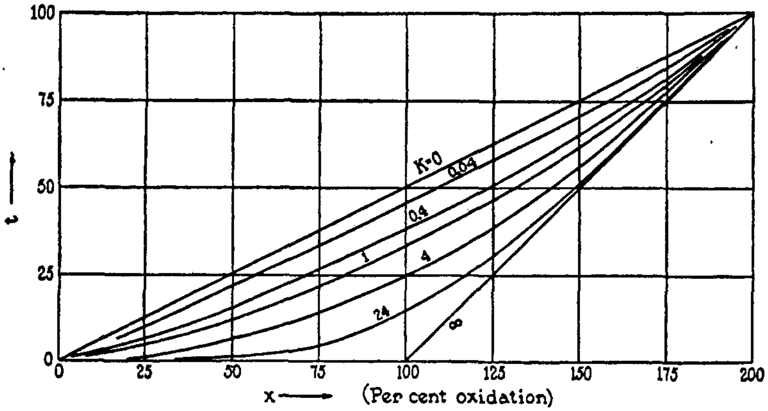

FIG. 3. t, the totally oxidized form (or quinoid, or holoquinoid form) in per cents of the total amount of the dye plotted against per cent of oxidation. When the scale of the abscissa is reversed, it is r, the reduced form plotted against per cent of oxidation.

These latter three functions are parabolic, whereas the others (11, 12, 13) are hyperbolic and consist, purely mathematically, of two branches of which only one has a physical significance, namely, to cover the range from $x = 0$ to $x = 2a$. So it is evident that on varying k, the value $k = 4$ will have some special significance, as will be shown in fact. The rather laborious evaluations of these functions for various values of k are plotted in figures 3 and 4. Figure 3 shows the amount of r, as a percentage of the total amount of the dye, plotted against per cent of oxidation, the whole oxidation being taken as 200 per cent (or, $a = 100$). When the abscissa is reversed according to the legend of the graph, we obtain the percentage of t. Figure 4 shows the percentage of s plotted against percentage of oxidation.

The values thus obtained for r, s, t can now be put in equations 6a, 6b, 6c in order to obtain the expressions for the potentials. Figure 5 is the result of such a calculation. As only differences of potentials are essential for the shape of the curves, the potential in the midpoint of titration ($x = 100$) for each curve has been arbitrarily taken equal to 0.

All that follows depends on a full understanding of this family of curves, which is to be discussed now in detail. Looking first at the curve $k = 100$ we recognize two steps, each one matching an ordinary titration curve of a one-electron system. The curve shows the value E_1, the normal potential of the first step, in the midpoint of the first half of the curve, and the value E_2 at the midpoint of the second half. These points are marked by circles. As k becomes greater the jump from the first step to the second is higher, or $E_2 - E_1$ becomes greater. As k becomes smaller, not only does this jump become smaller but also the circles are more and more shifted toward the middle, until for $k = 1$ all three circles coincide in the center of the figure. The curve $k = 4$ will be expected to be singular in behavior. This is shown by the fact that the two points of inflection, very distinct for high values of k, become less marked with decreasing k and completely vanish at $k = 4$. Here the curve as a whole has precisely the shape of an ordinary one-electron system. Neither the two-step nature of such a system nor the bivalent character of the oxidation could be recognized experimentally were there not the twofold change of color during the titration. Such a case is, for example, realized for pyocyanine at pH 5.0. When k becomes < 4, the curve becomes flatter until finally, at $k = 0$, it coincides with an ordinary two-electron system as represented by most reversible

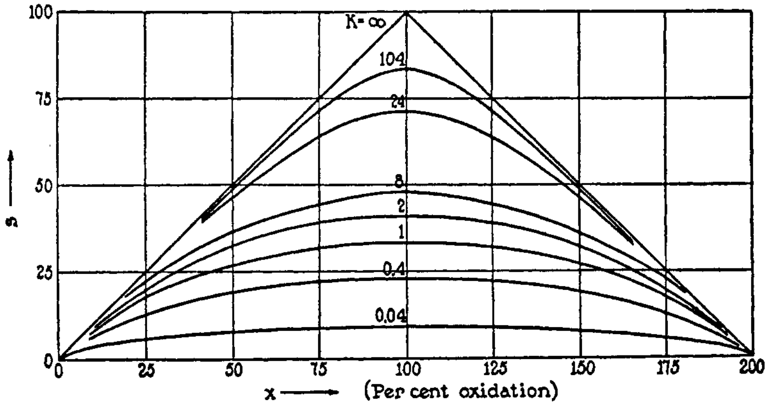

FIG. 4. s, the semiquinoid or intermediary form in per cents of the total amount of the dye plotted against per cent of oxidation.

quinoid dyestuffs. The circles indicating E_1 and E_2 are drawn only for curves with $k > 1$. At $k = 1$, E_1 and E_2 coincide with E_m, which lies always in the midpoint of each curve. For curves with $k < 1$ no circles have been drawn lest the diagram be crowded. For such curves, in contrast to the others, the circle for E_1 lies on the right-hand side of the center, and the one for E_2 on the left-hand side. This fact may be accounted for by saying that if $k < 1$, the oxidation of the reduced form leads more easily to the fully oxidized form than to the intermediary form, in the same way as, generally, the oxidation of copper leads more easily to the cupric state than the cuprous.[5] Finally, when $k = 0$, the intermediate form is not developed at all, and we have the situation of an ordinary quinoid dye having no semiquinone form at all.

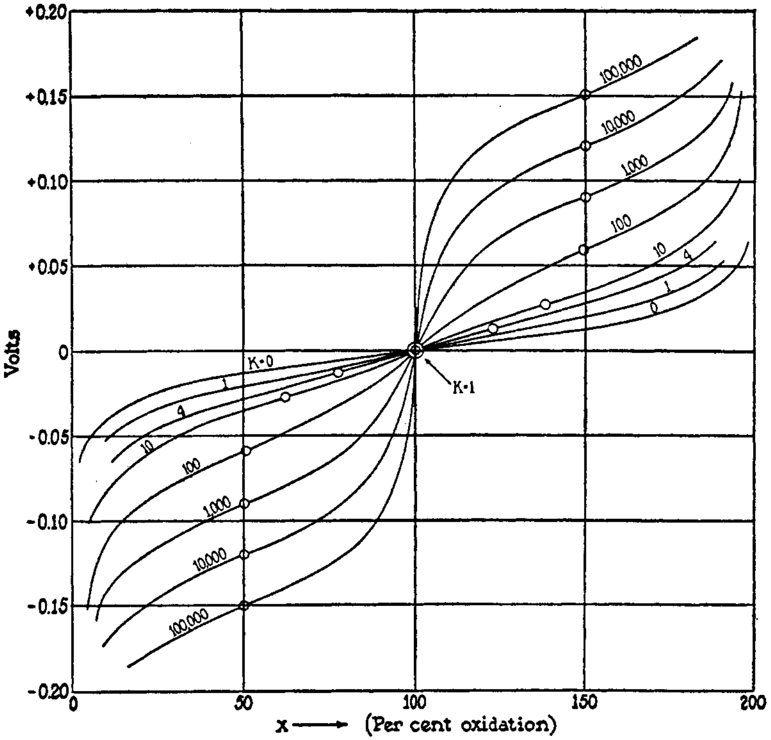

FIG. 5. The potential (in volts) plotted against per cent of oxidation

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[5] This case is not, however, quite comparable in every respect to the others, as the system—metallic copper plus a solution of copper salts—is a two-phase system, whereas we have to deal here with a homogeneous system.

This is the right place to look back at figure 4. The amount of semiquinone formed *in maximo* during the titration is always at the midpoint (100 per cent in figure 5). But even when k is as small as 1, in which case, according to the above reasoning, the normal potential of the lower step equals the one of the higher step, as much as 33.3 per cent of the dye is present as semiquinone at the midpoint of the titration.

DEPENDENCE OF k ON pH

To adapt the theory to actual conditions one has to consider that the equations 9 and 10 do not take into account that all these substances are acids (or bases), and that the difference between the oxidized and the reduced state may be not only an electron, but a full hydrogen atom. Each kind of molecule concerned may be present in several states of acidic dissociation. Equations 9 and 10 hold even then, provided we understand by r the total concentration of the reduced form in all its states of electrolytic dissociation, and the same holds for s and t. But equation 4a has to be modified. This equation holds only if we mean by r, s, t, just such forms of the three kinds of molecules as differ from each other only by electrons, not by full hydrogen atoms. When we pick out of all possible states of dissociation three particular ones r', s', t', which differ from each other only by electrons, equation 4a is valid. Now according to the mass law

$$r' = f_1\,(r, k_1, k_2, \ldots\ldots, \mathrm{pH})$$

where k_1, k_2, ... are the various successive dissociation constants of R, and f_1 is the symbol of some function the form of which is irrelevant for the time being. Analogous equations hold for s' and t'. These functions may be symbolized by f_2 and f_3. Equation 4a then reads

$$r \cdot t \cdot k \cdot \frac{f_1 \cdot f_3}{f_2^2} = s^2 \tag{16}$$

and taking

$$k \cdot \frac{f_1 \cdot f_3}{f_2^2} = K$$

the equation 16 has the same form as equation 4a, the difference being only that the constant k, which is independent of pH, is replaced by the "effective formation constant" K, which depends on pH.

For an individual dye K will vary with pH, and for this reason the shape of the titration curve of a dye may vary according to pH throughout all the possibilities as shown in figure 5. This is the explanation for the previously and preliminarily mentioned fact that a distinct separation of the two steps may be accomplished by working at a suitable pH. So, all the equations 11, 12, 13, 14, and 14a hold good, provided we apply K instead of k. We may designate k as the true formation constant of the semiquinone and K as the effective constant.

ADAPTATION OF THE THEORY FOR PRACTICAL APPLICATION

Heretofore we have started from a given value for K, taking it as the independent variable, and derived from it the shape of the titration curve. In practice, we have to deal with the reverse process. The experiment furnishes the titration curve, and we have to derive from it the value of K and of the three normal potentials. It is obvious that a strict and complete analysis of an experimentally obtained curve is not feasible on account of its complicated nature. However, since the various curves of figure 5 do not intersect except in the common midpoint, it is possible to match any experimentally obtained curve to a particular one of this family, and so to arrive at the value of K pertaining to it. To simplify this procedure, two methods have been recommended.

A. Michaelis' method

Let us define the magnitude E_i, the "index potential", by

$$E_i = E_{\frac{1}{2}} - E_{\frac{1}{4}} \tag{17}$$

where $E_{\frac{1}{2}}$ signifies the potential at the midpoint of the curve, at 50 per cent of the total oxidation (which is 100 per cent in the graph, figure 5), and $E_{\frac{1}{4}}$ is the potential at 25 per cent of the total oxidation (50 per cent in figure 5). On account of symmetry $E_i = E_{\frac{1}{2}} - E_{\frac{1}{4}} = E_{\frac{3}{4}} - E_{\frac{1}{2}}$. The occurrence of this symmetry in the experimental curve is a necessary criterion that the curve belongs to this family. Now it can be seen from figure 5 that K can be correlated with E_i. So we may set up an interpolation table from which for each value of E_i the corresponding value of K can be read. This is given in table 1, holding for a temperature of 30°C. It was first evaluated by the writer by means of graphical interpolation. A sufficient number of values were computed according to formulas 6 and 11 to 13, taking such values of K as to render the algebra easy, and the intermediate values were graphically interpolated. Later, Elema discovered a method of direct and strict calculation of any value of K from E_i. He arrived at the equation

$$K = \left(a - \frac{3}{a}\right)^2 \tag{18}$$

where $a = 10^{\frac{E_i}{0.0601}}$. This equation was derived by Elema from equation 21 of the next section. By this method the interpolation values formerly obtained could be checked and were found practically in agreement with the calculated, except for very slight errors, amounting at the worst to a few tenths of a millivolt, in the range from $K = 0$ to $K = 1$. This range is most sensitive to slight errors in the interpolation method. No practical consequence ensues from Elema's correction. In any case, the table given above has been corrected according to Elema's recalculation. We read from this table the value of log K correlated with any value of E_i. Experimentally we obtain E_i. From this, the table gives K, and hence $E_1 - E_2$, which is correlated with K in this way (compare formula 8)

TABLE 1

Interpolation table

LOG K	$0.0601 \log K = E_2 - E_1$	E_i
		volts
$-\infty$		0.0143
-3	-0.1803	0.0146
-2	-0.1202	0.0151
-1.5	-0.0901	0.0157
-1.3	-0.0781	0.0160
-1.0	-0.0601	0.0167
-0.8	-0.0481	0.0174
-0.6	-0.0361	0.0181
-0.4	-0.0240	0.0191
-0.2	-0.0120	0.0203
0	0	0.0218
$+0.2$	$+0.0120$	0.0237
$+0.4$	$+0.0240$	0.0258
$+0.6$	$+0.0361$	0.0286
$+0.8$	$+0.0481$	0.0320
1.0	$+0.0601$	0.0362
1.2	$+0.0721$	0.0404
1.4	$+0.0841$	0.0448
1.6	$+0.0962$	0.0494
1.8	$+0.1082$	0.0546
2.0	$+0.1202$	0.0601

Whenever $\log K > 2$, then $E_i = 0.03005 \log K$.

$$E_1 - E_2 = 0.06 \log K \tag{8a}$$

Besides, the experiment gives E_m, which is the potential at 50 per cent of the total oxidation. Now according to formula 7

$$E_m = \frac{E_1 + E_2}{2}$$

Herewith all data are available to arrive at E_1 and E_2. Having E_m, E_1, E_2, K, the problem is solved as to how to derive the characteristics of the dye, valid at the pH of the experiment, from the shape of the titration curve.

B. Elema's method

The middle part of any titration curve of this family is approximately a straight line, the slope of which will be correlated with K. The slope of the tangent at 50 per cent of the total oxidation is easily determined graphically. This tangent intersects the ordinate at 100 per cent of the total oxidation at a point which differs from the value of the ordinate at 50 per cent of the total oxidation. This difference can be determined graphically. Let it be v volts. Then, at 30°C.

$$K = (76.63v - 2)^2 \tag{19}$$

How to arrive at this formula will be mentioned in the next section. This equation permits of direct numerical evaluation of K for any value of v.

REVIEW OF SOME FURTHER MATHEMATICAL DETAILS

Although it does not seem appropriate to enter into the mathematical details of the theory more than necessary for practical application, some of the formulations should be at least briefly mentioned. The reason why it is desirable to have formulas of another type is this: All the above formulas are ambiguous with respect to the sign before the square root. The range of x of physical significance is only that between $x = 0$ and $x = 2a$, and so the problem arises whether these functions might be put into such a form that, at least within this range, no change of sign takes place. Secondly the foregoing formulas are of such a nature that for the case $K = 4$ a special formula must be used. So the problem also arises whether some formula may be found which can be applied generally for any value of K. As regards the first problem it cannot be solved for the formulas 11, 12, and 13 for r, s, t, but it is possible for the formula for E. Elema developed a formula fulfilling the first condition that it involve no change of sign:

$$E = E_m - \frac{RT}{F2} \ln \frac{KT_r - KT_o - 8T_r + \sqrt{(KT_r - KT_o - 8T_r)^2 + 16(K - 4)T_r^2}}{KT_o - KT_r - 8T_o + \sqrt{(KT_o - KT_r - 8T_o)^2 + 16(K - 4)T_o^2}} \tag{20}$$

Here T_r signifies the "total amount of the reductant", T_o the "total amount of the oxidant." These definitions may be easily misunderstood. A more precise definition is this:

$$T_r = \tfrac{1}{2}s + r$$

$$T_o = \tfrac{1}{2}s + t$$

s, r, and t having the same significance as in the above formulas 11, 12, and 13. Later the same author developed another formula which fulfills both of the mentioned conveniences and has besides the advantage that it can be relatively easily differentiated and so be used for the calculation of the slope of the midpoint tangent and hence for the establishment of formula 19. This formula reads:

$$E = E_m - \frac{RT}{F} \ln \frac{4\alpha}{(1 - 2\alpha)\sqrt{K} + \sqrt{K(1 - 2\alpha)^2 + 16(1 - \alpha)}} \tag{21}$$

where α is the "degree of reduction", or in terms of the previous definition,

$$\alpha = \frac{2a - x}{2a}$$

Herefrom an equation for the index potential E_i can be derived:

$$E_i = \frac{RT}{F} \log \frac{1}{2}(\sqrt{K} + \sqrt{K + 12})$$

When this is solved for K, one obtains the above-mentioned equation (18). Besides Elema's formulas, I may present the following:[6]

$$E = E_m + \frac{RT}{2F} \ln \frac{\xi}{2 - \xi} + \frac{RT}{2F} \ln \frac{\xi - 1 + \sqrt{(\xi - 1)^2 + 4\kappa\xi(2 - \xi)}}{1 - \xi + \sqrt{(\xi - 1)^2 + 4\kappa\xi(2 - \xi)}} \tag{22}$$

Here $\xi = \frac{x}{a}$ and κ is the dismutation constant, $= \frac{1}{K}$. This formula also fulfills both the desired conveniences and furthermore illustrates the function in so far as its first logarithmic term is the same as for an ordinary bivalent oxidation without step formation, whereas the second logarithmic term takes care of the correction for step formation when $K > 0$.

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[6] For the derivation of Elema's formula we may refer to his original papers. To arrive at formula 22, proceed as follows. Divide equation 9 by a, put $\frac{x}{a} = \xi$, $\frac{r}{s} = \rho$, $\frac{t}{s} = \tau$. Modify equation 10 in the same way. Divide the thus modified equation 10 by the modified equation 9. Rewrite equation 4a in the form $\rho \cdot \tau = \kappa$. Solve these three equations for ρ and τ and put $E = E_m + \frac{RT}{2F} \ln \frac{\tau}{\rho}$. Equation 22 has resulted from suggestions which the writer owes to Professor Albert Einstein.

PRACTICAL APPLICATION OF THE METHOD TO TWO EXAMPLES AND INTERPRETATION OF THE RESULT

1. *α-Oxyphenazine* (41)

A series of titration curves at constant pH was made, over a wide range of pH, either by oxidizing the reduced form, or by reducing the oxidized form. The oxidized form is lemon-yellow between about pH 1 to 11; it turns red at pH < 1, and cherry-red at pH > 11. The semiquinoid form in all those pH ranges where it exists is green. The reduced form is almost colorless, very slightly yellow. One example of a titration is figure 6, another figure 7. Similar titration experiments were performed in a wide range of pH and the values of E_m, E_1, and E_2 determined for each pH from these curves. The result of this set of experiments at varied pH is summarized in figure 8. Notice first of all the common point of intersection

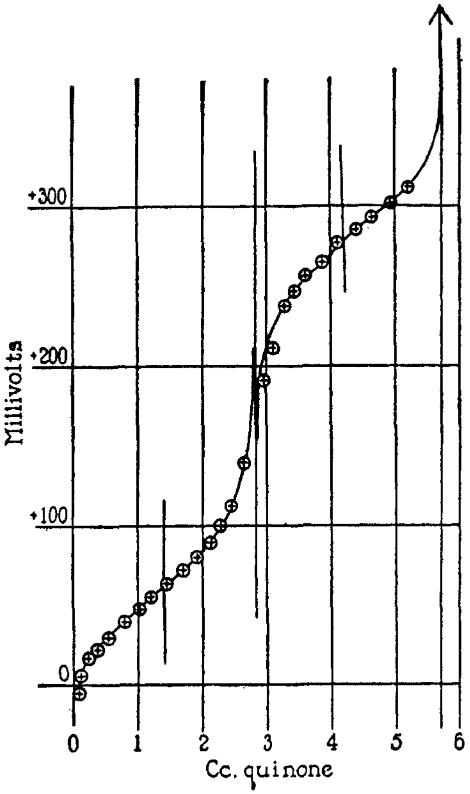

FIG. 6. Reduced α-oxyphenazine at pH 1.00 is titrated with quinone. Potentials referred to the normal hydrogen electrode.

of the three normal potentials E_m, E_1, E_2 at pH 3.2. Here $E_m = E_1 = E_2$ and therefore $K = 1$, and the maximum amount of semiquinone that can exist beside the other forms is 33.3 per cent of the total dye. This occurs when half the amount of oxidant necessary for complete oxidation has been added. To the left of this point of intersection, the separation of the two steps is greater.

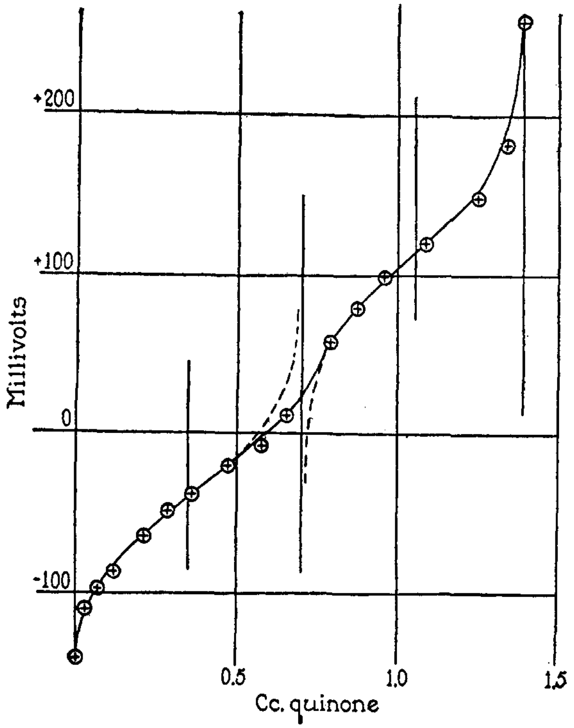

Fig. 7. Reduced α-oxyphenazine at pH 1.86 is titrated with quinone. Potentials referred to the normal hydrogen electrode.

Secondly, notice at pH 1.3 a bend in the E_m and in the E_1 curves, but none in the E_2 curve. To interpret these bends we apply the rules of W. M. Clark and B. Cohen (3), which may be expressed as follows: (1) Each bend is correlated with an acidic dissociation constant.[7] (2) When the curve becomes steeper this constant belongs to the oxidized form of that particular oxidation-reduction system; if flatter, to the reduced form. (3) Each dissociation constant alters the slope by 0.06 volt per pH unit, when

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[7] Applying Brönsted's definition, basic dissociation constants may be included among the acidic ones.

the system is a one-electron system; by 0.03 volt per pH unit, when it is a two-electron system. (4) In order to evaluate graphically the dissociation constant, extend the rectilinear parts of the curves to tangents, which then will intersect. Project the point of intersection on the abscissa. This gives pK.

On applying these rules to the E_1 curve we find at pH 1.3 a change of the slope from 0.06 volt per pH unit to 0.12, the change thus amounting to an increase in slope of 0.06 volt per pH unit. This signifies a pK = 1.3 for the oxidized form of a one-electron system. The system is here the T–S system, which really is a one-electron system, and the constant belongs to the oxidized form of this system. At the same pH we notice in the E_m curve a change of slope amounting only to 0.03 volt per pH unit, indicating a dissociation constant of a two-electron system. This is the T–R system, and this bend is a confirmation of the result obtained from the E_2 curve. The E_1 curve shows no bend at this pH. This curve belongs to the S–R system. The T form, whose constant becomes manifest at this pH in the two other curves, cannot have and does not have any influence on the slope of the E_1 curve. The existence of a pK = 1.3 is corroborated by the fact that around pH 1.3 the color of the oxidized form turns from yellow to red.

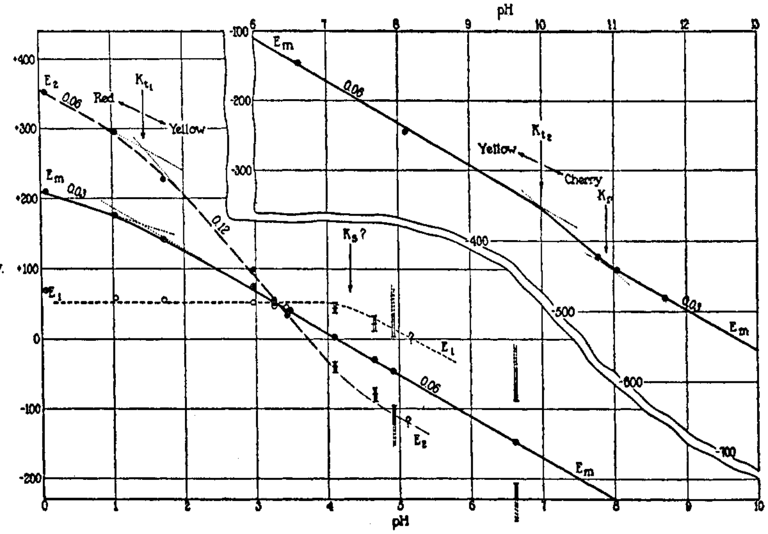


FIG. 8. The three normal potentials E_m, E_1, and E_2 of α-oxyphenazine plotted against pH (at 30°C.). The ordinate is the potential, in millivolts; the reference potential is a calomel electrode, which is +0.242 volt with respect to the normal hydrogen electrode. The upper curve is an extension of the lower one.

On pursuing the three normal potential curves to the right of the crossing, the limits of error in drawing the E_1 and E_2 curves become greater as these curves begin to diverge. For, from table 1 one can infer that, as the index potential approaches its minimum value of 0.0143 volt, an error of a few tenths of a millivolt in the index potential involves a conspicuous error in E_1 and E_2. Only E_m is unaffected with respect to the limits of error throughout all pH ranges. In figure 8 the influence of such a limit of error in E_1 and E_2 is symbolized, assuming the limit of error in E_1 to be ± 0.5 millivolt. Thus, it is of no use to extend the E_1 and E_2 curve too far beyond the point of intersection. It seems that at pH 4.2 these curves may have

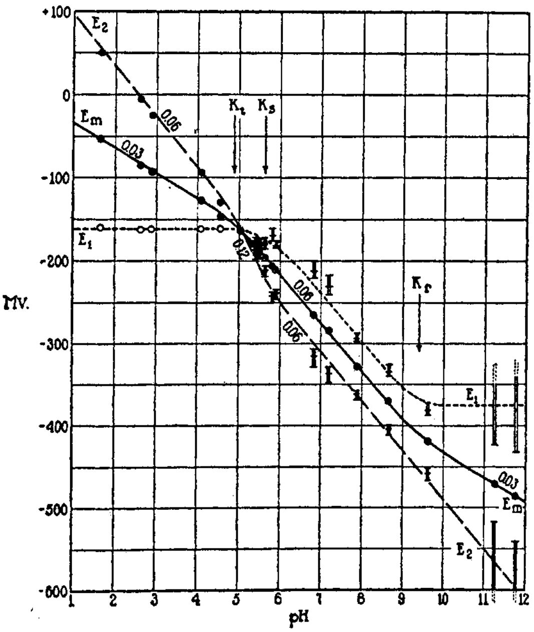


FIG. 9. The three normal potentials of pyocyanine plotted against pH. Designations as in figure 8.

a bend so as to make them, over a certain range, both parallel to the E_m curve. This would signify a dissociation constant of the semiquinone, pK = 4.2, if these bends be real.

A second example is that of pyocyanine (α-oxy-*N*-methylphenazine). The curves in figure 9 were established by the above method. Although here also the limits of error at the right-hand side of the point of intersection are appreciable, they are not quite so disturbing as in the foregoing case, and there is some justification in assuming that the bends in E_1 and E_2 at pH 5.6 are real, and that these curves both become parallel to the E_m

curve up to pH about 9. This agrees very well with the observation that, on oxidizing the colorless reduced form at pH 7 to 8, the color first turns greenish blue and on further oxidation a pure blue, whereas at pH 11 the color turns directly blue and thereafter only becomes more intensely so. In the interval of parallelism, $E_2 - E_1 = 0.070$ volt, approximately. This, according to table 1, would mean $K = 0.05$ approximately, and would allow, according to figure 4, for about 10 per cent of the dye to exist in the semiquinoid form in the midpoint of titration even in slightly alkaline solutions, a fact that may have a physiological bearing.

THE CONDITION FOR THE EXISTENCE OF SEMIQUINONES

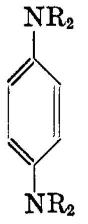
The occurrence of a semiquinone on partial reduction of a quinoid substance or on partial oxidation of a benzenoid substance may be interfered with for two very different reasons. In the first place the formation constant K may be extremely small or zero. Consequently, as K in general depends on pH, the semiquinone may exist at certain pH values, but not at others, as has been shown in the two examples just now presented. If K is very small but the semiquinone very little soluble, the semiquinone may exist only in the solid state, as is the case for quinhydrone. Also the semiquinoid form of chlororaphine is so difficultly soluble that in cultures of *Bacillus chlororaphis* it crystallizes within the liquid medium without the culture medium itself showing the green color of the semiquinone, as will be discussed in detail later on.

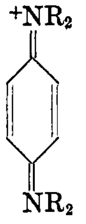
In the second place, the semiquinoid, being an unsaturated radical, may be liable to a more or less rapid irreversible rearrangement into substances different from the parent substances. The two conditions of existence may be distinguished as the thermodynamic condition and the stability condition. The first one may be stated thus: the dismutation constant must not be too large. The other may be stated as follows: the semiquinone must not be too fragile a molecule. All transitions may occur. So, as will be discussed in the special chemical part of this review, the lability of the semiquinones of *p*-phenylenediamine derivatives varies considerably according to substitutions in the amino groups. But among the three constituents of a two-step system it is not always the semiquinoid that exhibits the greatest instability. Sometimes it is the quinoid form, e.g., in the aromatic diamines. For this reason, Wurster's blue is perfectly stable in solution only in the presence of an excess of tetramethyl-*p*-phenylenediamine, which establishes the thermodynamic condition by preventing the dismutation of the semiquinone according to equation 4a. In the case of the γ,γ'-dipyridyl compounds, conversely, the oxidized form is perfectly stable, the semiquinoid forms relatively stable; the completely reduced one is labile.

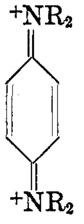
THE BEHAVIOR OF SEMIQUINOID RADICALS AS COMPARED WITH THAT OF OTHER ORGANIC RADICALS AND ITS INTERPRETATION

Two groups of organic radicals present themselves for comparison with the semiquinoids, those with tervalent carbon as represented by Gomberg's triphenylmethyl, and especially those with bivalent nitrogen as represented by Wieland's diphenylnitride. There are two essential differences. First of all, the compounds of these two groups have no existence in any aqueous solvent, whereas many semiquinones are perfectly stable in water under proper conditions of pH and oxidation–reduction potential. Secondly, the other types have a great tendency to abolish their unsaturated state either by dimerization or by eagerly combining with other reactive molecules, or certain specific substances. The triarylphenyl compounds combine with oxygen to a peroxide $(Ar)_3CO{:}OC(Ar)_3$; the diaryl nitrides add nitric oxide in this way, $(Ar)_2N + NO \rightarrow (Ar)_2NNO$. In the semiquinones, neither a dimerization nor any specific addition reaction has been found as yet. Several of the semiquinones are so perfectly stable even in aqueous solutions in certain pH ranges (of course, except for their sensitivity toward oxidants or reductants), that this contrast to the other types of radicals needs some explanation. The following suggestion is thought to be on the right track.

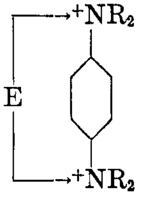
Taking tetramethyl-*p*-phenylenediamine as an example, we can formulate the three steps as follows:


I


II


III

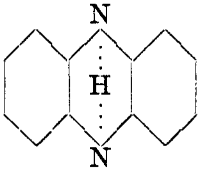
In formula II the lower nitrogen atom is of the character of a free ammonium radical. We now may imagine that the positive charge in II is not permanently at the upper nitrogen atom but oscillates between both nitrogen atoms, as symbolized in IIa, where the electron E is shown to oscillate between two positively charged nitrogen atoms so as to neutralize in rapid succession, now one, now the other charge. This may be considered a structure which might exhibit stability on the ground of the perfect symmetry.



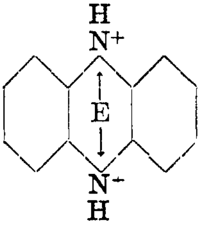
IIa

Each positively charged nitrogen means a nitrogen kernel with seven, instead of the usual eight, electrons in the outer shell, and the septets take turns in utilizing the odd electron to fill up the octet. Furthermore, as this molecule is a cation, in contrast to the electroneutral triphenylmethyl or diphenylnitride, electrostatic repulsion will prevent the formation of a double molecule. If we wish to speak of definite paths of the electrons we may say that the odd electron has a path encircling the two nitrogen atoms. As such a wide circuit for an electron appears unlikely, one may rather imagine that the electric oscillation takes place through the whole chain of atoms from one nitrogen to the other. Avoiding any mechanical model, one might simply say that the probability of the odd electron belonging to the one or to the other nitrogen is the same. We may also say that the odd electron is a chemical bond of the two nitrogen atoms, which is presented by a single electron instead of the customary pair of electrons. So the ordinary valence symbols are not suitable to formulate these compounds. The oscillating odd electron may be imagined to be an electric oscillator of especially low frequency and to account for the optical properties to be discussed later on.

In the phenazine group the shared electron may be imagined in two different ways, according to formulas I and II in which E is the electron:



I



II

In formula I one hydrogen atom stands between the two nitrogen atoms. The electron of the hydrogen atom completes alternately the septet, now of the one, now of the other nitrogen atom, to an octet. In this case we may also speak of a shared hydrogen atom. In the other form there is one electron shared by both nitrogen atoms. This formula differs from the other by a proton. The two forms may therefore be considered as two steps of acidic dissociation of the same substance. In fact, there is some evidence that the semiquinone in the phenazine group may exist in two states of acidic dissociation. For, as stated above, there seems to be a dissociation constant of the semiquinone of pyocyanine, pK = 5.2.

The occurrence of a dissociation constant in the semiquinoid form may have important consequences. In the case of pyocyanine, it brings about the parallelism of the three normal potentials over a certain pH range. Hence, the thermodynamic condition for the existence of the semiquinone remains constant within this pH range. According to circumstances, such a dissociation constant may have all kinds of peculiar consequences. One of them may be mentioned because later on a case will be shown where it seems to occur. Let us assume that the E_m curve has a 0.03 slope around the common point of intersection, and that the E_1 curve lies above the E_m curve to the left from this point of intersection (figure 10). When now the semiquinoid happens to have a dissociation constant, so that pK > the pH at the point of intersection, the bend brought about will cause once more an intersection. In such a case the thermodynamic condition for the existence of the semiquinone is restricted to a limited pH range and is at a maximum when pH = pK.

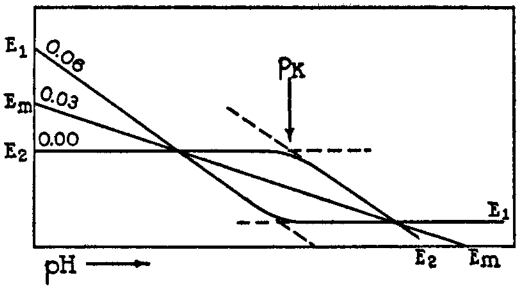
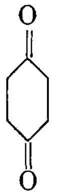


FIG. 10

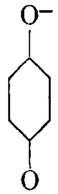
ANIONIC SEMIQUINONES

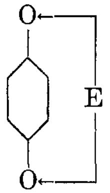
The above interpretation of the structure of a semiquinone holds for the case of its being a cation. The question will now be raised whether there are also anionic semiquinones. The simplest example imaginable would be that of quinhydrone (formulas I to III). This case is not accessible to a potentiometric investigation. The dark green crystals of quinhydrone when dissolved at pH < 7 do not show any color resembling that of the



I

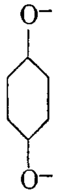
Quinone





IIa or IIb

Quinhydrone



III

Hydroquinone
(as ion)

crystals. When hydroquinone at pH < 7 is titrated with an oxidant, the titration curve is precisely the one of an ordinary two-electron oxidation without step formation. So no form intermediate between quinone and hydroquinone exists in an acid solution. This fact may also be stated in the following form. Quinhydrone dissolved in an acid solution dismutes completely into quinone and hydroquinone, the dismutation constant being extremely large. Since for cationic semiquinones the dismutation constant decreases with *decreasing* pH, one might infer that for anionic semiquinones this constant would decrease with *increasing* pH instead. In other words, the thermodynamic condition for the existence of an anionic radical would increase with increasing pH. In fact, alkaline solutions of quinhydrone exhibit a strong color, but unfortunately the stability condition is impaired by alkali. Secondary irreversible rearrangements take place. The color developed is not only that of the semiquinone, but is probably due even more to secondary reactions, and no constant potentials can be obtained in alkaline solutions. Concerning the solid crystalline quinhydrone, Foz and Palacios conclude from an x-ray analysis that the elementary unit of the quinhydrone crystal is the monomolecular radical, not any bimolecular unit.

It seems likely that the indigo dyes belong to this group and offer a better chance for a potentiometric investigation. What is known at the present time along these lines will be shown in the special chemical section of this review.

PRELIMINARY REVIEW ON THE OPTICAL PROPERTIES OF THE SEMIQUINONES

The odd electron supposedly present in all semiquinones and oscillating between two atoms intramolecularly renders these substances a desirable subject for spectroscopic studies. Although these are not far enough advanced for any theoretical treatment, yet the essential features may be mentioned. It seems that all semiquinones exhibit a remarkably distinct band spectrum. This is simplest in the viologen dyes. Here we have in the visible region a single distinct band which very likely will be suitable for a study of a finer structure. The maximum absorption lies in the red or yellow, drops rather steeply toward the longer wave lengths and more gradually toward the shorter wave lengths, forming here a secondary, less distinct maximum. In the hand spectroscope one has the impression of a strong band followed by a weaker one. Only in benzylviologen is the first band weaker than the second. This dye undergoes a very remarkable and reversible change of light absorption with temperature, a fact that was already known to Weitz and Piccard. The color appears violet at room temperature and blue at about 80°C. Spectroscopically, the change is not so much attended by a shift of the absorption maxima to different wave lengths but rather by a change in intensity of the two absorption maxima; the higher peak becomes the smaller one and vice versa. To a very small extent a similar phenomenon can be observed in the other viologens, but not in the violet dye produced from γ,γ'-dipyridyl itself.

Wurster's dyes, the oxidation products of the substituted phenylene diamines, show the same type of band, but this band is, in all its details, repeated in an interval of about 300 Ångström units, and this second band overlaps with the first. The semiquinones of the phenazine series, mostly green in color, have no definite band in the visible, but in the one example investigated by Hantzsch there is a double band in the ultra-violet. The semiquinoids of the indigo dyes show a very sharp single band in the visible spectrum.

It may also be expected that semiquinones may show paramagnetic properties, according to Taylor and Lewis (51). Kenyon and Sugden (27) also found paramagnetism in a colored compound of supposedly radical character, which however cannot be properly counted to the semiquinones. For these compounds the study of magnetic properties has still to be carried out.

SPECIAL CHEMICAL PART

1. p-Phenylenediamine group

The first product on oxidizing *p*-phenylenediamine in a moderately acid solution is too labile and not suitable for a detailed investigation. On oxidizing, e.g. with bromine, a very transient yellow arises, turning to green, red, and finally becoming colorless.

Substitution of the amino hydrogens of *p*-phenylenediamine leads to compounds having two distinctly different steps of oxidation. Table 2 has been established by Piccard (44). Of these compounds the asymmetrical dimethyl and diethyl, the tetramethyl and tetraethyl, and the symmetrical diphenyl derivatives have been subjected by the writer to a potentiometric study (42). The diphenyl compound is easiest to under-

TABLE 2

Oxidation steps of derivatives of p-phenylenediamine

LEUCO BASE	HOLOQUINOID SALT	MERIQUINOID SALT
$H_2N\cdot C_6H_4\cdot NH_2$	Colorless	Yellow
$H_2N\cdot C_6H_4\cdot NH(CH_3)$	Colorless	Red-yellow
$H_2N\cdot C_6H_4\cdot N(CH_3)_2$	Colorless	Red
$H_2N\cdot C_6H_4\cdot NH(C_6H_5)$	Yellow	Red
$H(CH_3)N\cdot C_6H_4\cdot N(CH_3)_2$	Colorless	Violet
$(CH_3)_2N\cdot C_6H_4\cdot N(CH_3)_2$	Yellowish	Blue
$(CH_3)_2N\cdot C_6H_4\cdot NH(C_6H_5)$	Yellow	Blue
$H(C_6H_5)N\cdot C_6H_4\cdot N(C_6H_5)H$	Red	Blue (greenish)
$(C_6H_5)_2N\cdot C_6H_4\cdot N(C_6H_5)_2$	Blue	Green

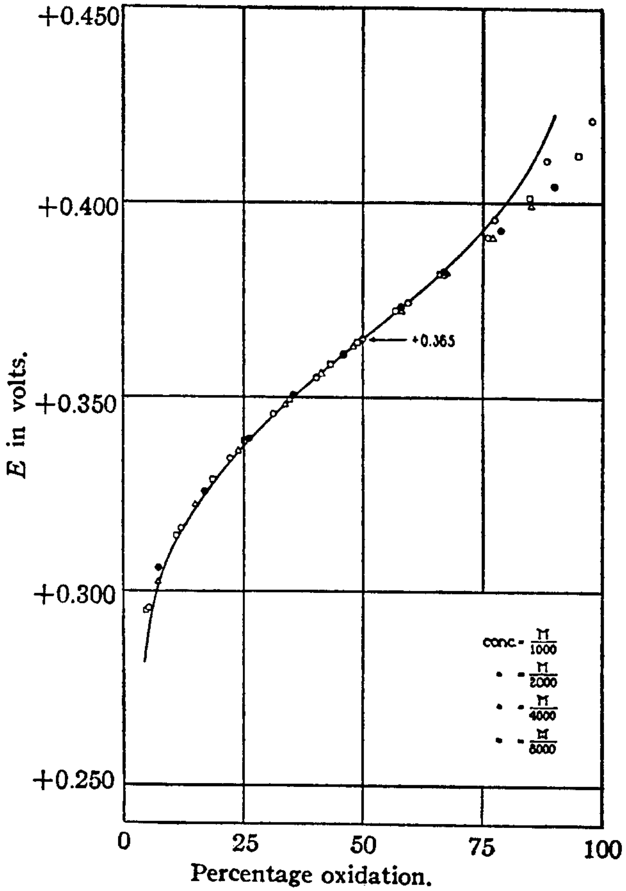


FIG. 11. Independence of the normal potentials of tetramethyl-*p*-phenylenediamine on the initial concentration. Potentials are plotted against percentage of oxidation; ordinate is potential in volts; abscissa is percentage of oxidation. Potentials referred to the normal hydrogen electrode. pH 4.620, varying no more than ±0.01 in the individual titration experiments.

stand, both steps of oxidation being stable compounds so that definitive and non-drifting potentials could be obtained using 90 per cent acetic acid + 10 per cent water as a solvent and bromine as oxidant. The two steps of oxidation are easily distinguished, being separated by a definite jump. The separation happens to be so great that very little overlapping of the two steps takes place, and each half of the curve shows the shape of an ordinary one-electron curve. The substance is unfortunately not sufficiently water-soluble to allow establishment of a set of titration curves in aqueous buffer solutions. This can be carried out with the tetramethyl or tetraethyl compound. Here, however, the holoquinoid form, a biquaternary iminium base, is an extremely labile compound, so the titration can be carried out only for the first step of oxidation and not even here to completeness. The two steps are not distinctly enough separated as to prevent some overlapping. Therefore the potentials are definite only up to about 60 to 65 per cent of the oxidation to the first step. On the other hand, this substance is very suitable for showing that the shape of the titration curve does not depend on the initial concentration of the substance, as shown in figure 11. In this figure the rudimentary titration curve, as

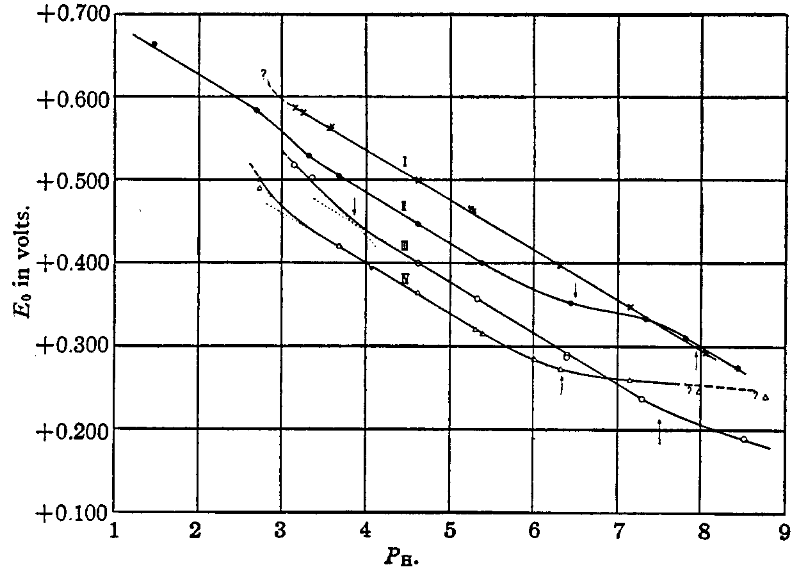
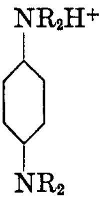


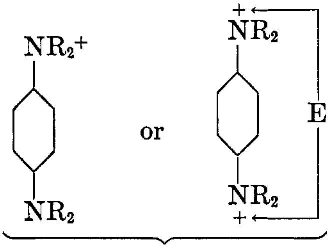
FIG. 12. Normal potentials are plotted against pH for the four alkylated diamines as follows: ×, diethyl-*p*-phenylenediamine; ●, dimethyl-*p*-phenylenediamine; ○, tetraethyl-*p*-phenylenediamine; △, tetramethyl-*p*-phenylenediamine. Ordinates, normal potentials in volts; abscissas, pH. Potentials referred to the normal hydrogen electrode.

obtained from the non-drifting part of the oxidation, has been extrapolated to a full theoretical curve. In the range of oxidation greater than 70 per cent it no longer fits the experimental data, in part because of the uncertainty of the measurements owing to the drift, in part because of the overlapping of the two steps which, although seemingly not very great, necessarily causes a deformation of the curve. Such titration curves can be obtained throughout a pH range of about 3 to 7. The normal potentials plotted against pH as shown in figure 12 lie on a straight line with a slope of 0.06 volt per pH unit. This indicates that the oxidized and the reduced form differ not only by an electron but by a full hydrogen atom. This is in agreement with the formulas 1 and 2 expressing the state of ionization of these compounds in a moderately acid solution. At pH approximately 6.5, the slope of the curve apparently is changed in such a way as to indicate a dissociation constant of the reduced form, the magnitude of which agrees with the dissociation constant directly determined. This part of the curve, however, cannot be pursued to higher pH values, as then the potentials show a drift even in the first part of the titration. Something similar takes place on the left-hand end of the curves of figure 12, owing to another dissociation constant.



I

Cation (primary) of
the reduced form



II

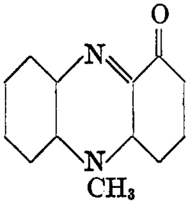
Cation of the
semiquinoid form

In the same figure there are also shown the results for the tetraethyl, the dimethyl, and the diethyl compounds. The dialkyl compounds can be titrated almost as well, but the potentials are not quite so stable as in the tetraalkyl compounds, even in the first part of the titration. This behavior is a transition to that of the unmethylated compound, which is not suitable for a titration at all, as stated above.

2. The phenazine group

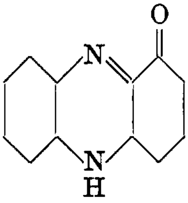
(*a*) *Phenazine.* Phenazine itself is not water-soluble enough for a complete study in aqueous buffer solutions. It gives most satisfactory results in 50 per cent acetic acid solution, titrated with quinone, dissolved in the same solvent. The curve shows two distinctly separated steps which agree perfectly in shape with two one-electron titration curves, the normal potentials amounting to -0.086 and $+0.254$ volt, in this solvent, which has an (apparent) pH of 1.26.

Several derivatives of phenazine are water-soluble enough to allow a complete set of experiments in aqueous buffer solutions over a wide range of pH.

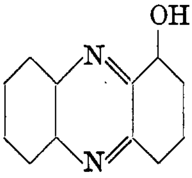


I

Pyocyanine

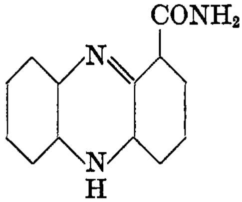


IIa



IIb

Two tautomeric formulas for α-oxyphenazine



III

Xanthoraphine
(or "oxychlororaphine")

(*b*) *Pyocyanine.* Pyocyanine, the blue pigment of *Bacillus pyocyaneus*, a saprophyte frequently encountered in purulent wounds, has been synthesized by Wrede and Strack (64, 65). They established the formula shown above except for the fact that they ascribed to it the double-molecular size, assuming that two molecules are bound together in such a way that the non-methylated nitrogen atoms are pentavalent and linked together by a double bond. For the completely reduced form they assume the monomolecular formula. The intermediate form escaped their attention, although they mention that on reducing the dye, a transient green color arises. The bimolecular formula of pyocyanine was based on a determination of the freezing point of the free base in glacial acetic acid. Without deprecating these authors' merit in the analysis and synthesis of this substance, their conclusion as to the molecular weight cannot be accepted.

We may consider this as another case of misleading results of a freezing point determination. It cannot be definitely excluded that in glacial acetic acid in such a high concentration of the dye as needed for the experiment a partial association may take place. It is equally possible that the large amount of dye dissolved in the solvent exceeds the solubility at freezing point temperature. In any case the bimolecular formula is untenable for a dilute aqueous solution. For in this case the equation of the reduction in a solution sufficiently alkaline to prevent the formation of the semiquinone would read

$$2\text{Re} \rightleftharpoons \text{Ox} + 4\epsilon$$

where Ox is the oxidized form and Re the reduced, which Wrede also assumes to be monomolecular. In this case the equation for the potential should be

$$E = \text{Const.} + \frac{RT}{4F} \ln \frac{[\text{Ox}]}{[\text{Re}]^2}$$

Whereas in reality the titration curve follows quite strictly the equation

$$E = \text{Const.} + \frac{RT}{2F} \ln \frac{[\text{Ox}]}{[\text{Re}]}$$

This necessarily leads to the chemical equation

$$1\text{Re} \rightleftharpoons 1\text{Ox} + 2\epsilon$$

and is in agreement only with the monomolecular form and entirely analogous to the other known phenazine compounds.

It has already been stated above that within the physiological pH range from 6–8 an appreciable amount of the semiquinone is capable of existence at a suitable oxidation–reduction potential in spite of the considerable overlapping of the two halves of the titration curves. The result of the potentiometric study of pyocyanine has already been shown in figure 9.

(*c*) *Chlororaphine.* *Bacillus chlororaphis Guignard* and *Sauvagneau* produces in its cultures a yellow pigment, xanthoraphine or oxychlororaphine, prepared by P. H. Lasseur (35). In the culture medium there is developed under certain circumstances a crystalline green pigment called chlororaphine. Kögl and Postowsky (28) succeeded in elucidating the constitution of these dyes. Oxychlororaphine was found to be phenazine-α-carbonamide. It could be reduced to chlororaphine and in a second step to a dihydro compound, yellow in color. When this dihydro compound and oxychlororaphine are dissolved in glacial acetic acid no color change occurs, but on addition of water green crystals of chlororaphine are precipitated.

The authors therefore ascribed to chlororaphine the constitution of a meriquinone in Willstätter's sense. In a later paper (30) under the influence of the results obtained on pyocyanine, as just discussed, they changed their point of view and accepted the monomolecular radical formula for chlororaphine. They determined the freezing point of a mixture of the fully oxidized and the fully reduced form in camphor and found in solutions from 3–7 per cent a molecular weight from 219 to 372, increasing with concentration, the theoretical value of the dimolecular compound being 448. Although here with increasing concentration quite obviously some kind of association takes place, no green color is developed in camphor solution. This shows that the intermediate compound does not exist in camphor, the dismutation constant being too high, and that the association at higher concentration has nothing to do with the green compound. The freezing point data, therefore, cannot be utilized to decide upon the molecular size of the green chlororaphine. They are rather due to the general occurrence of partial association of dissolved molecules, especially in organic solvents, at high concentrations. Once more it is shown how precarious is the interpretation of freezing point data. Their hypothesis of chlororaphine being a monomolecular semiquinone was entirely confirmed by Elema's (10) potentiometric studies. He showed that in a very acid aqueous solution at a sufficiently low concentration all three steps of oxidation–reduction are sufficiently soluble to allow a potentiometric titration in an homogeneous system. On reduction the color turns from light yellow to an intense emerald green and then to orange yellow, the latter being the color of the dihydro compound. Utilizing the theory of the two-step oxidation, in the way illustrated above, he arrived at the result shown in figure 13. This figure shows the crossing-point of the three normal potentials at pH 2.1, indicating that at this pH the formation constant = 1. The extension of the E_1 and E_2 curve towards the right-hand side of the crossing-point can only be approximately estimated for the reasons discussed above. Yet it seems that the E_1 and E_2 curves, which might have been expected to diverge to the right-hand side of the crossing-point, very soon begin to converge once more, so as to become parallel to each other and to the E_m curve. If this be so, it would indicate that the formation constant of the semiquinone at the right side from the crossing, although it may be small, remains constant over a certain pH range and so is compatible with the formation of the radical even at higher pH. The semiquinone being extremely difficultly soluble at this pH, the formation of its crystallized form in the cultures is explained.

(*d*) *Other phenazine compounds.* The case of α-oxyphenazine, a dye first synthesized by Wrede and Strack as an intermediate product during the synthesis of pyocyanine, has been already presented as an example for an especially illustrative instance of a two-step system (figure 8). In a great number of other phenazine compounds intermediate states of oxidation–reduction can be observed, although they have not been subjected to potentiometric study. Not only in the class of the phenazines proper, but also in the group of quaternary phenazonium compounds (safranines), it is remarkable that a semiquinone has never been observed in a compound containing one or more free amino groups as side chains to the benzene rings (42). The following compounds showed no intermediate form of reduction: symmetrical and asymmetrical diaminophenazine, triaminophenazine, *m*-methylrosinduline, *N*-dimethylisorosinduline, asymmetrical anilinoaminophenazine, aminoacridine, asymmetrical aminohydroxyphenazine, rosinduline, isorosinduline, phenylacridine. Semiquinones have been observed in phenazine, α-oxyphenazine, α-methoxyphenazine, α-oxy-*N*-methylphenazine (pyocyanine), α-carbonamidephenazine (chlororaphine), phenylnaphthazonium nitrate, phenylisonaphthazonium nitrate,

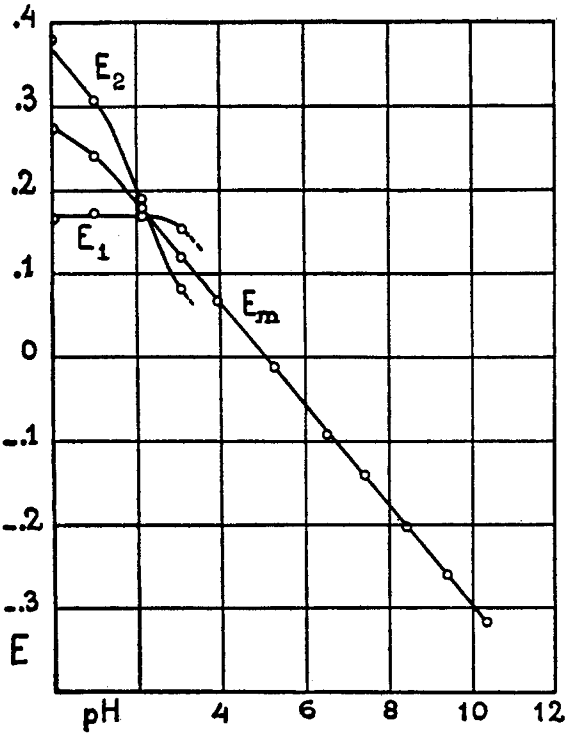
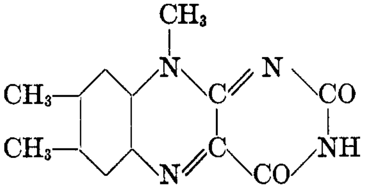


FIG. 13. The three normal potentials of the chlororaphine system plotted against pH, according to Elema.

methylnaphthazonium chloride, rosindonesulfonate, (rosinduline GG). In all phenazine and phenazonium compounds the semiquinoid is green, and in all naphthophenazine compounds, including rosindonesulfonate, it is red to violet. The oldest known green intermediate form is that of phenazine itself, observed as early as 1873 by Claus. The corresponding form for methylphenazonium compounds was the subject of a heated discussion between Kehrmann and Hantzsch, during which Hantzsch first advocated the radical formula.

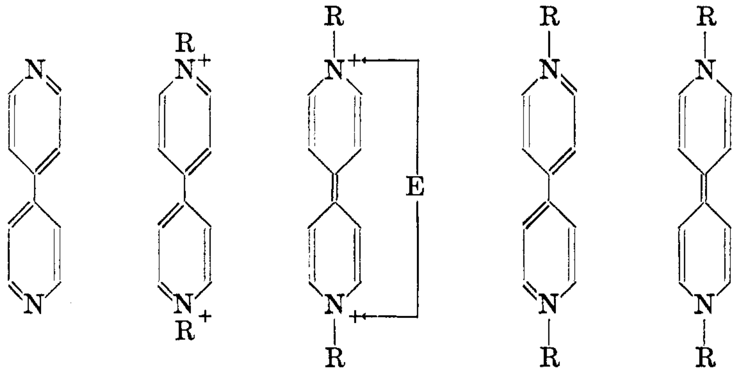
(*e*) *The flavin group.* Warburg and Christian (54) prepared a yellow compound, the "yellow respiration enzyme," from yeast, recently also in the crystallized state. This substance can be easily split into a colorless part, probably a protein, and a yellow pigment. This has now been recognized to be a dyestuff, called photoflavin, combined with a side chain consisting of a pentosephosphate ester (31, 32, 33; 52). Warburg discovered that this side chain is detached on illumination with visible light in alkaline solution. From this, free "photoflavin" can be extracted by chloroform. In the meantime R. Kuhn and his associates, Ellinger and Koschara (14), and K. G. Stern (48, 49) found representatives of this class of dyestuffs widely distributed in animal and vegetable tissues and fluids. The photoflavins derived from the representatives of this group seem to be alike or at least very closely related. R. Kuhn ascribes to lactophotoflavin, prepared from milk, the constitution of a substituted isoalloxazine (see formula). The resemblance of its structure



to pyocyanine is obvious. Homologous substances of similar properties have been synthesized by R. Kuhn and his associates, and by K. G. Stern and Holiday. These dyestuffs can be reversibly reduced to leuco compounds. Kuhn and Wagner-Jauregg discovered (32) that this reversible reduction, when performed in a strongly acid solution, passes through an intermediate stage of red color. They advanced the hypothesis that this is due to formation of a semiquinone. This problem has been studied potentiometrically by K. G. Stern (48) with photohepatoflavin and with photoyeastflavin, and by Barron and Hastings (1) with lactoflavin. The latter authors obtained distinct two-step curves in acid pH ranges, but they have not yet published the details. Stern found that in neutral and alkaline solutions the titration curve of the dye resembles very closely the one of an ordinary reversible organic dyestuff with the electron number 2. In the acid range, the slopes of the curves become increasingly steeper with decrease of pH, and in very acid solution, pH 0.4 to 0.2, a distinct step in the titration curve takes place, indicating all characteristics of semiquinone formation. On plotting the three normal potential curves against pH, a graph similar to that of pyocyanine is obtained, the common point of intersection lying at pH 2.2. To the right of this point, the E_1 and E_2 curves seem to converge so as to become approximately parallel to the E_m curve, as it is with pyocyanine. The data available in the range from pH 2.2 to 14 do not seem precisely enough known to allow accurate drawing of the E_1 and E_2 curves throughout this range. In this range, a slight error in the determination of the index potential involves a great error in E_1 and E_2, as has been shown above. The scarcity of the material then available was not favorable to obtaining quite precise determinations in this region. But the data available at least seemed to indicate that the thermodynamical existence condition of a semiquinone remains approximately constant throughout this range. So it is likely that the semiquinone is capable of existence even in neutral and alkaline solution, if only to a slight extent. Visible semiquinone formation is limited to the range of pH < 2. It should be mentioned that Bierich and Lang (2), working with a similar, or perhaps even identical pigment extracted from various organs, had obtained titration curves which showed no indication of any step formation.

(*f*) *The benzidine group.* Benzidine and tolidine can be oxidized in an acid solution to intensely green or blue compounds and, furthermore, to a second step of oxidation of brown color. The same holds for their derivatives such as those in which the hydrogen atoms of the benzene rings are substituted by halogens or those of the amino groups are substituted by alkyls. Schlenk (46) proved the blue or green compounds to be of the oxidation level of a meriquinone, and accordingly formulated them as bimolecular compounds. In general they are of very unstable nature, even in dry condition. A potentiometric study was attempted by Sullivan, Cohen, and W. M. Clark (50). The unstable nature and the ensuing drift of the potentials was unfavorable to an interpretation of the reactions involved. In very acid solution no intermediate green compound is formed; the oxidation leads directly to the second step, and in this case the authors obtained an ordinary two-electron curve. The writer attempted titration experiments with many benzidine derivatives without encountering any one in which the intermediate step of oxidation was sufficiently stable to obtain an interpretable titration curve. For this reason the molecular size of these products cannot yet be decided. There is no proof as yet that these compounds are of the same nature as those of the other groups described.

(*g*) *The dipyridyl group.* When γ,γ'-dipyridyl dissolved in diluted acetic acid is reduced by chromous chloride, a deep violet color is developed. On reduction with zinc dust the reduction passes through the same violet stage to a second colorless one. The violet dyestuff has been shown by Dimroth and Heene (8) to be on the oxidation level of a quinhydrone, intermediate between dipyridyl and dihydrodipyridyl. The violet color does not appear when the reduction is performed in a neutral or alkaline medium. Dipyridyl can easily be converted into a biquaternary base, each nitrogen adding, e.g., one methyl-halogen. These quaternary bases, especially dibenzyl-γ,γ'-dipyridylium diiodide, were already known to W. H. Hofmann, although he did not realize their true constitution. We shall refer to these quaternary bases as viologens, e.g., dimethyl-γ,γ'-dipyridylium dichloride will be designated methylviologen (43). All viologens can be reduced in a first step to violet dyes. Besides, substances of a further reduction level, namely, on the level of dihydrodipyridyl, are known. They have been prepared by E. Weitz, are light brown in color and very little soluble in water. He showed that they may be considered with the same justification as derivatives of dihydrodipyridyl or as free ammonium radicals as in formula IIc. The reduction of the quaternary bases of γ,γ'-dipyridyl leads to violet dyestuffs, not only in an acid solution



I IIa IIb IIc IId

I. γ, γ'-Dipyridyl.

IIa. Cation of a biquaternary compound of I (viologen).

IIb. The semiquinoid form obtained by partial reduction of IIa (E is an electron).

IIc and IId. Two tautomeric formulas of the second step of reduction; IIc is a double ammonium radical, the nitrogen atom being apparently "quadrivalent."

as is the case for free γ,γ'-dipyridyl, but also in alkaline solution. In this case sodium hydrosulfite can be used as reductant. The violet dye prepared by partial reduction of benzylviologen was isolated in crystalline form by E. Weitz. This is that particular semiquinoid compound in which he succeeded in establishing the molecular weight as mentioned above, by the boiling point method. He found it to be compatible only with the semiquinoid radical formula. It will be clear now even more so than above that this determination was favored by unusual circumstances. In order to obtain correct results by this method, the condition must be fulfilled that the semiquinone, dissolved in the solvent, undergoes no dismutation according to equation 4a. Such a possibility was not even considered by Weitz. However, the potentiometric method confirmed this result.

The titration experiment obtained by reducing the viologens (42) with sodium hydrosulfite showed potentials of a fairly stable nature (see table 3).

TABLE 3

Normal potential of viologens

(Independent of pH) at 30°C., referred to the normal hydrogen electrode

VIOLOGENS	NORMAL POTENTIALS
	volts
Methylviologen	−0.446
Ethylviologen	−0.449
Betaineviologen	−0.444
Benzylviologen	−0.359

The very slight drift of these potentials amounting to 0.1 to 2.0 millivolts per minute did not cause any serious trouble in the characterization of the titration curve obtained and left no doubt that the violet dye is a semiquinone of the same molecular size as the colorless viologen itself. The normal potentials are listed in table 3 and were independent of pH within the range accessible to experimentation, pH about 8 to 12. This range is limited, owing to the very negative level of the potential. For methylviologen, e.g., the normal potential approximately equals the potential of the hydrogen electrode (one atmosphere pressure of hydrogen) at the same pH. This normal potential, being independent of pH, therefore is in the hydrogen overvoltage range at pH < 7, and for this reason not suitable for a titration experiment in an acid solution. But even in distinctly acid solution viologen will be reduced by very strong reductants such as chromous chloride, providing that there is absent a catalyst such as colloidal palladium, which would immediately break down any hydrogen overvoltage potential. The viologens can therefore be used as oxidation–reduction indicators, even in a hydrogen overvoltage range, and are unique in this respect. As to the cause of the slight drift of the potential, the writer was inclined to the assumption that it is due to unavoidable minute traces of oxygen, even in the purified nitrogen used during the titration experiment, but now favors rather the assumption that the semiquinoid radical is not a perfectly stable molecule. This idea is suggested by the fact that γ,γ'-dipyridyl itself when titrated in an acid solution by chromous acetate shows very drifting potentials. Furthermore, that viologens containing long carbon chains as side chains always show strong drifts during the titration, and obviously furnish very labile reduction products. This was especially observed (unpublished experiment) in the following case: bromotetraacetylglucose, which, according to Emil Fischer (16), adds to pyridine to form a quaternary base, can also be attached to γ,γ'-dipyridyl. This compound can easily be freed from its acetyl groups. So, a glucose-viologen (or rather a glucosylviologen) is obtained which on reduction in alkaline solution also produces a blue dye. This, however, is much more labile than the others. Another very labile semiquinone is the one obtained by reduction of hydroxyethylviologen, prepared from γ,γ'-dipyridyl and iodoethyl alcohol. So it may be justifiable to assume that as to the lability of the semiquinone there is only a quantitative gradation according to the nature of the side chain.

The second step of the titration leading from the violet dye to the compounds of the oxidation level of dihydrodipyridyl cannot be followed potentiometrically. In the case of benzylviologen this second step of oxidation is practically insoluble, and so when the first step of titration has been completed, a continuation of the reduction will not change the potential any more. In the case of methylviologens and the others, the second step of reduction lies in too high a hydrogen overvoltage to be manageable.

(*h*) *The indigo group.* Willstätter some years ago drew the writer's attention to the fact that indigo dissolved in organic solvents, under certain conditions on reduction shows an intermediate red color. Shaffer found that the indigosulfonates when dissolved in a strongly alkaline aqueous solution, on reduction pass through a cherry-red intermediate state. This has been published so far by Preisler and Shaffer only in the mimeographed abstracts of the meeting of the American Chemical Society.[8] I owe more information about his experimental data to a personal communication and can confirm these authors' main results by my own experiments. When indigodisulfonate is reduced at pH up to about 10, ordinary potential curves are obtained as in most organic dyestuffs, as has been al-

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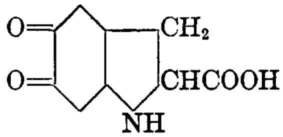
[8] Meeting held in Chicago, April, 1933.

ready published by Sullivan, Cohen, and Clark (50). When the dye is reduced at pH > 10.5, a new phenomenon arises that escaped the attention of the latter authors. An intermediate red color is developed, and at the same time the shape of the titration curve is distorted so as to indicate a two-step reduction. The intermediate form may be either a bimolecular meriquinone, or a semiquinone; in this case there is even a third possibility. Considering the formula of indigo which consists of two perfectly symmetrical halves held together only by a double bond between two carbon atoms, one may be inclined to consider the intermediate form as a radical generated by dissociation of the molecule into two halves by breakage of the bond between the carbon atoms. However, two observations can be pointed out which are compatible only with a semiquinone of the same molecular size as indigo itself. The first, shown by Loeffel in Shaffer's laboratory (36), is this: The semiquinones of the indigo dyes have a distinct absorption spectrum consisting of one single distinct band different for the mono-, the di-, the tri-, and the tetra-sulfonate. Now, if the intermediate form were a molecule of half molecular size, in the case of the trisulfonate the spectrum of the purple radical would be an overlapping of that of disulfonate and the tetrasulfonate radical. This is, however, not the case; the trisulfonate compound has a spectrum of its own. I may add another reason. The shape of the titration curve even at such a pH where the step formation is distinct, is independent of the initial concentration of the dye. This is, as stated above, compatible only with the assumption that the intermediate form has the same molecular size as the oxidized or the reduced one.

As for the details, I do not wish to anticipate Shaffer's publication, but only to state the main features as I could see them from some preliminary experiments of my own. The three normal potentials can be plotted in the same way as shown above for α-oxyphenazine. The common crossing-point of the three normal potentials lies for indigodisulfonate at about pH 11. Around this pH the slope of E_m is a 0.03 one, that of E_1 is a 0.06 one, and that of E_2 is a 0.00 one. Whereas for oxyphenazine E_1 is more negative than E_2 at pH smaller than that of the crossing-point, this is the case for indigo at pH greater than that of the crossing-point. This involves the fact that the thermodynamic condition of existence is improved with increasing pH, in contrast to the case of the phenazines. This is reminiscent of the behavior of quinhydrone, as discussed above, and suggests the idea that the indigo radical is an anionic one. In order to account for such an assumption, one would have to assume that leucoindigo, like hydroquinone, contains two hydrogen atoms of a weakly acidic character. The hydrogen atoms of the sulfonic groups can play no rôle in this respect, as they are very strongly acidic. It is likely that the two hydrogen atoms attached to indigo on reduction are slightly acidic, and in this respect complete the analogy with those two hydrogen atoms of hydroquinone which are detached on oxidation.

On increasing the pH beyond 12 the stability of the system is impaired. The drift of potential is, however, not large enough to prevent the establishment of approximate titration curves and estimations of the index potentials. From these it can be seen that the E_1 and E_2 curves first diverge from the E_m curve after the common point of intersection. Thereafter they appear to converge again so as to intersect once more. We cannot yet be quite sure about this assertion. The instability of the system at pH > 12, involving drifts of the potentials, may lead to erroneous conclusions. If the second intersection of the curve be real, it can, in the writer's opinion, be only explained by a dissociation constant of the semiquinone, pK about 12. Here the case seems to be realized, referred to above, that the existence of the semiquinone is restricted to a limited, rather narrow pH range.

(*i*) *Hallachrome.* A case of semiquinone formation quite unique as yet has been recently described. Mazza and Stolfi (36a) isolated from a marine worm of the gulf of Naples, a polychaete, named *Halla parthenopea*, a dyestuff, hallachrome, to which they ascribe the formula



It can be reversibly reduced to a leuco compound which is slightly yellow. E. A. H. Friedheim (20) subjected this dye to a potentiometric investigation. Oxidative or reductive titrations give stable and well reproducible potentials between pH 0.19 and about 10.0. Throughout this whole pH range any titration curve at constant pH is too steep for a two-electron system and too flat for a one-electron system. The curve has throughout this pH range an index potential of 20 millivolts, varying no more than within the limits of error. At pH > 10 the system begins to become labile, but it can be recognized that the index potential becomes > 20 millivolts. The formation constant K of the semiquinone, can be accordingly calculated to be 0.56, and the difference, $E_2 - E_1 = -18$ millivolts (the limits of error estimated ± 6 millivolt), throughout the pH range 0.1 to 10. The negative sign corresponds to the fact that $K < 1$. The maximum amount of semiquinone capable of existence in equilibrium with the other forms of the dye is as little as about 10 per cent or so. For this reason the pure color of the semiquinone is never to be observed, but only a mixed color.

The oxidized form is red at pH < 8.53, and green at pH > 8.53. Why at this pH the E_m curve shows no bend is not clear and needs further investigation. The slope of the E_m curve (and also E_1 and E_2) is 0.06 volt per pH unit. The change of color on oxidizing the reduced form is yellow–brown–red, or yellow–brown–green, according to the pH. The potential range of this dye is very close to that of pyocyanine.

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