[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF WAYNE UNIVERSITY]

The Reduction Potentials of Organic Systems. I. The Dimolecular Reduction of Thioindigo Disulfonate

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W. M. Clark and his collaborators[1] were pioneers in the development of oxidation–reduction indicators. They determined the normal potentials of a large number of dyestuffs and found in each case that two electrons were involved in the reduction of one molecule. Other investigators subsequently added to this list of indicators. No exception to Clark's observation that two electrons were involved per molecule was forthcoming until Michaelis and Friedheim[2] and later Elema[3] showed that in acid solutions pyocyanine is reduced in two steps, each involving one electron per molecule. The intermediate "semiquinone" is of the same molecular size as the pyocyanine and is stable in solution. Further work by Michaelis and his collaborators[4] and by Elema[5] showed that the phenomenon was more general than at first supposed, and the whole subject was put on a satisfactory mathematical and theoretical basis. According to this theory ordinary quinhydrone is not a meriquinone but a semiquinone whose dismutation constant is so large that it cannot exist in solution. Apparently there are no cases on record where potentiometric investigation has shown the presence of a true meriquinone in solution. Michaelis in his review also mentioned work reported by Preisler and Schaffer at the 1933 meeting of the American Chemical Society at Chicago. They found that when indigo disulfonate is reduced at *p*H values greater than 10.5, a red semiquinone is formed as an intermediate product.

The present investigation of thioindigo disulfonate was undertaken in the hope that it would prove to be a satisfactory oxidation–reduction indicator whose potential would be higher than any of the indigo sulfonates and hence might be used to replace one of the relatively unstable indophenols. When the reduction potential of the system formed by the dye and its leuco compound was measured in this Laboratory it was found, much to our surprise, that the dye underwent a dimolecular reduction, involving but one electron per molecule of dye. The experimental data conform with the following mathematical formulation and establish this as the first system observed to undergo a thermodynamically reversible, dimolecular, oxidation–reduction reaction. No evidence of a second step was found.

Mathematical Formulation

The thermodynamic equations for the reduction potential of this system may be most easily formulated by the method and symbols of Clark.[6] If $2\text{Ox} + 2\text{e} \rightarrow \text{Red}^{=}$ (I) we may follow Clark's mathematical procedure exactly and get the equation

$$E_{\text{h}} = E_0 - \frac{RT}{2F} \ln S_{\text{R}}/S_0^2 - \frac{RT}{2F} \ln \frac{K_1K_2}{K_1K_2 + K_1[\text{H}^+] + [\text{H}^+]^2} \quad \text{(II)}$$

If the *p*H is held constant equation II becomes

$$E_{\text{h}} = E_0'' - RT/2F \ln S_{\text{R}}/S_0^2 \quad \text{(III)}$$

If a moles of dye are reduced and then titrated by the addition of x moles of potassium ferricyanide, and if at any stage of the titration v is the volume of the solution in liters, then

$$S_{\text{R}} = (a - x)/2v \text{ and } S_0 = x/v$$

Introducing these values into equation III we get at 25°

$$E_{\text{h}} = E_0' - 0.0295 \log [(a - x)/x^2] - 0.0295 \log v \quad \text{(IV)}$$

Experimental Part

Preparation and Analysis of Dye.—Fifty grams of Ciba Pink B (Rowe No. 1207) were added to 500 cc. of fuming sulfuric acid (15–20% excess SO_3) and heated in an oil-bath at 115–125° until a test sample completely dissolved in water (about one hour). It was then allowed to cool somewhat, thrown onto ice and the resultant solution almost neutralized with sodium hydroxide, the neutralization being completed with sodium carbonate. The relatively insoluble purple precipitate (probably the monosulfonate) was filtered off and the filtrate was worked up by concentration and fractional recrystallization, first from

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(1) Clark, Cohen, Gibbs, etc., U. S. Public Health Service, Hyg. Lab. *Bull.* No. **151** and subsequent papers.

(2) Michaelis and Friedheim, *J. Biol. Chem.*, **91**, 355 (1931).

(3) Elema, *Rec. trav. chim.*, **52**, 569 (1933).

(4) Michaelis, *J. Biol. Chem.*, **92**, 211 (1931); **96**, 703 (1932); Michaelis, Hill and Schubert, *Biochem. Z.*, **255**, 66 (1932); Michaelis and Hill, THIS JOURNAL, **55**, 1481 (1933); Michaelis, "Cold Spring Harbor Symposium Quant. Biol.," **1**, 224 (1933); *Chem. Rev.*, **16**, 243 (1935).

(5) Elema, *Rec. trav. chim.*, **54**, 76 (1935); *J. Biol. Chem.*, **100**, 149 (1933).

(6) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928, Chap. 18.

TABLE I

COMPOSITIONS OF BUFFER SOLUTIONS

No.	1	2	3	4	5	6	7	8
$M/5$ KCl, cc.	250	250	250	100	100	190	40	250
Constituent	..	Potassium acid phthalate			KH_2PO_4			..
Cc., $M/5$	..	250	250	250	250	250	250	..
Constituent	HCl	HCl	HCl	KOH	KOH	KOH	KOH	KOH
Cc., $M/5$	30	130	50	150	150	60	210	250
Water, cc.	220	370	450	500	500	500	500	500

75% aqueous ethanol and later from water until a test sample no longer gave a sulfate test.

The final sample of dye (sample No. 1) was analyzed for sulfur by the Parr bomb sodium peroxide fusion method: sulfur (calcd. for disulfonate), 25.63%; sulfur (found), 23.67, 23.43; purity of disulfonate, 92.3, 91.4%.

That the compound is really the disulfonate and contains no appreciable amount of reducible impurities is evidenced by the electrometric titrations (*cf.* Table II) in which the actual end-point usually varied by not more than a few tenths of a cc. from the end-point calculated on the basis of the above per cent. purity. The compound is probably the 5,5'-disulfonate.[7]

Mr. Ira Avrin kindly furnished us with a small sample (No. 2) of the disulfonate which he reported as being 100% pure on the basis of a sulfur analysis. In this case, however, the electrometric end-points averaged 1.5 cc. higher than the calculated end-points, showing the presence of a reducible impurity.

Apparatus.—Potentiometer, Gray Inst. Co., Queen Potentiometer No. E3040; galvanometer, Leeds and Northrup No. 2500e; standard cell; Weston type, checked frequently against cells at The Detroit Edison Co., which in turn were frequently checked by the U. S. Bureau of Standards; temperature regulation, oil-bath at 25.00 ± 0.05° thermometer, checked against standard thermometers at The University of Chicago through the kindness of Dr. Hugh A. Shadduck.

Materials.—The buffer solutions were modelled after those of Clark.[8] They are listed in Table I. Ordinary C. P. reagents were used and no particular care was taken in their preparation since the *p*H of each was to be determined electrometrically.

Two samples of potassium ferricyanide were used. They were the analyzed grades of the Baker and Kerr Companies, respectively. Both samples gave the same results in electrometric titrations. An analysis of the Baker sample by reduction and subsequent titration with permanganate indicated that it was practically 100% pure.

Procedure.—In each case except run 13 a weighed sample of dye was dissolved in 150 cc. of buffer solution. A small amount of platinized asbestos was added and a rubber stopper (carrying the necessary inlet and outlet tubes, an agar–potassium chloride bridge connecting with the reference cell, and two platinum electrodes, freshly cleaned in hot aqua regia) was inserted in the mouth of the beaker. Hydrogen was then run through the solution until it was yellow. The hydrogen was purified by passing over copper shavings heated to 560–630° in an electric tube furnace and was then passed through a wash bottle containing the pure buffer solution. Nitrogen was substituted for the hydrogen and bubbled through the solution for eighty minutes. To test for complete removal of hydrogen, 0.1 cc. of potassium ferricyanide solution (made by dissolving a weighed amount in a definite volume of buffer) was run in from a buret. The solution reddened, and persistence of the color for five minutes was taken as evidence of complete hydrogen removal. The titration was carried on with the same ferricyanide solution.

During the course of the investigation several saturated calomel electrodes were used as reference cells. Each one was standardized against a hydrogen electrode in 0.05 *N* potassium acid phthalate solution, prepared from U. S. Bureau of Standards phthalate. The potential of this hydrogen electrode was taken arbitrarily to be −0.2350 volt at 25.00°, referred to the normal hydrogen electrode as zero. The *p*H of the buffer solutions was determined by means of a hydrogen electrode, using a calomel electrode as a reference cell. In all cases the potentials of the hydrogen electrodes were corrected for aqueous tension and for atmospheric pressure. European electrochemical conventions were used and the potentials of liquid junctions neglected.

Discussion

Table II contains the complete data for one typical run. Table III summarizes the results of the entire investigation. The symbols have the meanings ascribed to them in the mathematical development.

Run 13 was the first run made and was performed with the use of a nitrogen-filled buret, essentially as described by Clark.[9] In this case no platinized asbestos was present in the reaction chamber. This was followed by runs 14 and 15 using the method described in the experimental part. The agreement of the average values of E_0' indicates that the presence of platinized asbestos has no deleterious effect. The essential agreement in results obtained by using two samples of dye is evidence that the impurities present caused no appreciable error.

It becomes evident upon examination of the values of E_0' (Table II) that they decrease as the titration proceeds. This was found always to be the case. Furthermore, the value of E_0' is always a linear function of the volume of ferricyanide

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(7) Friedländer, *Ber.*, **49**, 959 (1916).

(8) Ref. 1, p. 71.

(9) Ref. 1, p. 35.

TABLE II

REDUCTION POTENTIALS AT 25.00°, RUN 5

Wt. of dye, 0.1039 g.; $K_3Fe(CN)_6$, 0.01219 *M;* *p*H, 2.90; $a = 1.951 \times 10^{-4}$ *M;* (from end-point = 16.0 cc.)

$K_3Fe(CN)_6$, cc.	v	E_h	E_0'
5.00	0.155	0.2189	0.3294
6.00	.156	.2241	.3289
7.00	.157	.2283	.3279
8.00	.158	.2321	.3267
9.00	.159	.2360	.3260
10.00	.160	.2398	.3252
11.00	.161	.2439	.3246
		Avg.	.3269

TABLE III

SUMMARY OF REDUCTION POTENTIALS AT 25.00°

Run	Dye sample	Buffer no.	10^4a moles/l.	*p*H	Av. E_0'
1	1	1	1.247	2.02	0.3903
2	1	2A	2.082	2.82	.3294
3	1	2A	1.386	2.82	.3359
4	1	2B	1.704	2.88	.3311
5	1	2C	1.951	2.90	.3269
6	1	2C	1.216	2.90	.3320
7	1	3A	1.228	3.32	.2975
8	1	3B	1.160	3.48	.2869
9	1	4	1.300	5.30	.2199
10	2	4	1.593	5.30	.2197
11	1	5	1.844	6.97	.1607
12	2	6	1.522	7.35	.1596
13	1	7	1.135	7.61	.1495
14	1	7	1.259	7.61	.1509
15	1	7	1.286	7.61	.1482
16	1	8	1.161	12.59	....

added, individual points almost never falling more than 0.5 millivolt from the line. Since the concentration effect predicted by theory had already been corrected for by the last term in equation IV, and since the values of E_0' change in a direction opposite to that which would be produced by increased acidity (due to the conversion of the ferricyanide into the acid ferrocyanide) the observation was baffling. Cohen and Preisler[10] found a similar effect in the study of ethyl nile blue which they ascribed to the colloidal behavior of their dye. There is no indication, however, that thioindigo disulfonate forms colloidal solutions. To determine whether the ferricyanide was totally responsible, run 17 (Table IV) was performed. The dye was reduced in the usual manner, partially reoxidized by air, and titrated with deaerated buffer stored in the nitrogen-filled buret. From Table IV it is apparent that the potential increases with the dilution at constant percentage oxidation. The relationship is linear. Since in an ordinary run the potential decreases linearly with an increase in the concentration of the oxidant, it is evident than run 17 is brought into qualitative accord with the others by ascribing the concentration effect to the oxidant only. The effect observed in run 17 is also different in magnitude and direction from that predicted by equation IV.

TABLE IV

CONCENTRATION EFFECT, RUN 17

Buffer No. 2A; *p*H, 2.82; wt. dye, 0.1127 g.

v	0.1500	0.1680	0.1788
E_h	.2256	.2292	.2313

Again the concentration effect is evidenced by correcting the average values of E_0' from runs 2–6 to a common value of *p*H and plotting them against the initial concentration. They fall on a straight line within a maximum error of 1.5 mv. Obviously the values of E_0' could be corrected empirically to give better agreement, but since the correction factor appears to be a function of the *p*H, it seems better not to become involved with such empirical corrections.

Note that at every *p*H value except 6.97, at least one run was made in which a had a value rather close to 1.2×10^{-4}. Hence the results should be fairly comparable without correction. When these average values of E_0' from Table III are plotted against the *p*H the points fall on two straight lines intersecting at *p*H 3.9, which is the *pK* value for the first phenolic dissociation of the reductant. The slope of the line below *p*H 3.9 is 0.068 and above this *p*H is 0.030; the theoretical slopes are, respectively, 0.059 and 0.0295. Considering the error introduced by the concentration effect, the agreement is satisfactory.

In run 10 the titration was continued beyond the end-point. The potentials corresponded to those of the ferricyanide–ferrocyanide system.

Run 16 is worthy of special mention. The dye dissolved in the alkaline buffer with a yellow color; the red color did not return when acidified. The yellow solution was reduced for the usual length of time without color change. When ferricyanide was added to this reduced solution a momentary red color was produced. Potential drifts were bad and calculated values of E_0' were not constant. However, a reasonably sharp end-point was obtained which corresponded accurately to a dimolecular reduction, as before.

Although the dye has excellent tinctorial powers and good solubility, the anomalous concentration

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(10) Cohen and Preisler, U. S. Pub. Health Rep., Suppl. No. 92, p. 8.

effect detracts from its usefulness as an oxidation–reduction indicator. Accordingly the chief interest in this investigation lies in the discovery that thioindigo disulfonate undergoes dimolecular reduction. Since in the range of acidity investigated there were no signs of a second step of reduction, and since the E_0'–*p*H curve for hydrogen does not parallel that for the dye, it seems improbable that we are dealing with a meriquinone. Moreover, meriquinones would presumably be deeply colored while our compound is yellow. The mathematical formulation rules out the possibility of a semiquinone. As far as we are aware this is the first case of a thermodynamically reversible dimolecular oxidation–reduction reaction reported in the literature. The discovery has added interest because it has commonly been assumed that the leuco compounds of thioindigo and its derivatives have structures similar to indigo white. We are not yet prepared to hazard a guess as to the structure of the leuco compound. Work is now in progress in this Laboratory in an attempt to answer this question.

Summary

1. The reduction potentials of the system thioindigo disulfonate–leuco compound have been measured from *p*H 2.02 to 7.61 and at 12.59.

2. The potentials were found to be a linear function of the concentration of the oxidant at constant percentage oxidation.

3. In all cases except in strongly alkaline solution the results were shown to conform to the thermodynamic equations developed for a one step dimolecular reduction involving one electron per molecule of dye. In alkaline solution also the reduction is dimolecular.

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[Contribution from the Chemistry Laboratory of the University of Michigan]

Organic Deuterium Compounds. Acetic, Malonic and Succinic Acids

By J. O. Halford and Leigh C. Anderson

Two methods of approach are available for the synthesis of organic compounds containing deuterium. The first, the direct transfer by means of the exchange reaction, has been used, for example, in the preparation of benzene-d_6.[1] It has the obvious disadvantage of requiring the depletion, in deuterium, of relatively large quantities of heavy water, and of making the removal of the last traces of ordinary hydrogen very difficult. The second method is the direct hydrolysis, hydrogenation or hydration of appropriate carbon compounds. Examples of this type of approach are the preparation of acetylene-d_2[2] and of methane-d_4[3] by the hydrolysis of metallic carbides, and the hydration of carbon suboxide to malonic-d_2 acid-d_2[4] as shown by the equation

$$C_3O_2 + 2D_2O \longrightarrow CD_2(COOD)_2$$

When Wilson's description of malonic-d_2 acid-d_2 and of acetic-d_3 acid-*d* appeared, the present authors had been studying the synthesis of these acids by the same method and had samples of both substances available, although the details of the method had not yet been worked out in an entirely satisfactory manner. Wilson's excellent procedure leaves nothing to add, except that the use of a benzene solution of carbon suboxide, rather than the pure liquid, may not be necessary.

The present contribution is concerned with these acids, together with acetic acid-*d*,[5] acetic-d_3 acid, and three new succinic acids. Melting points of the seven compounds are compared with those of the respective "parent" substances and measurements of the vapor pressures of the several acetic acids are presented.

The preparation of succinic-d_4 acid-d_2 employed the reduction of potassium acetylene dicarboxylate by means of a dilute sodium amalgam and deuterium oxide

$$C_2(COO)_2^{--} + 4Na + 4D_2O = (CD_2COO)_2^{--} + 4Na^+ + 4OD^-$$

followed by acidification with DBr in D_2O

$$(CD_2COO)_2^{--} + 2D^+ = (CD_2COOD)_2$$

Succinic-d_4 acid and succinic acid-d_2 were prepared by treating succinic-d_4 acid-d_2 and succinic acid, respectively, with water and deuterium

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(1) Bowman, Benedict and Taylor, This Journal, **57**, 960 (1935).

(2) Clemo and McQuillen, *J. Chem. Soc.*, 851 (1935); Herzberg, Putat and Spinks, *Nature*, **33**, 951 (1934).

(3) Urey and Price, *J. Chem. Phys.*, **2**, 300 (1934).

(4) Wilson, *J. Chem. Soc.*, 492 (1935).

(5) Lewis and Schutz, This Journal, **56**, 493 (1934).