RECENT STUDIES ON REVERSIBLE OXIDATION-REDUCTION IN ORGANIC SYSTEMS

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INTRODUCTION

Previous to 1920 there had been several attempts to extend potentiometric methods to the study of organic, oxidation-reduction systems. Abegg, Auerbach, and Luther in their practically complete summary of potential measurements mention a few such studies which had found their way into the literature up to 1915. But significant as they are they are not comparable in quality with the more exact of the measurements on inorganic systems and they form but a diminutive body of data compared with the inorganic. Yet it must have been obvious from the first that if reversible cells could be constructed with organic solutes their unlimited variety could be made the means of attacking numerous fundamental problems. Why then had there been so little success?

Such a question can be answered adequately only by one who is familiar with those unpublished discussions which I believe must have enlivened the laboratories of a generation or so ago. I can only suggest some possible reasons for the scarcity of published data.

For one, the attractiveness of preparing organic compounds by electrolytic processes inevitably diverted attention from equilibrium states to a variety of dynamical problems of seemingly more direct practical importance. Thus, even the study by Haber and Russ (1904), which is often quoted now that the equilibrium data for the quinone-hydroquinone system have become of great interest, was more or less incidental to a study of a problem in electrolysis.

Second, the failure to obtain equilibrium potentials with certain organic systems burdened the whole subject with an incubus which might equally well have arisen from inapt choice of inorganic material. The signal success of potentiometric methods applied to metal-metal ion systems has not been paralleled by an equal success of such methods applied to many common inorganic oxidation-reduction systems.

Third, requirements for the stability of most inorganic systems and for the action of many reagents used for the oxidation or reduction of organic compounds led in general to familiarity with strongly acid and strongly alkaline solutions. It required the necessities of the living cell to force upon the biochemist familiarity with the intermediate range of hydrion concentrations. Then there arose a general appreciation of those controlled conditions which we shall see are essential to the potentiometric study of many organic systems.

Fourth, a mistaken concept was responsible for failures with certain organic systems which we now know are amenable to potentiometric measurement. In some of the older papers there will be found attempts to measure the potential-difference between an electrode and a solution containing what was presumed to be the oxidant alone or the reductant alone. We shall see that in such a case the potential-difference becomes indefinite and that any approach to constancy indicates a finite ratio between oxidant *and* reductant. The heroic efforts to measure the potential of a pure solution of a reductant are evidence of the tenacity of a preconception which demands explanation. Clark (1922, pages 255–256) suggests it was "due to the emphasis which had been placed upon the final, working form of the equation for the difference of potential between a metal and a solution of its ions." In obtaining the final form of this equation certain assumptions have been made and the potential difference is made to appear as if it were dependent only upon the concentration of *one species*, namely, the metal ions.

Last, and perhaps most important of all the reasons for scarcity of data, is the difficulty which will be briefly discussed at the conclusion of this article.

I shall not attempt to review the older literature. Much of it is valuable but requires detailed discussion to develop the points of interest. That part of the recent work to which I shall for the most part confine this review admits concise formulation.

In 1920 three groups of investigators quite independently of one another published upon the problem which had long been neglected.

The dissertation of Granger, a student of Nelson (cf. Granger and Nelson, 1921) dealt with equilibrium potentials of quinone and hydroquinone in equilibrium with quinhydrone, demonstrating a good stability and reproducibility of potentials in acid solutions and data in fair agreement with the van't Hoff isotherm.

At the same time Clark (1920) brought to the problem the modern methods of hydrion control which greatly simplify the experimental segregation of variables. The oxidants and reductants were kept at concentrations relatively very small compared with those of a buffer system and thus the ratio of oxidant to reductant could be varied with almost negligible variations of acid-base dissociation. Measurements were then repeated at various pH values to reveal the effects of acidic or basic dissociations of reductant and oxidant. Thus there were obtained preliminary data on methylene blue-methylene white mixtures and on the mixtures of an indigo sulphonate with its reduction product.

The effect of hydrion concentration upon the electrode potential-difference was Biilmann's (1920) chief theme in another paper on the quinone-hydroquinone system. Since quinhydrone in acid solutions furnishes a definite ratio between oxidant and reductant, and since the reductant is a diacidic acid, it follows (as will be shown in detail later) that an electrode in contact with a quinhydrone solution exhibits a potential-difference which in acid, buffer solutions is a linear function of pH. Therefore, as Biilmann demonstrated, the "quinhydrone electrode" can be used for the determination of pH values in acid, buffered solutions.

Since the publication of these three independent papers in 1920 there have been many interesting developments. LaMer and Baker, Conant and his coworkers, Biilmann and his coworkers, the investigators at this laboratory and several others whose important contributions I shall discuss have been developing the subject rapidly.

I shall illustrate experimental methods by means of only one case which will suffice to show the more important principles. Then I shall outline one of several procedures for the development of equations. With a convenient method of formulating the somewhat complex relations which will be encountered we can proceed rapidly. We shall not only gain a better understanding of how it is that accurate data on organic systems are now being obtained but we shall also see that the potentiometric methods are furnishing precise data on free energy relations, opening new methods of analysis, broadening the methods of determining hydrion concentrations, aiding in the solution of problems in structural chemistry, furnishing valuable data on the effects of substitution, and suggesting new approaches to fundamental problems of biological oxidation-reduction.

EXPERIMENTAL METHOD

So many experimental procedures are available that I shall attempt only to illustrate main principles by a specific case.

Let it be proposed to determine the oxidation-reduction characteristics of indigo carmine.

In the next section it will be made clear by means of equations that the potential-difference between an electrode and a solution containing indigo carmine and its reductant, leuco indigo carmine, is determined both by the ratio of the oxidant to reductant and by the hydrion concentration. Therefore it simplifies the problem if in one case the hydrion concentration is kept constant while the ratio of oxidant to reductant is varied and in the other case the ratio is kept constant while the hydrion concentration is varied.

The hydrion concentration can be kept practically constant by means of buffers if the dye which is added be kept at relatively very low concentration.

A buffer solution is drawn into the bulb S of figure 1 making liquid junction there with the saturated KCl-solution of the calomel half-cell C. The vessel A is rinsed with fresh buffer solution and put into place with 50 cc. of buffer solution. This is then deaerated with a stream of oxygen-free nitrogen in order to prevent air-oxidation of the leuco indigo carmine which will presently be added.

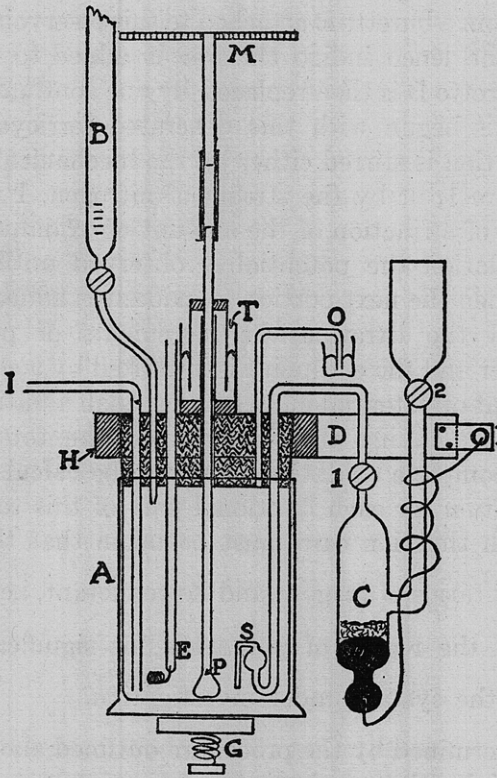

Fig. 1. Electrode Vessel

There will now be determined the effect on the gold electrode E of variations in the ratio of indigo carmine to leuco indigo carmine. Several procedures are available. Separate solutions of oxidant and reductant prepared in deaerated samples of the same buffer as that contained in A can be added to A in measured amounts. This is called the method of mixtures. The reductant can be added to A and titrated with an oxidizing agent, or the oxidant can be added to A and titrated with a suitable reducing agent. Let us titrate the reductant.

The indigo carmine is reduced by hydrogen and platinized asbestos. The reduced solution is then forced through a filter into a reservoir which has been washed with pure nitrogen and there residual hydrogen is blown out by a vigorous stream of nitrogen. From a burette B attached to this reservoir a measured quantity of the leuco indigo carmine is added to the buffer in vessel A. Burette B is then replaced by one containing K_3FeCy_6 and titration is begun with this deaerated ferricyanid solution while the solution is stirred either by the mechanical stirrer MP, shown in figure 1, or by the stream of nitrogen, I to O. After each addition of a fraction of the amount of oxidant required for complete oxidation the potential is observed until it becomes constant. Then the next portion of oxidant is added. Near the completion of the titration the increments of potential will become greater and the end-point is indicated by a comparatively large increment of potential induced by a small additional amount of ferricyanid. Having thus determined the total ferricyanid required for complete oxidation there can be calculated the percentage oxidation for each fractional part of this total.

In any such titration care must be taken that the potentials for significant ratios of reagent and its reductant, i.e., $\frac{\text{ferricyanid}}{\text{ferrocyanid}}$ do not lie in the region of potentials for significant ratios of $\frac{\text{oxidant}}{\text{reductant}}$ of the system under investigation.

Having determined by the procedure outlined above the variation of potential with variation in the ratio of reductant to oxidant at constant pH, the experiment can be repeated in buffers of different pH. Or there can be added to buffers of different pH a fixed mixture of oxidant and reductant. This second set of experiments reveals the effect of variation of pH at constant ratio of oxidant to reductant.

It is sometimes advantageous to employ a hydrogen electrode in the same buffer mixture as that employed for the oxidation-reduction system and to substitute this for the calomel half-cell C. We shall see in the next section the conditions under which such a chain gives the same E.M.F. independent of the buffer used.

It is significant that in practically all the cases examined the same results have been obtained with electrodes of platinum, gold, gold-plate, thin coats of platinum deposited on glass, "rhotanium" alloy and pure mercury. However, slight differences between electrodes of the same material are sometimes found and it is always best to use two.

The vessel A and calomel half-cell C are arranged so that they can be immersed in a constant-temperature oil-bath and are rigidly joined to the same support HD to lessen the danger of disturbing the liquid-junction in S.

If the liquid-junction can be made in a reproducible manner it is safe to neglect the small, residual potential-difference since it cancels in most of the comparative data.

There being no way to measure directly the pH of buffer + dye, the dye and titrating agent are kept at very low concentration in solutions of the same buffer composition and the pH of this buffer as measured with the hydrogen electrode is assumed to be the same as the mixture with corrections which can be made when the dissociation constants of the added materials have been determined.

Potential differences are measured with a potentiometer. Standards and precautions are discussed in The Determination of Hydrogen Ions (Clark, 1922) and in other texts.

EQUATIONS AND SOME TYPICAL APPLICATIONS

For the sake of conciseness it will be well to maintain a uniform procedure in developing the equations we shall have to employ. Several devices are now in use. They all lead to the same final working equations. This is evidence not only of lack of agreement upon an explanation of electrode mechanism but also of the fact that the devices postulated are to the actual mechanism like a convenient staging to the survey and construction of a building. When the final equations are built the staging disappears. There is left a structure of thermodynamic relations but their verification tells nothing *directly* of the mechanisms involved.

For this reason some writers prefer to throw the components of a cell reaction directly into an electromotive force equation; but postulated mechanisms still have their uses.

Recognizing this it has seemed to me that the procedure outlined in the second edition of The Determination of Hydrogen Ions, chapter 16 (Clark 1922)[1] is somewhat more useful to the present purposes than some of the other procedures since it involves a device of considerable aid to the maintenance of orientation amid the complexities to be encountered.

We shall assume that in formulating the equilibrium state of an oxidation-reduction system we can include an electron activity a_{es} as in equation (1).

$$a_{es}^{n} = K \frac{a_r}{a_o} \tag{1}$$

Here n is the number of electrons required for the transformation of oxidant of activity a_o to reductant of activity a_r.

Let us now immerse an electrode, the material of which (e.g., platinum) does not readily enter into equilibrium with the materials of the solution. If a_{em} is the intrinsic electron activity of the "unattackable" electrode, there will be established a difference of potential E between electrode and solution in accordance with the equation

$$E = C - \frac{RT}{F} \, ln \frac{a_{es}}{a_{em}}$$

Ignoring the complex problem (involving the Volta effect) of why it is that the equations applicable to working conditions make it *appear* as if a_{em} were a constant, let us so regard it. Then

$$E = C_2 - \frac{RT}{F} \, ln \, a_{es} \tag{2}$$

Equation (2) we shall call our fundamental electrode equation.

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[1] Compare Clark and Cohen, 1921 and 1923.

Substituting (1) in (2) we have

$$E = C_3 - \frac{RT}{nF} \ln \frac{a_r}{a_o} \tag{3}$$

Equation (3) with an important interpretation presently to be noted is the general form for an oxidation-reduction system; but we must now adopt a standard of reference for electrode potential-difference. That of the so-called normal hydrogen electrode will be used.

Applying (1) to the reaction $2H^+ + 2e \rightleftharpoons H_2$ and substituting in (2) we have

$$E = C_4 - \frac{RT}{F} \ln \frac{\sqrt{a_{H_2}}}{a_{H^+}}$$

or using hydrogen pressure, P,

$$E = C_5 - \frac{RT}{F} \ln \frac{\sqrt{P}}{a_{H^+}}$$

By definition in the commonly accepted convention regarding the normal hydrogen electrode, $E = 0$ when $P = 1$ atmosphere and $a_{H+} = 1$. (The normal hydrogen electrode.)

Then C_5 is zero and the cell

$$\begin{array}{l|l|l} \text{normal} & \text{oxidant} & \\ \text{hydrogen} & & \text{Pt} \\ \text{electrode} & \text{reductant} & \end{array}$$

has an E.M.F. equal to E of equation (3).

We shall consistently use the normal hydrogen electrode standard of reference, and shall designate single potential-differences so referred by the symbol E_h.

In giving signs to single potential-differences we shall call a single potential difference "+" when, if the half-cell A involved were joined by liquid junction to a normal hydrogen electrode half-cell, the metal of the half-cell A would appear to be positive to the metal of the normal hydrogen electrode.

Now having chosen, for purposes of schematic presentation, to orient the relation of reductant to oxidant as a difference of electrons we shall have to be consistent, and when dealing with particular cases in which we employ oxidants and reductants which can dissociate we shall have to carefully identify the species used as oxidant and reductant in equation (3).

There are many possible cases, as

$$\begin{gathered}\mathrm{Ox} + \mathrm{e} \rightleftharpoons \mathrm{R\overset{-}{e}d} \\ \overset{+}{\mathrm{O}}\mathrm{x} + 2\mathrm{e} \rightleftharpoons \overline{\mathrm{Red}} \\ \mathrm{Ox} + 2\mathrm{e} \rightleftharpoons \overset{-\,-}{\mathrm{Red}} \\ \text{etc.}\end{gathered}$$

In the last case we identify the reductant as the anion of a diacidic acid. Such identification is the key to the formulation of effects due to alteration of hydrion activity.

To simplify the next steps we shall temporarily regard activities to be proportional to concentrations. Thus, if we continue with the case under discussion we have:

$$E_h = C - \frac{RT}{nF}\, ln \frac{[\overset{-\,-}{\mathrm{Red}}]}{[\mathrm{Ox}]} \tag{4}$$

Also we have the sum (5) of all forms of reductant and the dissociation equilibrium equations (6) and (7).

$$[S_r] = [\overset{-\,-}{\mathrm{Red}}] + [\mathrm{H\overline{R}ed}] + [\mathrm{H_2Red}] \tag{5}$$

$$\frac{[\mathrm{H\overline{R}ed}]\,[\mathrm{H}^+]}{[\mathrm{H_2Red}]} = K_1 \tag{6}$$

$$\frac{[\overset{-\,-}{\mathrm{Red}}]\,[\mathrm{H}^+]}{[\mathrm{H\overline{R}ed}]} = K_2 \tag{7}$$

Combining (5) (6) and (7) and substituting in (4) we obtain (8) where constants that can properly be assembled are united in E_o.

$$E_h = E_o - \frac{RT}{2F}\, ln \frac{[S_r]}{[S_o]} + \frac{RT}{2F}\, ln\, [K_1K_2 + K_1[\mathrm{H}^+] + [\mathrm{H}^+]^2] \tag{8}$$

In (8) we have let $[S_o]$, the concentration of total oxidant, be identical with [Ox] of (4).

A series of similar hypothetical cases with their characteristic last terms are tabulated and illustrated by Clark and Cohen (second paper, Studies on Oxidation-Reduction).

Equation (8) shows clearly the conditions for experimental segregation of the effects of the independent variables. If oxidant and reductant are kept at such low concentration that they have a negligible effect upon the hydrion concentration of a buffer solution, and if [H+] is kept at a definite value by this buffer solution, the last term of equation (8) becomes a constant for the given condition and (8) becomes (9).

TABLE 1

Titration of leuco o-cresol-indophenol with ferricyanid at pH 8.65. 30°.

K_3FeCy_6	OXIDATION	$0.03006 \log \frac{[Sr]}{[So]}$	Eh OBSERVED	E'_0	E'_0 CORRECTED	DEVIATION FROM +0.0837
cc.	*per cent*		*volts*			
1	4.31	+0.0405	+0.0432	+0.0837	0.0836	−0.0001
2	8.62	+0.0308	0.0526	0.0834	0.0833	−0.0004
3	12.93	+0.0249	0.0587	0.0836	0.0834	−0.0003
4	17.24	+0.0205	0.0633	0.0838	0.0835	−0.0002
5	21.55	+0.0169	0.0671	0.0840	0.0836	−0.0001
6	25.86	+0.0137	0.0702	0.0839	0.0835	−0.0002
7	30.17	+0.0110	0.0732	0.0842	0.0836	−0.0001
8	34.48	+0.0084	0.0759	0.0843	0.0836	−0.0001
9	38.79	+0.0059	0.0785	0.0844	0.0836	−0.0001
10	43.10	+0.0036	0.0809	0.0845	0.0837	0.0000
11	47.42	+0.0014	0.0834	0.0848	0.0839	+0.0002
12	51.73	−0.0009	0.0858	0.0849	0.0839	+0.0002
13	56.04	−0.0032	0.0882	0.0850	0.0839	+0.0002
14	60.35	−0.0055	0.0904	0.0849	0.0837	0.0000
15	64.66	−0.0079	0.0929	0.0850	0.0837	0.0000
16	68.97	−0.0104	0.0955	0.0851	0.0838	+0.0001
17	73.28	−0.0132	0.0984	0.0852	0.0838	+0.0001
18	77.59	−0.0162	0.1016	0.0854	0.0840	+0.0003
19	81.90	−0.0197	0.1054	0.0857	0.0842	+0.0005
20	86.21	−0.0240	0.1097	0.0857	0.0841	+0.0004
21	90.52	−0.0295	0.1154	0.0859	0.0843	+0.0006
22	94.83	−0.0380	0.1242	0.0862	0.0845	+0.0008
23	99.14	−0.0620	0.1486	0.0866	0.0848	+0.0011
23.2	100					

$$E_h = E'_o - \frac{RT}{2F} \ln \frac{[S_r]}{[S_o]} \tag{9}$$

For example, Cohen, Gibbs and Clark (1924) give the data of table 1 for the titration with ferricyanid of leuco o-cresol indophenol. The temperature was 30° and hence equation (9) becomes

$$E_h = E'_o - 0.03006 \log \frac{[S_r]}{[S_o]} \tag{10}$$

Since in this case, even with buffer, there is an appreciable change of acidity during the transformation, there is applied in the next to last column of table 1 a correction experimentally determined. The resulting constant is quite satisfactory as shown by the deviations in the last column of table 1.

LaMer and Baker (1922) have published some accurate data for cases to which equation (9) applies. Their data illustrated by figure 10, page 164 will be referred to again. Another illustration of the typical curves given by equation (10) are shown in figure 2, together with experimental data for three of the indigo sulphonates studied by Sullivan, Cohen and Clark (fourth paper, Studies on Oxidation-Reduction). In this figure the curve for the monosulphonate was located by means of experimental data obtained at other values of pH.

These cases will suffice to show the conformity of experimental data with equation (9) which is applicable only when the hydrion concentration is constant.

We shall next consider the methods of completing the data for equation (8) which involves the variable $[H^+]$.

In equation (8) if $\frac{[S_r]}{[S_o]} = 1$ we have (11).

$$E_h = E_o + \frac{RT}{2F} \ln [K_1K_2 + K_1 [H^+] + [H^+]^2] \tag{11}$$

But we have already seen that at any particular value of $[H^+]$ the mid-point of a titration curve is the condition that $\frac{[S_r]}{[S_o]} = 1$. Consequently by repeating the first series of measurements in

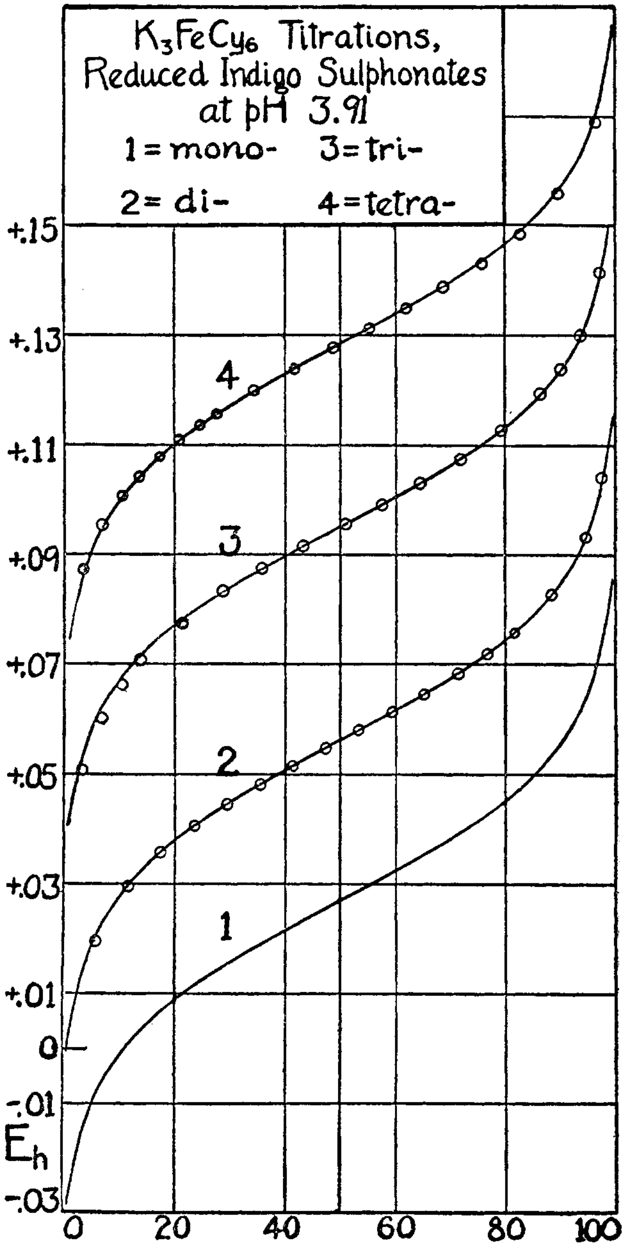


FIG. 2. RELATION OF ELECTRODE POTENTIAL, E_h (ORDINATE) TO PERCENTAGE OXIDATION (ABSCISSA)

buffers of different hydrion concentration we obtain the data to solve (11). Involving fewer sources of experimental error is the method in which a *fixed* mixture of total reductant and oxidant is added to a series of buffers of various pH values. This fixed mixture need not be that of $\frac{[S_r]}{[S_o]} = 1$ since the previous set of measurements has determined E_0' for a given value of $[H^+]$. It is necessary, however, for the purpose of orientation, that this

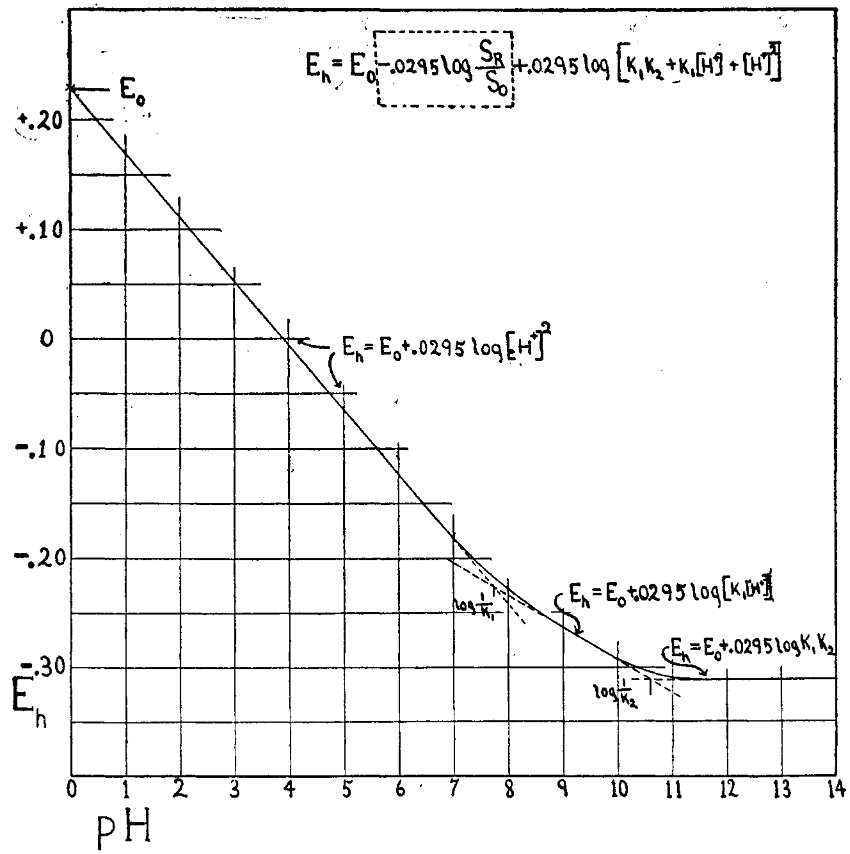


FIG. 3. VARIATION OF POTENTIAL WITH pH WHEN TOTAL OXIDANT AND TOTAL REDUCTANT ARE KEPT CONSTANT AT $\frac{S_r}{S_o} = 1$

Anthraquinone, 2,7-disulphonic acid and its reductant at 25°

second set of measurements include one in which $[H^+]$ is the same as in the first set.

If now either K_1 or K_2 comes within the experimental range of $[H^+]$ it can be measured. This is best shown graphically by figure 3 drawn with the aid of data for 25° on anthraquinone, 2,7-disulphonic acid supplied by Conant, Kahn, Fieser and Kurtz (1922).

These authors give $K_1 = 2 \times 10^{-8}$ and $K_2 = 3 \times 10^{-11}$. It is obvious that at high values of $[H^+]$ (low pH) both K_1K_2 and $K_1 [H^+]$ are negligible. Consequently the curve has a slope $\frac{-dE}{dpH} = 0.059$. When $[H^+] = K_1$ and $\log \frac{1}{K_1} = pH$, the curve is midway in its inflexion to the section where K_1K_2 and $[H^+]^2$ are negligible compared with $K_1 [H^+]$. Consequently the slope characteristic of this section is $\frac{-dE}{dpH} = \frac{0.059}{2} = 0.0295$. When $[H^+] = K_2$ and $\log \frac{1}{K_2} = pH$ the curve is midway in its inflexion to the section where $K_1[H^+]$ and $[H^+]^2$ are negligible compared with K_1K_2. There $\frac{-dE}{dpH} = 0$.

Conversely the intersections of projections of the several sections of the curves fall at pH values corresponding numerically with what are called the pK_1 and pK_2 values, namely and respectively $\log \frac{1}{K_1}$ and $\log \frac{1}{K_2}$.

In figure 4 are the E_0':pH curves of the indigo sulphonates for which the E_h: % oxidation curves are shown in figure 2. Some discrepancies to be noted in the more alkaline solutions were traced to a specific effect of borate buffers. (See the original paper.)

It will be noted that within the experimental range of pH covered by the curves for the sulphonated anthraquinone and the sulphonated indigos there is no sign of the influence of dissociation in the sulfonic acid groups. This is chiefly because no acidic group which is *common* to both oxidant and reductant makes its presence felt in such E_0':pH curves *unless* the "strength" of this group *changes* in the transformation of oxidant to reductant. In case this change does occur the equation can easily be developed by consistently following the plan outlined. (See Clark and Cohen, 1923.)

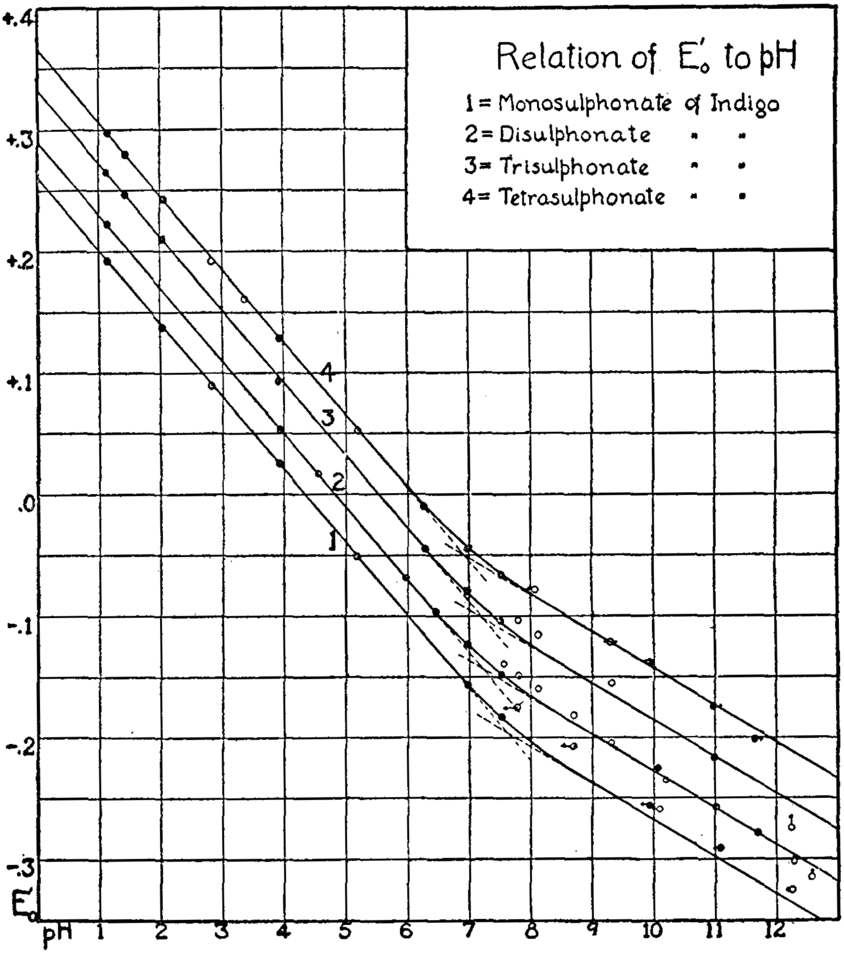
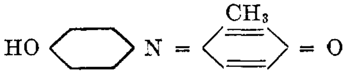
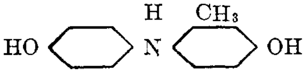


FIG. 4

Specific instances of this occurrence are found among the indophenols. For instance in the oxidant (I) and the reductant (II) of the methyl indophenol,


(I)


(II)

the dissociation constant of the common phenolic group shifts in value and there has to be taken into account both K_o of the oxidant and K_r of the reductant each applying to the same structural group. The displacement E-C and the intersections of

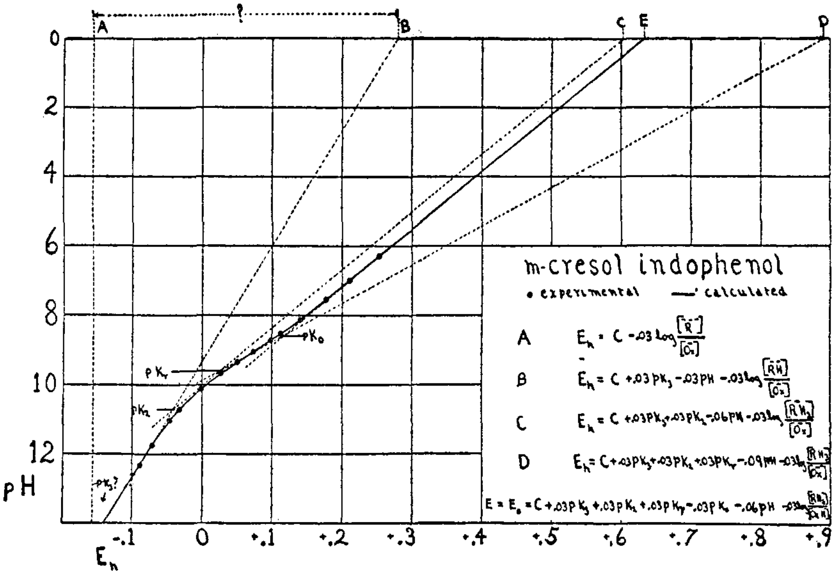


FIG. 5

D, E and C illustrated in figure 5 are used to determine the values of these constants.

In the case of the systems now under discussion the absence of any zero value for $\frac{-dE}{dpH}$ indicates that one hydrogen is fixed—presumably that on the bridging N of the reductant (II). Neglecting the inappreciable value of this dissociation and developing the equation in accordance with the scheme previously outlined we have, at the temperature (30°) of the measurements, equation (12) in which K_o is the dissociation constant of the oxidant, K_r the dissociation constant of the same group as it occurs in the reductant and K_2 is the dissociation constant of the phenolic group created by reduction.

$$E_h = E_o - 0.03006 \log \frac{[S_r]}{[S_o]} + 0.03006 \log [K_r K_2 [H^+] + K_r [H^+]^2 + [H^+]^3]$$
$$- 0.03006 \log [K_o + [H^+]] \qquad (12)$$

With the values

$$K_o = 2.8 \times 10^{-9}$$
$$K_r = 2.7 \times 10^{-10}$$
$$K_2 = 2.2 \times 10^{-11}$$
$$E_o = + 0.632$$

there is found the curve as drawn in figure 5. With this, the experimental data are shown to agree very well.

In this curve there are four sections for which the determining values of $\frac{-dE}{dpH}$ are in order from low to high pH: 0.06, 0.09, 0.06, and 0.03. The "0.09-slope" is not very distinct in this instance but is very distinct in the case of 2.6-dibromophenol indophenol.

With the constants characteristic of the 2.6-dibromophenol indophenol system Cohen, Gibbs and Clark (1924) have constructed the isometric figure 6 which illustrates the type of surface determined by equation (12).

The recognition of details such as those which reveal the "0.09-slope" in the indophenol system, and the use of a consistent method of formulating equations have been imperative prerequisites to the full appreciation of such remarkable cases as that of the methylene blue-methylene white system.

Here as shown by the E_o' : pH curve of figure 7 we have a situation which seems at first very difficult to analyze. However, with help from structural chemistry a satisfactory formulation is reached directly.

The Bernthsen formula for methylene blue is supported by a very beautiful series of syntheses. If we write it with conventional symbols for electronic configuration (figure 8) preserving the rule of eight and the rule of two (see Lewis, 1923) we observe that one of the terminal nitrogens in the oxidant should be "polar" and comparable with that of a substituted ammonium.

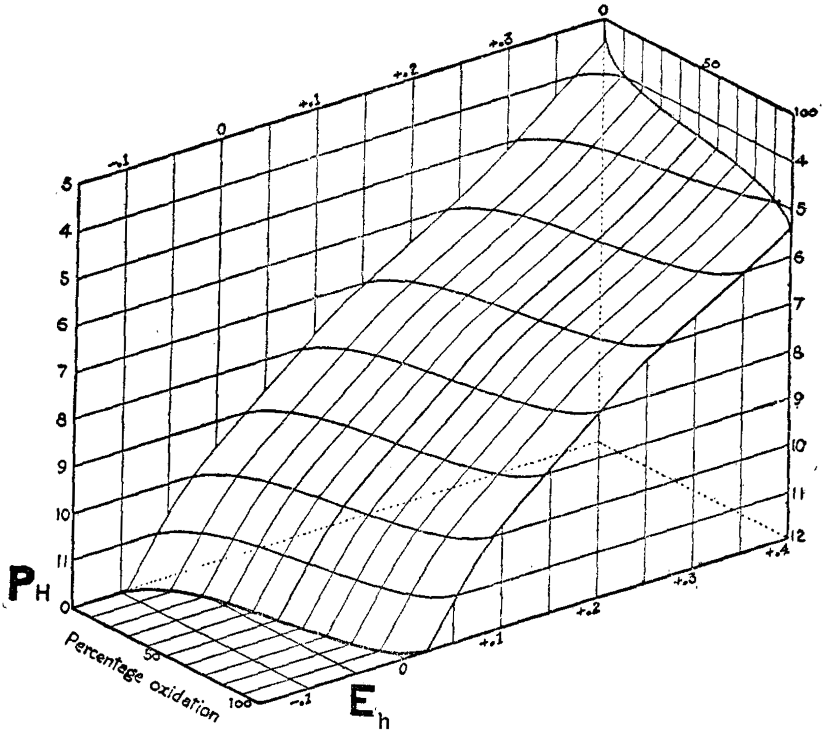


Fig. 6. Isometric Drawing of the Surface Descriptive of the System Composed of 2–6 Dibromophenol-indophenol and Its Reductant and [H+] at Different Values of pH

Now for the unmethylated analogue of methylene blue, Lauth's violet, we find an inflexion of the E_0':pH curve at pH = 11 (see fig. 7) indicating a strong basic group, $K_b = 10^{-3}$. By analogy with substituted ammoniums we should expect the introduction of methyl groups to increase the "strength" of the base. In methylene blue no inflexion of the E_o:pH curve occurs in the alkaline regions studied. If our reasoning is correct this experimental fact means that methylene blue base is comparable in "strength" to an alkali hydroxide and since the chloride suffers

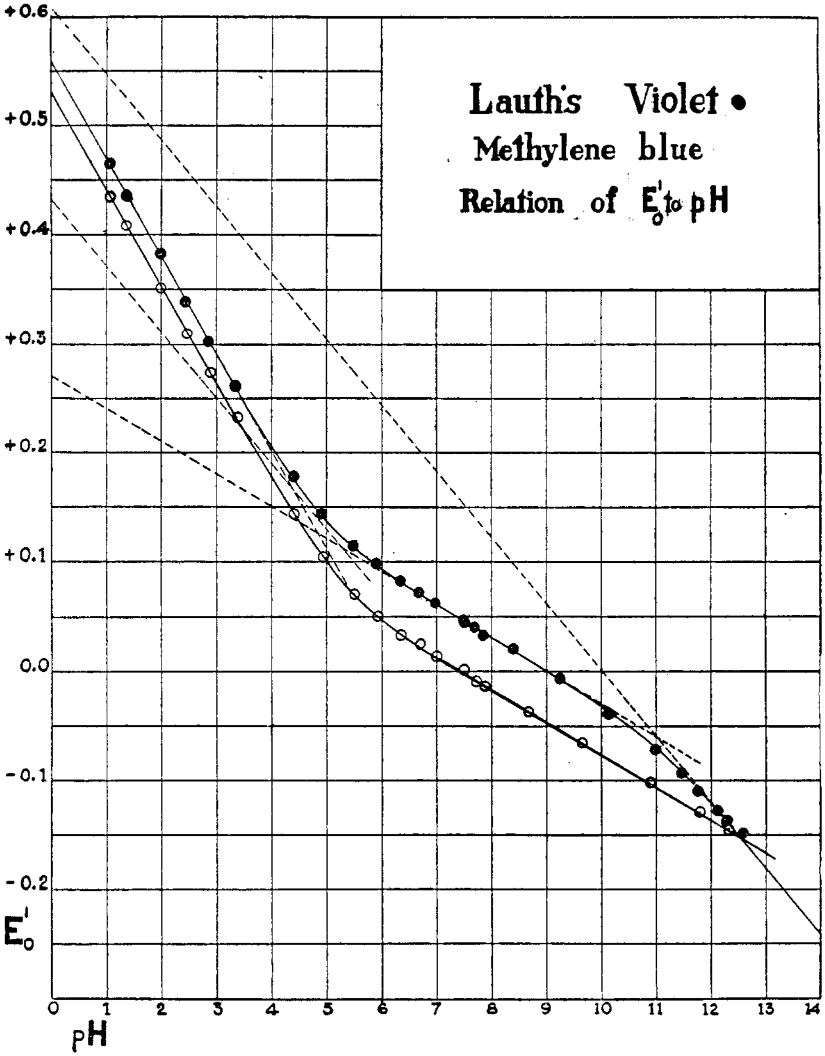


FIG. 7

no hydrolysis within the range of pH used, the constant eludes measurement. Abundant supplementary evidence of this is to be found in the literature.

Clark, Cohen and Gibbs (1925) starting with this interpretation and the orienting reaction

$$\text{Oxidant}\overset{+}{\text{ }}\text{cation} + 2e \rightleftarrows \text{Red}\overset{-}{\text{uc}}\text{tant anion}$$

were led, by the procedure described, directly to a satisfactory equation (see original paper) in accordance with which the curves of figure 7 are drawn.

Briefly the interpretation is as follows. The polar group of methylene blue is so strong that its K_b value is not measured; that of Lauth's Violet has a K_b value of 1.89×10^{-3} (inflexion at pH 11). The opposite dimethylamino group of methylene blue and amino group of Lauth's violet is each so weak that its K_b value is not measured. The convenient form of the reaction given above indicates that the reductant is an anion. At the point concerned, which may be assumed to be the bridging nitrogen, a hydrogen ion is bound so firmly that there is established a lower limit of 0.03 for $\frac{-dE}{dpH}$. At the same time the reduction, by destroying the polarity of the strong basic group has turned it into an ordinary substituted amino group which acquires weak basic properties either by addition of H^+ or by hydration and dissociation of $O\overline{H}$. The now symmetrically placed, substituted amino group common to oxidant and reductant has become of appreciable basic strength. These changes

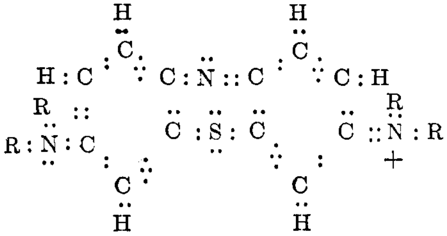


FIG. 8. CONVENTIONAL ELECTRONIC STRUCTURE OF A THIAZINE

listed in table 2, establish the not unique "0.09-slope" over a remarkably wide zone of pH.

These few illustrative examples have been treated in some detail in order to emphasize several matters of considerable importance.

In the first place it is the detailed working out of such specific cases that is a necessary prerequisite to the mastering of more difficult problems which are continuously arising.

In the second place it is alone the charting of specific cases that can give us a proper appreciation of the remarkable way in which alteration of pH affects the relation of one system to another.

TABLE 2

Ionization constants, centers of inflexion of E'_0:pH curves and characteristic potentials of Lauth's violet- and methylene blue systems at 30°

GROUP	LAUTH'S VIOLET		METHYLENE BLUE	
	Basic constant	Inflexion at pH	Basic constant	Inflexion at pH
Oxidant's polar	1.9×10^{-3}	11.0	Too high to measure	None
Oxidant's amino	Negligibly small	None	Negligibly small	None
Reductant's bridging N	Fixes H^+	None	Fixes H^+	None
Reductant's first amino	3.8×10^{-9}	5.30	1.35×10^{-8}	5.85
Reductant's second amino	4.5×10^{-10}	4.38	6.3×10^{-10}	4.52
E'_0, potential at pH = 0	+0.563		+0.532	

It has not yet been made clear to many writers that certain statements regarding the effect of acid or alkali upon oxidation-reduction can have no meaning unless the systems concerned are specified. Several well defined cases are now known in which one system having a reducing potential relative to a second system becomes oxidative with respect to the second on change in the pH of the solution and subsequently reductive again on further change in pH.

Third, the details reveal characteristic constants for each system and these are valuable in quantitative studies on substitution as will be shown in a later section.

DATA ON ENERGY CHANGES

It is well known that potentiometric measurements, when applicable, furnish precise data for certain energy changes.

Free energy changes involved in the organic reactions now under review are implicit in the equations we have been discussing. For instance let equation (8) be recast to the following form.

$$2FE_h = 2FE_o - RT\ ln \frac{[S_r]}{[S_o]} + RT\ ln\ [K_1K_2 + K_1\ [H^+] + [H^+]^2]$$

If K_1 and K_2 are small in relation to $[H^+]$ and if $[H^+] = 1$

$$2FE_h = 2FE_o - RT\ ln \frac{[S_r]}{[S_o]}$$

Since the use of E_h values implies the coupling of the half-cell with that of the normal hydrogen electrode, $2FE_h$ is the yield of free energy, or $- \Delta F$ in volt-coulombs, of the reaction:

Oxidant (1 molar) + H_2 (1 atmos) → Hydrogenated Reductant (1 molar)

Thus in the case of indigo carmine $E_o = + 0.291$ and consequently if the reaction proceeds in the direction indicated above there would be yielded under conditions of maximum work the free energy of 56.16 kilo joules or 13.413 mean gram calories.

When K_1 and K_2 are large in relation to $[H^+]$ and $\frac{[S_r]}{[S_o]} = 1$

$$2FE_h = 2FE_o + RT\ ln\ K_1K_2$$

Thus in the case of anthraquinone 2, 7-disulphonic acid (see figure 3), Conant, Kahn, Fieser and Kurtz (1922) give

$$K_1 = 2 \times 10^{-8} \quad K_2 = 3 \times 10^{-11} \quad \text{and } E_o = 0.229 \text{ at } T = 298$$

Hence RT $ln\ K_1K_2 = -103.9$ kilo joules, and $2FE_o = 44.2$ kilo joules.

Consequently the cell made up of the hydrogen half-cell and the oxidation-reduction half-cell (liquid-junction potential-differences being neglected) would run of itself in the direction

$$\text{Oxidant (1 molar)} + H_2 \text{ (1 atmos)} \rightarrow \text{Hydrogenated Reductant (1 molar)}$$

at $[H^+] = 1$ yielding 44.2 kilo joules; and in the opposite direction, namely

$$\text{Reductant anion (1 mol)} + 2H^+ \text{ (2 mols)} \rightarrow \text{Oxidant (1 molar)} + H_2 \text{ (1 atmos)}$$

when the oxidant and reductant are at high alkalinities then yielding 59.9 kilo joules.

There are valuable additional data which may be gained from a study of temperature effects. If the increase of potential with increase of temperature $\frac{dE_0}{dT}$ is determined, the Gibbs-Helmholtz equation,

$$-\Delta H = 2FE_0 - 2FT\frac{dE_0}{dT}$$

permits the calculation of the total energy change $-\Delta H$ and the latent heat

$$-T\frac{d(\Delta F)}{dT}.$$

Biilmann (1920) has thus found the total energy change for the hydrogenation of benzoquinone and toluquinone, Biilmann and Lund (1923) for the hydrogenation of alloxan and Conant and Fieser (1922) for several anthraquinones, naphthoquinones and quinones. The last authors find that, while anthraquinones differ markedly from quinones and naphthoquinones in their values of ΔF and ΔH, the latent heats of reduction are of the same order of magnitude for all of these systems.

There are thus being accumulated some fundamental data on energy relations which are far more precise than some of the existing thermal data. Incidentally we might note that the variation of free energy change with variation of the pH of the solution could not have been very easily taken into consideration in certain of the older types of measurement. The potentiometric methods reveal free energies of ionization and dilution of H^+

which are implicit in the equations given above but which have not been detailed in this brief sketch.

NEW METHODS OF DETERMINING pH

Any of the systems we have been considering may be used under proper conditions to determine pH. Consider for instance any case for which equation (8) holds.

$$E_h = E_o - \frac{RT}{2F} \, ln \, \frac{[S_r]}{[S_o]} + \frac{RT}{2F} \, ln \, [K_1K_2 + K_1 \, [H^+] + [H^+]^2] \qquad (8)$$

Let the ratio $\frac{[S_r]}{[S_o]}$ be unity and let attention be confined to the zone of hydrion concentration where $[H^+]$ is large in relation to K_1 and K_2. Then

$$E_h = E_o + \frac{RT}{F} \, ln \, [H^+]$$

or at 20°,

$$pH = \frac{E_o - E_h}{0.058}$$

Now quinhydrone in acid solution fulfills the conditions. The dissociation constants of hydroquinone are very low and are for present purposes negligible. The hydroquinone and quinone resulting from the dissociation of quinhydrone are fixed in ratio. Consequently $\frac{[S_r]}{[S_o]}$ is established. Furthermore air oxidation is slow in acid solution.

Developing his equations from a different point of view Biilmann (1920) realized the implications above described and devised his so-called quinhydrone electrode for the determination of pH. It is a very simple arrangement. A little solid quinhydrone is mixed with the solution to be tested and placed in a vessel such as is used for a calomel half-cell. A bright platinum electrode is then dipped into the mixture and liquid junction is made with a standard half-cell. Knowing the value of this standard and having measured the E.M.F. of the chain,

E_h is found at once. Then since E_o has already been established pH is determined.

The following are values of E_o at different temperatures:

AUTHOR	TEMPERATURE	E_0
Biilmann and Krarup (1924)	0	0.717
Conant and Fieser (1922)	0	0.713
Biilmann (1920)	18	0.704
Veibel (1923)	18	0.704
Biilmann (1920)	25	0.699
LaMer and Baker (1922)	25	0.699
Biilmann and Krarup (1924)	37	0.690
Conant and Fieser (1922)	40	0.682

Biilmann and Krarup propose the equation

$$\pi_r = 0.7175 - 0.00074t$$

where π_r is the value of our E_o at temperature t.

One advantage of the quinhydrone electrode is the rapidity with which the equilibrium potential is attained. Another advantage over the classic hydrogen electrode is this: The finely divided metal (platinum or palladium black) necessary to the operation of the hydrogen electrode is easily "poisoned" by many substances or else will so catalyze the reduction of a substance as to alter the system under study. In the quinhydrone electrode the hydrogen and the finely divided catalyst are eliminated. Its potential adjusts rapidly, very often much more rapidly than certain classes of reagents affect the constituents of the oxidation-reduction system. Consequently it is possible to use the quinhydrone electrode in solutions incompatible with the hydrogen electrode and even in systems which will ultimately oxidize hydroquinone. For instance Biilmann (1921) has employed it in dilute solutions of nitric acid, fumaric acid, etc., and a number of other investigators have found it useful in a variety of instances. (Compare Bodforss, 1922; Schreiner, 1922–1924; Larsson, 1922; Pring, 1924; Kolthoff, 1923; Harris, 1923; Darmois and Honnelaitre, 1924; Hugonin, 1924.) It has also been applied to the measurement of pH in soils (Biilmann, 1924) in milk (Lester, 1924) in beer (Kolthoff, 1923).

Its limitations in alkaline solution are two-fold. As equation (8) (page 136) will indicate, corrections have to be made for dissociation of hydroquinone. See LaMer and Parsons (1923). More serious is the oxidizing effect of air. LaMer and Rideal (1924) have made an interesting study of this. Compare Meunier and Queroix (1924).

Perhaps at this point it may be well to add a note to this subject. It is very proper that investigators who have dealt with organic systems have attempted to maintain conditions for constancy of electrode potential-differences over long periods of time. Thus Granger's dissertation presents an admirable study of long-time experiments on the quinhydrone electrode. On the other hand the evidence is growing that many of the organic systems, such as the quinone-hydroquinone system, give their significant and reproducible potentials much more rapidly than students of the hydrogen electrode and of metal electrodes are accustomed to, and that subsequent changes of potential are due to decompositions the effects of which should not be allowed to cloud the main issue. Now, while no one would care to depend in important cases upon initial potentials which immediately begin to drift, I would like to call attention to the fact that reasonable measurements in alkaline solutions may be made by first deaerating the solution, then adding the quinhydrone as a small amount of saturated solution and taking the initial potentials. Incidentally I have made fair estimates of the dissociation constants by this procedure.

In acid solutions the quinhydrone electrode has a good stability and Veibel (1923) has proposed its use as a standard which he finds to be as reproducible as his 3.5 N-calomel electrodes and more reproducible than his 0.1 N-calomel electrodes. On the other hand Sørensen and Linderstrøm-Lang (1924) have pointed out that since the permanence of the quinhydrone electrode is not equal to that of the calomel electrode the latter should remain standard.

In any case where the quinhydrone or a similar electrode is used to determine pH there must be taken into consideration the so-called "salt-effects." Because of its theoretical importance the salt-effect will be discussed in a separate section where treatment of the quinhydrone electrode will be continued.

EFFECTS OF SALT AND SOLVENT

It will have been noticed that in developing equations used to outline the main features of the subject we assumed that the ratio between activity of oxidant and activity of reductant could be replaced by the ratio of the concentrations. This distinctly limited the treatment to a first approximation. If persisted in, certain effects would be classified as "salt-errors."

Sørensen, Sørensen and Linderstrøm-Lang (1921) have examined such "errors" in the case of the quinhydrone electrode.

If we assume for simplicity that the quinhydrone electrode is being operated at constant hydrion activity in acid solution we have the equation

$$E_h = E_o - \frac{RT}{2F} \ln \frac{a_h}{a_q}$$

where a_h and a_q represent activities of hydroquinone and quinone respectively. Representing concentrations by C and activity coefficients by f we can write

$$E_h = E_o - \frac{RT}{2F} \ln \frac{C_h f_h}{C_q f_q}$$

For the special case where $\frac{C_h}{C_q} = 1$ as determined by quinhydrone in absence of excess of either constituent the ratio $\frac{f_h}{f_q}$ determines the potential. But this ratio should be determinable by means of solubilities. This the Danish authors did obtaining a good correspondence between potentials found at different salt concentrations and that calculated with the aid of the solubility data on activity coefficients. Fortunately the effects of salt concentrations are not large until the salt concentration becomes large.

Linderstrøm-Lang (1924) has extended the work to a variety of salts and has charted certain corrections which are useful to those who apply the quinhydrone electrode in accurate determinations.

Biilmann and Lund (1921) applying the principles developed by Sørensen, Sørensen and Linderstrøm-Lang (1921) have used the so-called quino-quinhydrone and hydro-quinhydrone electrodes, in the one case the solution being saturated with quinone in addition to quinhydrone and in the other the solution being saturated with hydroquinone as well as with quinhydrone. These electrodes should be free from salt-effect because the solid phases establish a constant ratio of the activities of oxidant and reductant.

An interesting development in the same direction is found in an observation by Conant and Fieser (1923). They make use of the fact that tetrachlorobenzoquinone and its reductant do not form sufficient of the corresponding quinhydrone to prevent simultaneous saturation of solutions with oxidant and reductant. Now let us confine attention to acid solutions and the equation

$$E_h = E_o - \frac{RT}{2F} \mathit{ln} \frac{a_r}{a_o} + \frac{RT}{F} \mathit{ln}\ a_{H^+}$$

By the definition of activity the presence of the solid phases fixes the activities of the oxidant and reductant in the solvent independently of the nature of the solvent. Therefore

$$E_h = E_o'' + \frac{RT}{F} \mathit{ln}\ a_{H^+}$$

where E_o'' will vary with the solvent. If a fixed reference value of a_{H^+} were to be established for each solvent so that E_o'' for that solvent could be determined, we would have in the chloranil electrode a simple device for correlating experimentally the hydrogen ion activities of different solvents. Unfortunately there is as yet no perfectly satisfactory way of relating the hydrogen ion activity in one solution to that in another, but the time is not distant when the use of electrodes such as those here described will have developed for each of the more important solvents much more extensive data than we now possess.

Finally it may be suggested that the significance of the new developments lies not so much in the properties of a particular system as it does in the possibilities of devising, from the still untouched stores of organic chemistry, systems adapted to special cases, and new experimental methods for the study of "salt-effects."

ANALYTICAL METHODS

The equations we have been using with the constants they involve are concise definitions of certain characteristics of each oxidation-reduction system. The establishment of these characteristics by accurate potentiometric measurements of pure compounds is the preliminary to an obvious method of later identification.

The analyst uses potentiometric methods to determine end-points in oxidation-reduction titrations; but if he confines his attention to end-points he may overlook valuable information furnished by a complete "titration-curve." In their study of the sulphonates of indigo, Sullivan, Cohen and Clark were able to establish fairly accurate characteristic constants for each system and consequently could predict the conduct of mixtures during titration. Thus they were able to approximately estimate by graphic methods the quantity of over-sulphonated or under-sulphonated indigo in some of their products. Even when the characteristic constants of a pure material have not been accurately established there may still be determined the order of magnitude of the percentage impurity provided this impurity is active. Thus in our recent studies at the Hygienic Laboratory we have established at least to our own satisfaction the presence of small percentages of active impurity in every sample of methylene blue at our disposal. Of course if an impurity is electromotively active in a zone of potential distinct from the zone characteristic of the chief constituent its detection is easy. Thus a titration of a commercial safranin with $TiCl_3$ revealed a large percentage of material active in a region very much more positive than the safranin itself.

The establishment of constants for a large number of different systems will give us not only data useful for identification but also a variety of titrating reagents from which to choose those suitable to specific purposes. When such a series of reagents is assembled we shall have begun the systematization of oxidation-reduction titrations and can then carry over to this subject many of the principles developed by A. A. Noyes, Bjerrum and others for acidimetric and alkalimetric titrations.

Moreover, just as certain acid-base mixtures have characteristic pH values useful in tests of identity by the rapid colorimetric method of determining pH, so many oxidation-reduction systems have characteristic reduction intensities which would be measurable by a colorimetric method if we had a series of oxidation-reduction indicators.

The beginning of such a series will be described in the next section.

Another interesting application of potentiometric methods is in determining the ratio of oxidant to reductant during progressive changes. Thus, for example, Biilmann and Blom (1924) used this method in their determination of velocity constants of semidine or benzidine rearrangement, Clark, Cohen and Gibbs (1925) in following the photodynamic regeneration of methylene blue from methylene white, and Conant and Lutz (1923) in following the effect of irreversible upon reversible oxidation-reduction systems.

OXIDATION-REDUCTION INDICATORS

The detection of reduction by dye decoloration is a very old practice which was introduced to the chemist by the artisan of the indigo vat. Through histology came the indophenols and methylene blue. The latter has been used as an indicator of reduction in such a diversity of problems that it may be compared with litmus, the universal acid-base indicator of a now historic age.

Prior to Ehrlich's classic, *Das Sauerstoffbedürfniss des Organismus* (1885), little was known of any order for the reduction of the different dyes. Ehrlich's use of living tissues as supposedly graded, titrating reagents established a semblance of order and inspired a number of researches. The results might have been harmonious had it not been that reduction capacity, reduction intensity, equilibrium state and rate of reaction were jumbled in disharmony.

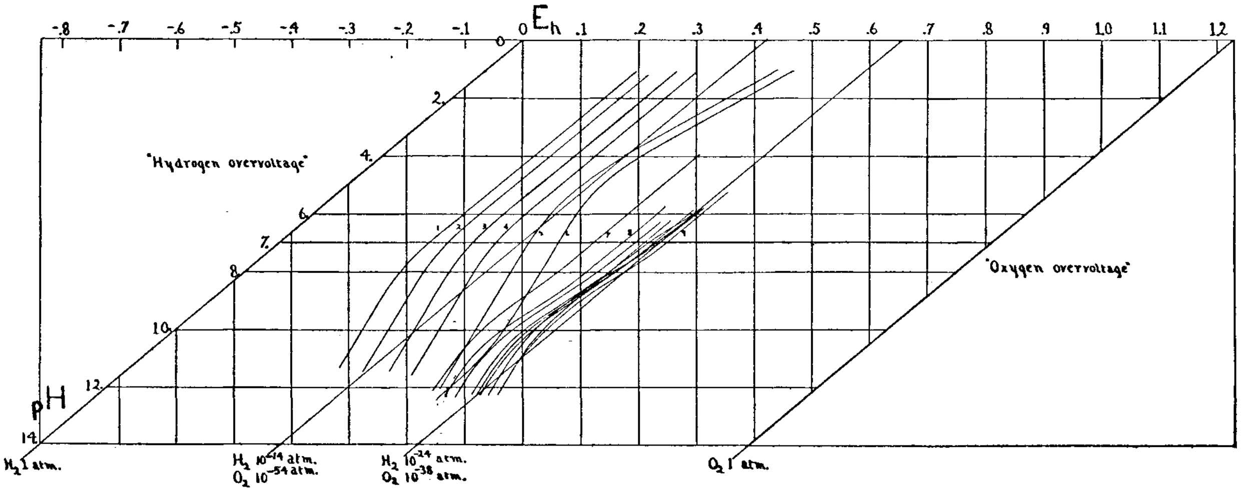


FIG. 9. E'_0:pH CURVES OF A FEW OXIDATION-REDUCTION INDICATORS

1. Indigo monosulphonate
2. Indigo disulphonate
3. Indigo trisulphonate
4. Indigo tetrasulphonate
5. Methylene blue
6. Lauth's violet
7. 1-naphthol 2-sulfonic acid indophenol

8–9. Indophenols

There is now little excuse for such confusion and with the potentiometric studies begun by Clark (1919) there is being built up a series of oxidation-reduction indicators suitable for the measurement of oxidation-reduction "intensity." These are comparable to the acid-base indicators used for the measurement of acid-base "intensity."

The methods employed have been described and data on such systems as those of the indigo sulphonates and methylene blue have been quoted. Therefore it remains for this section to summarize and to describe the chief features.

TABLE 3

Corrections to be applied to E_h as found on E_0':pH curves to obtain E_h at given percentage reduction, 30°

REDUCTION	CORRECTION
per cent	*volts*
5	+0.038
10	+0.029
20	+0.018
30	+0.011
40	+0.005
50	0.000
60	−0.005
70	−0.011
80	−0.018
90	−0.029
95	−0.038

In figure 9 are shown the E_0':pH curves of a few of the indicators studied in detail by the investigators at this laboratory.

Although it is practicable to present on one chart only a pair of the variables as in the E_0:pH curves, the uniformity of the percentage reduction:E_h curves makes it easy to estimate the potential at any percentage reduction from the chart and the data of table 3.

Since it is difficult to distinguish between the lower degrees of reduction an indicator is chiefly useful at 70 to 95 per cent reduction.

Let it be found for instance that indigo tetrasulphonate in a solution of pH 6.0 is 80 per cent reduced. The chart shows that

50 per cent reduction corresponds to $E_h = 0.007$. Hence at 80 per cent reduction $E_h = -0.011$.

Among the systems shown in figure 9 several must be rejected from practical use. Thus indicators such as Lauth's violet and indigo monosulphonate must be rejected because of their low solubilities. The same would apply to the methylene blue system because of the very low solubility of methylene white at neutral reaction were it not for the fact that the intense tinctorial power of the dye permits its use in extreme dilutions. Among the indophenols are many which have such low dissociation constants that the extraordinary blue of the dissociated compound does not appear in neutral solutions. However, by introduction of halogen substituents the dissociation constant can be increased until in 2, 6-dichlorophenol indophenol pK_a is 5.7. This compound gives brilliantly blue neutral solutions.

No indicators suitable for potentials more positive than those of the m-bromophenol indophenol system (9 of fig. 9) are yet available, and it may be that the chemist will look in vain to the biochemist for efforts to fill the gap between this and the region of oxygen over-voltage. The reason is that every living cell so far tested reduces these indophenols almost instantaneously. Consequently only the incentive of special problems yet to be defined will encourage the biochemist to search for more positive indicator systems.

In the extreme negative zone figure 9 shows a large gap and this gap is of particular interest to the bacteriologist because it is attained in cultures of many bacteria. A portion of this gap will doubtless be covered by compounds now under investigation at this laboratory.

It is of course obvious that the charting of indicator characteristics is the beginning of indicator theory as applied to titrations. It is also obvious that the possession of such definite values as are shown in figure 9 enables one to tell rapidly the approximate oxidation-reduction intensity of any system which can enter into simultaneous equilibrium with one of the listed indicators.

Later I shall touch briefly upon certain biochemical applications of these indicators but shall refer the reader to forthcoming papers for details.

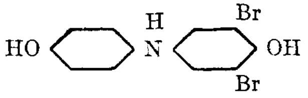
In the meantime let the reader remember that hundreds of applications of methylene blue-reduction have been made without the quantitative interpretations now available.

THE EFFECTS OF SUBSTITUTION

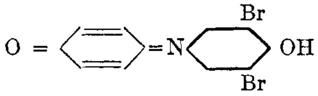
If we consider a case to which equation (8) applies and for which the numerical values of K_1 and K_2 fall within the experimental range of $[H^+]$ there can be established not only the "normal potential," E_o, but the values of K_1 and K_2. An acidic or basic group common to oxidant or reductant will not be apparent unless the dissociation constant of such a group *changes* when the substance is oxidized or reduced. Then, if the change is of sufficient magnitude, both K-values are determinable as already illustrated in a previous section (see page 143). Thus it is often possible to establish several characteristic constants for a given system.

If then a substitution in the molecule is made, the influence of this substitution upon the values of several constants can be established. Thus we have a beautiful and exact set of data on substitution.

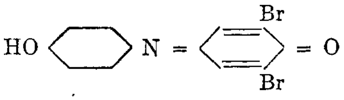
However, there often remain difficulties of interpretation. Conant, Kahn, Fieser and Kurtz (1922) in discussing a case where equation (8) was applied remark: "There is nothing to indicate that the two constants . . . . are the real dissociation constants of the reduced form except that the equation can be developed on this basis." Their meaning is not very clear, for it can be shown that if the same basis of reference could be reached in handling different methods (colorimetric, potentiometric, conductimetric, etc.) the constants would be the same whatever the method of approach. On the other hand, there is nothing in the method under discussion which helps us in the allocation of the constants. For this we must depend upon structural chemistry. It is just here that structural chemistry presents a most serious difficulty. In a reductant such as (I) is the first hydrogen to dissociate that adjacent to the bromines or that of the other phenolic group, and does the replacement of bromine by methyl alter whatever may be the order of dissociation in (I)? In the oxidant is the predominant structure (II) or (III) and if (II) does it become wholly or partially of type (III) or remain type (II) on replacing bromine by methyl?



(I)



(II)

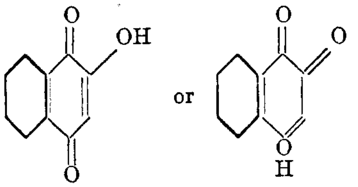


(III)

Certain hints may be obtained such as those noted in the case at hand by Cohen, Gibbs and Clark (1924) but perfectly definite evidence is lacking.

However, some problems are in a fair way to definite settlement. For instance Heller (1912) claimed that two different indophenols result from the condensation in the one case of p-amino phenol with m-cresol and in the second case from the condensation of (p-amino-m-cresol) 6-amino-3-hydroxy-1-methyl benzene with phenol. Cohen, Gibbs and Clark (1924) proceeding with different chloroimides which should have given Heller's isomers obtained compounds which acted the same within the limits of error of potentiometric measurements; as might well have been expected if we regard Heller's isomers as tautomers. More positive evidence could be obtained with substituents of greater effect than that of methyl.

The methods under discussion will not reveal the presence of tautomers in mobile equilibrium but they will definitely reveal stable isomers when such isomers have different characteristic constants. Consequently Conant and Fieser (1924) were able to show for hydroxy-naphthoquinone



that either only one isomer was present or else that the two forms are tautomers in mobile equilibrium.

These instances may suffice to suggest on the one hand a number of cases where potentiometric measurements can aid in the solution of problems of structural chemistry, and on the other hand the kind of problem still to be solved by structural chemistry before the allocation of substitution effects of the kind we are discussing can become definite.

The space available does not allow an adequately detailed review of the numerical values now available on substitution effects. Moreover a quantitative formulation of the data must await either sufficient material for statistical analysis or else a key constructed without *ad hoc* assumptions.

Nevertheless, it is interesting to note that among the limited types of compound investigated by Biilmann, LaMer and Baker, Conant and his coworkers and the investigators at the Hygienic Laboratory, halogens increase and alkyls decrease potentials; sulphonic acid groups and carboxyl increase and hydroxyl, methoxy, and phenyl decrease potentials.

In figure 2 (page 139) there have already been shown the effects of one series of substitution. Figure 10, taken from the dissertation of Baker, shows graphically the general relations among a series of substituted quinones accurately studied by LaMer and Baker (1922). In each of tables 4, 5 and 6 are some comparable data, which are given only as samples of much more extensive data to be found with interesting comments in the original papers by Conant and his coworkers and by the investigators at this laboratory.

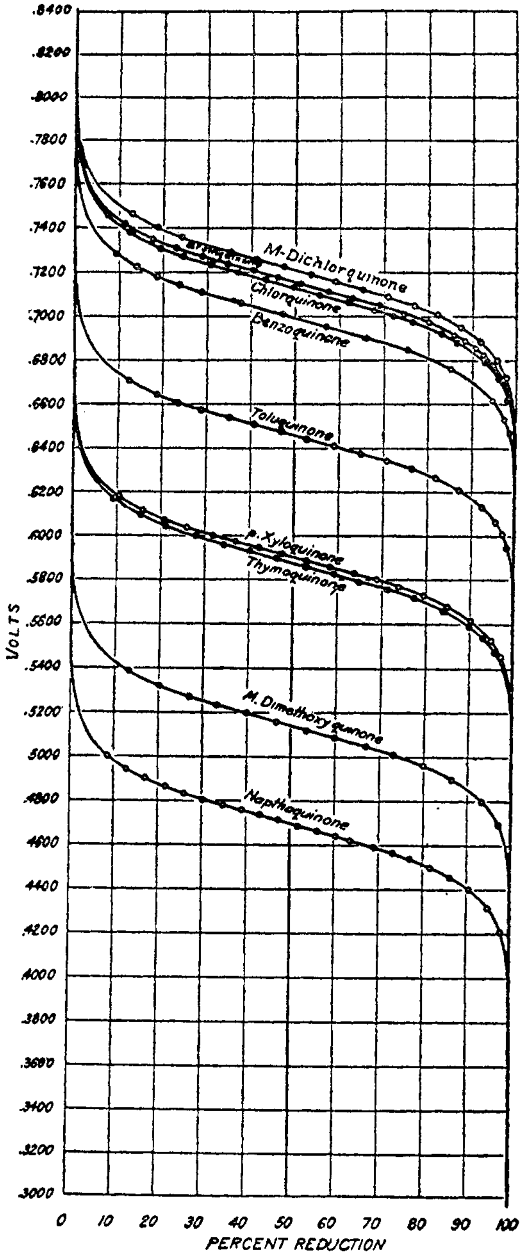


FIG. 10. RELATION OF ELECTRODE POTENTIAL TO PERCENTAGE REDUCTION SHOWING EFFECTS OF SUBSTITUTION

After Baker

Conant and Fieser (1923) finding that some of the systems they wished to compare were not adapted to measurements in aqueous solutions used the principle outlined in a previous section in their study of aqueous and alcoholic solutions. They were able to show that parallel data of value can be obtained by measurements in aqueous and alcoholic solution. This was an important advance in that it broadened the available material and led to a

TABLE 4

Compiled from Conant's data

E.M.F. of cell: H_2 (1 atmos.) Pt | 0.5 M HCl in 95 per cent ethanol at 25° | 0.5 M HCl + $S_o = S_r$ in 95 per cent ethanol | Pt^+

S_o/S_r SYSTEM NAMED BY OXIDANT	E.M.F.
1,2 naphthoquinone	0.580
1,4 naphthoquinone	0.495
2 chloro-1,4 naphthoquinone	0.510
2,3 dichloro-1,4 naphthoquinone	0.499
2 bromo-1,4 naphthoquinone	0.508
1,4 napthoquinone-2-sulfonic acid	0.553
2 hydroxy-3 chloro-1,4 naphthoquinone	0.349
2,3-diphenoxy-1,4 naphthoquinone	0.456
1,2 anthraquinone	0.492
9,10 anthraquinone	0.156
2 methyl-9,10 anthraquinone	0.152
1 chloro-9,10 anthraquinone	0.175
2 chloro-9,10 anthraquinone	0.202
9, 10 anthraquinone-2-carboxylic acid	0.213
methyl-9,10-anthraquinone-2-carboxylate	0.224
ethyl-9,10 anthraquinone-2- carboxylate	0.223

TABLE 5

Normal potentials, E_0, and dissociation constants of groups formed by reduction. 25° (after Conant, Kahn, Fieser and Kurtz, 1922)

SYSTEM NAMED BY OXIDANT	E_0	K_1	K_2
9,10 anthraquinone 1 sulfonic acid	0.195	4×10^{-9}	
9,10 anthraquinone 1,5 disulfonic acid	0.239	3×10^{-12}	
9,10 anthraquinone 1,8 disulfonic acid	0.206	7×10^{-9}	
9,10 anthraquinone 2 sulfonic acid	0.187	1×10^{-8}	5×10^{-12}
9,10 anthraquinone 2,6 disulfonic acid	0.228	8×10^{-9}	3×10^{-11}
9,10 anthraquinone 2,7 disulfonic acid	0.229	2×10^{-8}	3×10^{-11}

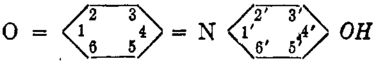
later illuminating study (Conant and Fieser, 1924) of some 35 additional systems.

Although the limitations of the method and the inherent errors leave something to be desired, the most accurate applications of the method to the determination of acidic and basic dissociation constants are those of the Hygienic Laboratory workers. These authors have shown how necessary is a fair accuracy in the estimation of such constants if there is to be avoided the confusion due to unrecognized crossings of E'_o:pH curves.

TABLE 6

Effects of substitution on E_0 and pK values of indophenols

Type compound called phenol indophenol:



SYSTEM NAMED BY OXIDANT*	E_0	pK_o	pK_r	pK_2
Phenol indophenol	0.649	8.1	9.4	10.6
2-methyl-phenol indophenol	0.616	8.4	9.5	10.9
3-methyl-phenol indophenol	0.632	8.6	9.6	10.7
2 isopropyl-5 methyl-phenol indophenol	0.592	8.8	9.9	10.8
2 methyl-5 isopropyl-phenol indophenol	0.593	8.9	9.9	10.7
2-bromo-phenol indophenol	0.659	7.1	8.5	10.2
3-bromo-phenol indophenol	0.670	7.8	9.0	10.3
2-chloro-phenol indophenol	0.663	7.0	8.4	10.3
3′-5′ dichloro-phenol indophenol	0.668	5.7	7.0	10.1
3′-5′dibromo-phenol indophenol	0.668	5.7	7.0	10.1
2 chloro-3′, 5′-dichloro-phenol indophenol	0.668	5.8	7.1	8.8
2 methyl-3′,5′-dichloro-phenol indophenol	0.639	5.5	7.1	10.4

* For discussion of tautomerism, see page 162.

Indeed there is involved a very interesting question which will arise when attempts are made to compare systems of different type. What is to be the standard state for comparison? While certain hydroquinones can be brought to the fully dissociated state other reductants cannot. On the other hand certain compounds such as methylene blue cannot be brought to the undissociated state.

It seems to me that there is more than a mere question of convention involved and I may suggest what I have in mind by an interesting fact.

Among the quinones halogen substitution increases the *normal* *potential* much less than alkyl substitution decreases, taking benzoquinone as a standard of comparison. The same tendency in less marked degree is observed among simple indophenols. On the other hand among the indophenols the effect of halogen substitution upon K_o-*dissociation constants* tends to be greater than the effect of alkyl substitution.

Thus one set of data, for instance "normal potentials" may not give as full an expression of the effects of substitution as can readily be obtained.

Now undoubtedly what we are providing in these data is some sort of comparative measurement of the electron constraints within the molecule. Cohen, Gibbs and Clark (1924) have therefore suggested that possibly the most illuminating comparisons of potentials would be found among completely ionized systems could a series of these be obtained. Conant and his coworkers give some good reasons for choosing the undissociated state of hydroquinones as the standard state.

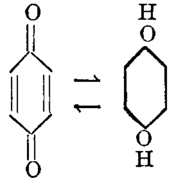
The real difficulty is not in finding a *convenient* state for the comparison of one group of compounds but in the element of relativity which permeates the whole subject. Of this the interpreter of the substitution data must take full account.

In the meantime the basic facts of the quantitative effects of substitution are being found to have very considerable value in a number of directions. It is hoped that they will soon receive an illuminating interpretation.

TYPES OF COMPOUND SUCCESSFULLY STUDIED

It is important for the advancement of the subject to know its limitations as suggested in the types of compound which have yielded good or fairly reliable potentiometric data.

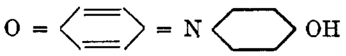
Foremost in the number of cases examined are quinone-quinol systems of which benzoquinone-benzohydroquinone



is the prototype. Including in this group the naphthoquinones and anthraquinones we find data on about 50 systems in the papers of Biilmann (1920) LaMer and Baker (1922) and Conant and his coworkers (1922–24), the latter group of workers having contributed the greater number of data.

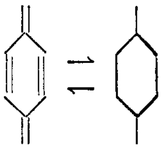
The corresponding diamines are being studied in this laboratory.

The indophenols of which the simplest member is



may be considered as oxidants of substituted amino phenols. Clark and Cohen (1923), Cohen, Gibbs and Clark (1924) and Gibbs, Cohen and Cannan (1925) have successfully measured some 26 of these systems. In addition they have unpublished data for certain indamines.

The change

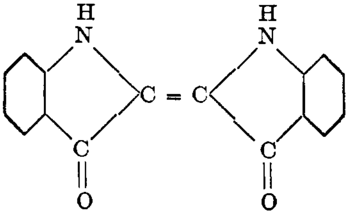


evident in the above cases is still evident in the transformation methylene white ⇌ methylene blue and in the corresponding system of Lauth's violet. Clark, Cohen and Gibbs (1925) have measured these systems.

In the quinones there is the conjugated system

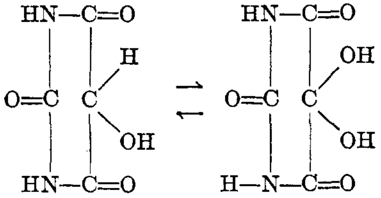
$$O=C-C=C-C=O$$

which may be found in the following disputed formula of indigo



The indigo sulphonates have been reported upon by Sullivan, Cohen and Clark (1923) and Conant and Lutz (1924) have reported preliminary data on two sulphur indigoes and alizarin indigo.

Adopting Biilmann's formulae we have Biilmann and Lund's (1924) data on the dialuric acid-alloxan system



and a methylated pair of derivatives.

By allowing for potential drifts which could easily be ascribed to a semidine or benzidine rearrangement of their hydrazo compounds Biilmann and Blom (1924) have found reasonable data for two azo-hydrazo systems.

Conant and Lutz (1923) describe nitroso benzene and nitroso-β-naphthol as yielding reliable potentials in their reversible reductions to phenylhydroxyl amine and 1-hydroxylamino-2-naphthol respectively.

In addition there is the highly important study by Conant (1923) on hemoglobin. The equilibrium between this blood pigment and oxygenated hemoglobin seems not to be susceptible to potentiometric measurement. On the other hand Conant finds that the hemoglobin-methemoglobin system can be followed potentiometrically. While the experimental data leave much to be desired in accuracy the equation

$$E_h = E_o - \frac{RT}{F} \ln \frac{\text{Hemoglobin}}{\text{Methemoglobin}}$$

is fairly well satisfied at constant pH. There is a change of E_0' with change of pH such as might be expected from a change in the dissociation constants of one or more groups common to oxidant and reductant. The one-equivalent concerned in the reduction of methemoglobin to hemoglobin is in marked contrast to the two equivalents which Conant's preliminary data indicate are concerned in the reduction of hematin to hemochromogen. These considerations suggest that the hemoglobin-methemoglobin system involves the one equivalent necessary in a ferrous-ferric transformation.

Although somewhat out of place in our scheme of presentation the later papers by Conant and Fieser (1925) on methemoglobin which have just come to hand may be mentioned here. In the first paper the simultaneous equilibrium between the hemoglobin-methemoglobin system, the hemoglobin-oxyhemoglobin system and the ferro-ferricyanid system have been studied in more detail, confirming the main conclusions of the earlier paper and giving additional data of value to the analysis of oxyhemoglobin solutions. In the second paper the new data are shown to lead to a simple method for the determination of methemoglobin in the presence of its cleavage products.

Whether Dixon and Quastel's (1923) study of cysteine can properly be included is questionable. Were I reviewing the entire subject I should like to discuss this highly interesting case but shall now arbitrarily classify it as belonging outside the realm which I am reviewing.

SIDE-LIGHTS ON MECHANISM

It should of course be perfectly well recognized that potentiometric measurements reveal nothing definite regarding mechanisms of oxidation-reduction and that equations which imply any mechanism are purely formal.

At the same time certain *comparative* data furnished by the potentiometric measurements provide material for *intuitive* deductions.

The more accurate data on reduction of substances having a quinoid structure have left not the slightest doubt that the two equivalents concerned are in some way *paired* in their energetics. There is no trace of stepwise reduction corresponding *structurally* (as Conant has pointed out to me in conversation) to an intermediate between a quinoid and a quinol, or analogous to the stepwise dissociation of acids and bases. From the point of view of the *electronic configurations* of the molecule this corresponds to no stable intermediate between the configuration of quinone and that of quinol with its *two additional electrons.*

On the other hand the hydrogens which might be regarded as the more directly concerned equivalents involved in the transformation have been shown, in a variety of indigoes, indophenols and thiazines, to have very greatly different ionization constants and hence very different free energies of ionization. Furthermore the indophenol systems lie in a region of potential which by the application of theoretical relations for the oxy-hydrogen gas cell can be shown to indicate physically insignificant partial pressures of hydrogen in equilibrium with the systems. The implication which has been discussed by Cohen, Gibbs and Clark (1924) is that the reduction in these cases consists essentially in the transfer of an *electron pair* accompanied or not accompanied by hydrogen ions according to the state of acid-base equilibrium in the solution. This is the kind of implication which cannot definitely disprove Wieland's very hypothetical mechanism of biological oxidation by hydrogen-transport which is now quite in vogue and which he supports by citing the conduct of reducible dyes, but it doubtless would have directed speculation into other channels had it been appreciated earlier.

The types of compounds which have furnished reliable potentiometric data are comparatively few. To generalize upon this is very dangerous but it is interesting to guess the significance.

In most if not all of the cases for which we have reliable data there is at least one hydrogen associated with the change of oxidant to reductant which ionizes within the experimental range of pH leaving at least one unguarded charge upon practically all the molecules at high pH and upon a few at other regions of pH.

Secondly there is frequently a nucleus such as we picture in the quinone-quinol rings, or in the electron shells of nitrogen and of iron where the gain or loss of electrons unaccompanied by neutralizing ions is possible without complete loss of the system's stability.

Accordingly the criterions of a reversible system capable of potentiometric measurement with the aid of electrodes should be first a system in which ionization uncovers at least one vulnerable point and second a system containing what may be called a reservoir effect, i.e., an electronic configuration not instantly made unstable by loss or gain of electrons and therefore capable by itself of taking up or releasing electrons as they flow to and from an electrode in the course of potentiometric balancing.

It has long since been found impossible to treat the *actual* conduct of the electrode under the conditions now being discussed as if it were that of a hydrogen electrode, although this, as a formalistic treatment, is sometimes convenient.

These guesses lead directly to the consideration of those systems which have no very definite effect upon the electrode and to the so-called irreversible oxidation-reductions. No review such as this would be at all adequate if it failed to mention this vast subject. Here let me remind the reader that in the introduction to this review I promised to mention what is perhaps the most important of all the reasons which may be advanced for the scarcity of potentiometric data on organic systems. It is simply that the systems regarded as most important by the organic chemist and the physiologist have resisted the methods of approach we have been discussing.

Recent adventures in this important realm will be discussed by Dr. Conant and I shall try not to trespass upon the review he has promised. But I know he will not object if in my final remarks I touch very briefly upon certain studies of a peculiar kind of so-called irreversible system in which I have been particularly interested.

BIOLOGICAL STUDIES AND CONCLUDING REMARKS

The processes of oxidation-reduction which are peculiar to the chemistry of the living cell have received a vast amount of attention. The greater part of this has been centered upon the mechanisms by which molecular oxygen enters into the chemistry of life. I would emphasize the difficulty incident to the study of a set of oxidative processes continuously being opposed by that powerful trend toward higher and higher reductive intensities which is uncovered whenever the cell is isolated from extraneous oxidants. Upon the cause of this trend it would be idle to speculate but the trend is far more generally characteristic of the living cell than respiration without which many organisms do very well (cf. review by Clark, 1924).

In the study of the reductive trend will the electrode be of service? Gillespie's (1920) work suggests that it will. After extensive experiments which confirmed the main features of Gillespie's observations on bacterial cultures, I concluded that an answer worthy of the importance of the question could be found only after a better understanding of simple and reversible systems. This was the origin of potentiometric studies on oxidation-reduction indicators (Clark, 1919–1920).

I believe there should now be no doubt that the establishment of equilibrium constants makes it possible to reinterpret quantitatively in terms of potential the intensities of reduction revealed by the decoloration of indicators such as methylene blue which have been very extensively used in a purely qualitative way to build elaborate theories. Now it is of course obvious that, having shown a mixture of methylene blue and methylene white to affect an electrode in a perfectly definite way, we cannot gain much that is new by using an electrode in a suspension of cells containing partially reduced methylene blue. On the other hand we have shown that with the electrode *in the absence* of any indicator it is possible to follow in much greater detail the course of reduction in the Schardinger reaction, in the reduction test of milk, in the putrescibility test of sewage. The diagnostic differential reduction by different species of bacteria is recorded by potentiometric measurements quite as well and perhaps better than by indicators. Phenomena of washed and unwashed cell-suspensions in the presence and absence of metabolites, which have hitherto been studied chiefly with the aid of methylene blue, can be followed in greater detail by potentiometric measurements.

In short the cumulative results, reproducing as they do in terms of electrode potential-difference all the chief features of dye reduction, may be considered as evidence that the cautiously interpreted potentiometric measurements have at least as much significance as dye-reduction.

However we must proceed with caution. We encounter in these cell-suspensions no equilibrium having a mobility comparable to that of an ordinary acid-base system. Consequently poising[2] action is either obscured by slow rates of adjustment or is absent and the activity of the uncontrolled partially cytolysed cells induces a continuous change in electrode potential. Thus the type of problem to be considered is very different from that of the acid-base equilibria in living tissue. A drifting potential may be due to so many causes that unless we can give a reasonable interpretation, as Biilmann and Blom have done for their azo-hydrazo systems, we would ordinarily reject our data. But in the case at hand the importance demands persistent investigation of the facts.

Among the many interesting facts are the following. We never find E_h potentials more positive than about $+0.2$ at pH 7 as measured *both* by indicators and electrode even after air has been bubbled through the cell-suspension! Cultures of certain anaerobic bacteria, as measured by electrodes and independently by such indicators as are available for rough estimates, can not only induce the potential of the hydrogen electrode but also a definite although slight overvoltage! Within the intermediate range of potential, cell-suspensions act as if there were present at any moment sufficient active material to prevent serious polarization in the balancing of potentials and yet insufficient to prevent serious change of potential when *active* oxidants or reductants are added. A more adequate discussion will appear in a forthcoming paper from this laboratory.

In short the compounds present in these cell-suspensions seem to be activated sufficiently to affect indicators and electrodes in a comparable way which is certainly suggestive but short of satisfactory. This casts the problem back again to the study of more definite and more simple systems which we still have to understand better. That this would become the main problem was

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[2] Clark, 1923, suggests the term "poising action" as the oxidation-reduction analogue to acid-base buffer action.

apparent to me at the beginning of my biochemical studies in 1919 but at that time relations were quite obscure which have now become plain by the study of the reversible systems discussed in this review. I have no doubt that while definite limitations of the methods will be found it will be realized that there is no sharp line of demarkation between systems amenable to these methods and systems which are not. The problem is to find how far the methods will be of use. But here I am encroaching upon a field beyond the scope of this review.

In closing I may indicate the significance of the recent studies on reversible systems by suggesting that the organic chemist with the infinite variety of structure at his disposal may soon discover why it is that so many inorganic systems have resisted potentiometric measurement. If the full force of this suggestion be appreciated it will be realized that the significance transcends the importance of the immediate problem cited.

REFERENCES

Papers of the series, Studies on Oxidation-Reduction:

I. Introduction. W. Mansfield Clark. Public Health Reports, 1923, **38**, 443. (Reprint No. 823.)

II. An analysis of the theoretical relations between reduction potentials and pH. W. Mansfield Clark and Barnett Cohen. Public Health Reports, 1923, **38**, 666. (Reprint No. 826.)

III. Electrode potentials of mixtures of 1-naphthol-2-sulphonic acid indophenol and the reduction product. W. Mansfield Clark and Barnett Cohen. Public Health Reports, 1923, **38**, 933. (Reprint No. 834.)

IV. Electrode potentials of indigo sulphonates each in equilibrium with its reduction product. M. X. Sullivan, Barnett Cohen, and W. Mansfield Clark, Public Health Reports, 1923, **38**, 1669. (Reprint No. 848.)

V. Electrode potentials of simple indophenols, each in equilibrium with its reduction product. Barnett Cohen, H. D. Gibbs, and W. Mansfield Clark. Public Health Reports, 1924, **39**, 381. (Reprint No. 904.)

VI. A preliminary study of indophenols: (A) Dibromo substitution products of phenol indophenol; (B) Substituted indophenols of the ortho type; (C) Miscellaneous. Barnett Cohen, H. D. Gibbs, and W. Mansfield Clark. Public Health Reports, 1924, **39**, 804. (Reprint No. 915.)

VII. A study of dichloro substitution products of phenol indophenol. H. D. Gibbs, Barnett Cohen, and R. K. Cannan. Public Health Reports, 1925, **40**, 649.

VIII. Methylene blue. W. Mansfield Clark, Barnett Cohen, and H. D. Gibbs. Public Health Reports, 1925.

Abegg, R., Auerbach, F., and Luther, R. 1911–1915 Messungen elektromotorischer Kräfte galvanischer Ketten. *Abhandlungen deut. Bunsen-Gesellschaft* Nos. 5 & 8.

Baker, L. E. 1922 The effect of substitution on the free energy of reduction of benzoquinone. Dissertation. Columbia University, N. Y.

Biilmann, E. 1920 Kinhydroners Brintning. Ann. University, Copenhagen. (Cited.)

Biilmann, E. 1920 Sur l'hydrogénation des quinhydrones. *Ann. Chim.*, **15**, 109.

Biilmann, E. 1924 Oxidation and reduction potentials of organic compounds. *Trans. Faraday Soc.*, **19**, 676.

Biilmann, E. 1924 On the measurement of hydrogen-ion concentrations in soil by means of the quinhydrone electrode. *J. Agricultural Sci.*, **14**, 232.

Biilmann, E., and Blom, J. H. 1924 Electrometric studies on azo- and hydrazo-compounds. *J. Chem. Soc.*, **125**, 1719.

Biilmann, E., and Krarup, I. 1924 The temperature coefficient of the quinhydrone electrode. *J. Chem. Soc.*, **125**, 1954.

Biilmann, E., and Lund, H. 1921 Sur l'électrode à quinhydrone. *Ann. de Chim.*, **16**, 321.

Biilmann, E., and Lund, H. 1923 Sur le potentiel d'hydrogènation des alloxanthines. *Ann. Chim.*, **19**, 137.

Bodforss, S. 1922 Über die Beeinflussung von verschiedenen chemischen Reaktionen durch Substituenten. *Z. physik. Chem.*, **102**, 41.

Clark, W. M. 1919 Reduction potential in its relation to bacteriology. *Proc. Soc. Am. Bacteriologists* in *Abstracts Bact.*, **4**, 2.

Clark, W. M. 1920 Reduction potentials of mixtures of indigo and indigo white and of mixtures of methylene blue and methylene white. *J. Washington Acad. Sci.* **10**, 255.

Clark, W. M. 1922 The Determination of Hydrogen Ions. 2d edition. Baltimore.

Clark, W. M. 1924 Life without oxygen. *J. Wash. Acad. Sci.*, **14**, 123.

Clark, W. M., and Cohen, B. 1921 Some elementary aspects of the putrescibility and similar tests. *Proc. Soc. Am. Bact.* in *Abstracts Bact.*, **6**, 3.

Clark, W. M., and Zoller, H. F. 1921 A series of oxidation-reduction indicators. *Science*, **54**, 557.

Conant, J. B. 1923 An electrochemical study of hemoglobin. *J. Biol. Chem.*, **57**, 401.

Conant, J. B., and Fieser, L. F. 1922 Free and total energy changes in the reduction of quinones. *J. Am. Chem. Soc.*, **44**, 2480.

Conant, J. B., and Fieser, L. F. 1923 Reduction potentials of quinones. I. The effect of solvent on the potentials of certain benzoquinones. *J. Am. Chem. Soc.*, **45**, 2194.

Conant, J. B., and Fieser, L. F. 1924 Reduction potentials of quinones. II. The potentials of certain derivatives of benzoquinone, naphthoquinone and anthraquinone. *J. Am. Chem. Soc.*, **46**, 1858.

Conant, J. B., and Fieser, L. F. 1925 Methemoglobin. *J. Biol. Chem.*, **62**, 595.

Conant, J. B., and Fieser, L. F. 1925 A method for determining methemoglobin in the presence of its cleavage products. *J. Biol. Chem.*, **62**, 623.

Conant, J. B., Kahn, H. M., Fieser, L. F., and Kurtz, S. S., Jr. 1922 An electrochemical study of the reversible reduction of organic compounds. *J. Am. Chem. Soc.*, **44**, 1382.

Conant, J. B., and Lutz, R. E. 1923 An electrochemical method of studying irreversible organic reductions. *J. Am. Chem. Soc.*, **45**, 1047.

Conant, J. B., and Lutz, R. E. 1924 The irreversible reduction of organic compounds. I. The relation between apparent reduction potential and hydrogen-ion concentration. *J. Am. Chem. Soc.*, **46**, 1254.

Darmois, E., and Honnelaitre, A. 1924 Electrometric study of the acidity of mixtures of malic and molybdic acids. *Compt. rend.*, **178**, 2183. (Cited.)

Dixon, M., and Quastel, J. H. 1923 A new type of reduction-oxidation system. I. Cysteine and glutathione. *J. Chem. Soc.*, **123**, 2943.

Ehrlich, P. 1885 Das Sauerstoffbedürfniss des Organismus. Berlin.

Gillespie, L. J. 1920 Reduction potentials of bacterial cultures and of water-logged soils. *Soil Science*, **9**, 199.

Granger, F. S. 1920 Oxidation and reduction in organic chemistry from the standpoint of potential differences. Dissertation. Columbia University, N. Y.

Granger, F. S., and Nelson, J. M. 1921 Oxidation and reduction of hydroquinone and quinone from the standpoint of electromotive-force measurements. *J. Am. Chem. Soc.*, **43**, 1401.

Haber, F., and Russ, R. 1904 Über die elektrische Reduktion. *Z. physik. Chem.*, **47**, 257.

Harris, L. J. 1923 Use of the quinhydrone electrode for the estimation of amino-acids and of acid and basic functions. *J. Chem. Soc.*, **123**, 3294.

Heller, G. 1912 Über die einfachsten Indophenole und Indamine. *Ann. Chem.*, **392**, 16.

Hugonin, G. 1924 The use of the quinhydrone electrode for measuring hydrogen-ion concentration in tan liquors. *J. Soc. Leather Trades Chem.*, **8**, 537. (Cited.)

Kolthoff, I. M. 1923 Die Verwendung der Chinhydron- statt der Wasserstoffelektrode bei potentiometrischen Aciditätsbestimmungen. *Rec. trav. chim.*, **42**, 186.

LaMer, V. K., and Baker, L. E. 1922 The effect of substitution on the free energy of oxidation-reduction reactions. I. Benzoquinone derivatives. *J. Am. Chem. Soc.*, **44**, 1954.

LaMer, V. K., and Parsons, T. R. 1923 The application of the quinhydrone electrode to electrometric acid-base titrations in the presence of air, and the factors limiting its use in alkaline solution. *J. Biol. Chem.*, **57**, 613.

LaMer, V. K., and Rideal, E. K. 1924 The influence of hydrogen concentration on the auto-oxidation of hydroquinone. A note on the stability of the quinhydrone electrode. *J. Am. Chem. Soc.*, **46**, 223.

Larsson, E. 1922 Bestimmung der zweiten Dissoziationskonstanten einiger Säuren aus elektrometrischen Messungen. *Z. anorg. allgem. Chem.*, **125**, 281.

LESTER, V. 1924 On the measurement of hydrion concentration in some dairy products by means of Biilmann's quinhydrone electrode. *J. Agricultural Sci.* **14**, 634.

LEWIS, G. N. 1923 Valence. Chemical Catalog Co., N. Y.

LINDERSTRØM-LANG, K. 1924 On the salting-out effect. *Compt. rend. trav. lab. Carlsberg,* **15**, 1.

MEUNIER, L., AND QUEROIX, M. 1924 Time changes in quinone solutions. *Cuir tech.,* **13**, 520. (Cited.)

PRING, J. N. 1924 Determination of the affinity constants of bases by the hydrogen and quinhydrone electrodes. *Trans. Faraday Soc.,* **19**, 705.

SCHREINER, E. 1922 Die Hydration des Wasserstoffions. *Z. anorg. allgem. Chem.,* **121**, 321.

SCHREINER, E. 1922 Der Dissoziationszustand von mittelstarken Säuren besonders der Dichloressigsäure, in Wasser und in Salzlösungen. *Z. anorg. allgem. Chem.,* **122**, 201.

SCHREINER, E. 1924 Hydration of univalent ions. *Z. anorg. allgem. Chem.,* **135**, 333. (Cited.)

SØRENSEN, S. P. L., AND LINDERSTRØM-LANG, K. 1924 On the determination and value of π_0 in electrometric measurements of hydrogen ion concentrations. *Compt. rend. trav. lab. Carlsberg* **15**, No. 6.

SØRENSEN, S. P. L., SØRENSEN, M., AND LINDERSTRØM-LANG, K. 1921 Sur l'"erreur de sel" inhérente à l'électrode de quinhydrone. *Compt. rend. trav. lab. Carlsberg,* **14**, 1; *Ann. de Chim.,* **16**, 283.

VEIBEL, S. 1923 The quinhydrone electrode as a comparison electrode. *J. Chem. Soc.,* **123**, 2203.