6. Both reactions have equal energies of activation, the value of which is 34,000 calories.

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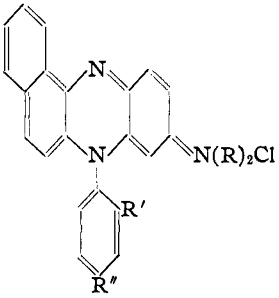
Studies on Oxidation–Reduction. XIX. Aposafranines

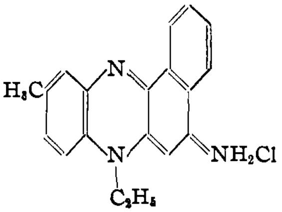
BY ROBERT D. STIEHLER[1] AND W. MANSFIELD CLARK

In the previous paper[2] of this series there were reported the equilibrium potentials of oxidation–reduction systems the oxidants of which are simple safranines. Data for several other azine systems, which may be designated by the names of the following oxidants, are now reported.



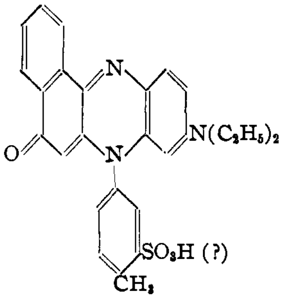
I Neutral blue (Rowe 832): $R = CH_3$; $R' = H$; $R'' = H$
II Isorosinduline No. 1: $R = C_2H_5$; $R' = H$; $R'' = H$
III Isorosinduline No. 2: $R = C_2H_5$; $R' = H$; $R'' = CH_3$
IV Isorosinduline No. 3: $R = C_2H_5$; $R' = CH_3$; $R'' = H$

V



Induline Scarlet (Rowe 827)

VI



Sulfonated Rosindone

We are indebted to Dr. H. A. Lubs and Dr. P. W. Carleton of E. I du Pont de Nemours and Company for authentic samples of all the dyes except induline scarlet

The rosindone is particularly interesting because it provided material with which to study the so-called "concentration effect" discussed in the previous paper. The results of this study support the suggestion of

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[1] R. D. Stiehler presented the details of this study in a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, The Johns Hopkins University.

[2] Stiehler, Chen and Clark, THIS JOURNAL, 55, 891 (1933).

Cohen and Preisler[3] that such a concentration effect is due to a reversible aggregation of a dye.

In the potential maesurements we encountered much the same sort of difficulties that were discussed in the previous paper. In particular it may be said that there remains an element of selection in using the minimal potentials attained after each addition of reducing agent. However, we now have to report the following significant fact. A phosphate was made with resublimed phosphorus pentoxide and potassium hydroxide prepared electrolytically by use of a mercury diaphragm. When the oxidation–reduction systems were placed in buffers made with this material, the usual reversal of potential drift was almost or entirely eliminated; the attainment of a steady potential was slower, and in all cases the amount of reducing agent used before the dye was attacked was appreciably smaller. Spectrograms failed to reveal the difference between this phosphate and the previously used phosphate, which had been prepared from recrystallized U. S. P. phosphoric acid and electrolytic sodium hydroxide. A limited series of titrations made with the "purified" buffer components and involving no reversal of potential drift gave the same constants as titrations in which minimal potentials were used.

Other aspects of electrode behavior, the special precautions, the more general methods and the standards of reference have been adequately described in previous papers.

In accordance with the scheme outlined by Clark and Cohen[4] we may derive for systems I–V the following equation relating electrode potential E_h (referred to the hydrogen standard), concentration of total reductant $[S_R]$, concentration of total oxidant $[S_O]$, hydrion activity (H^+) and apparent dissociation constants of reductant, K'_{r_1} and K'_{r_2}. E_0 is implicitly a function of the activity coefficients of the species involved and can be considered a "normal potential" only when the environment is constant.

$$E_h = E_0 - \frac{RT}{2F}\ln\frac{[S_R]}{[S_O]} + \frac{RT}{2F}\ln\,[K'_{r_1}K'_{r_2} + K'_{r_1}(H^+) + (H^+)^2] \qquad (1)$$

The oxidants of systems I–V possess the highly polar group $=NR_2^+$ throughout the range of P_H considered. Therefore the dissociation constants of the oxidants may be neglected.

Equation (1) applies when no association takes place. In the case of system VI there is evidence of association of the dye in aqueous solution. This was tested by a comparison of spectrophotometric data, potentiometric measurements and the distribution coefficient for the partition of the rosindone between water and amyl alcohol.

Let it be assumed that the unimolecular species OX^- is distributed between water and amyl alcohol in accordance with:

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[3] Cohen and Preisler, Public Health Reports, Supplement 92 (1931).

[4] Clark and Cohen, *Public Health Reports*, **38**, 666 (1923).

$$\frac{[\mathrm{OX}^-]_{\mathrm{alc.}}}{[\mathrm{OX}^-]_{\mathrm{w}}} = \frac{\gamma_{\mathrm{OX}^-\text{ in w}}}{\gamma_{\mathrm{OX}^-\text{ in alc.}}} = k' \tag{2}$$

Assuming a limited range of conditions we may replace the ratio of activity coefficients by the usual distribution coefficient, k'. The same sort of approximation is used in (3). In water let there be equilibrium between unimolecular and bimolecular forms.

$$\frac{[\mathrm{OX}^-]^2_{\mathrm{w}}}{[\mathrm{OX}_2{}^{--}]_{\mathrm{w}}} = K\,\frac{\gamma_{\mathrm{OX}_2{}^{--}}}{\gamma^2_{\mathrm{OX}^-}} = K' \tag{3}$$

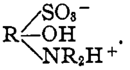
Let $C_{\mathrm{alc.}}$ and C_{w} be the total concentrations of dye in alcohol and water phases, respectively. Then

$$\frac{K'}{2} = \frac{C^2_{\mathrm{alc.}}}{(k')^2C_{\mathrm{w}} - k'C_{\mathrm{alc.}}} \tag{4}$$

In applying this equation it will be assumed that the two solvents do not change their mutual solubilities as the concentration of the dye in each phase changes and, for the purposes of approximation, changes in activity coefficients will be neglected. We shall also neglect higher polymers the consideration of which would greatly complicate the equation.

In the case of the potentiometric measurements with the rosindone, equation (3) must be combined with the ordinary, basic equation similar to (1). There is then obtained equation (5).

$$E_{\mathrm{h}} = E_0 - \frac{RT}{2F}\ln\frac{[\mathrm{S_R}]}{[\mathrm{S_O}]}\,\frac{K' + \sqrt{K'^2 + 8K'[S_{\mathrm{O}}]}}{2K'} + \frac{RT}{2F}\ln\,[K'_{\mathrm{r_1}}K'_{\mathrm{r_2}}(\mathrm{H}^+) + K'_{\mathrm{r_1}}(\mathrm{H}^+)^2 + (\mathrm{H}^+)^3] \tag{5}$$

$K'_{\mathrm{r_1}}$ and $K'_{\mathrm{r_2}}$ are the apparent dissociation constants of the phenolic and substituted ammonium groups of the reductant,  $\mathrm{R}\begin{cases}\mathrm{SO_3}^-\\ \mathrm{OH}\\ \mathrm{NR_2H}^+\end{cases}$. The sulfonic acid is assumed to be completely dissociated in solutions of oxidant and reductant. The range of experimental conditions was such that no change in the dissociation of the oxidant was observed.

Experimental

Neutral Blue (Rowe 832).—The sample was recrystallized from ethylene dibromide. The aqueous solution had a minimal transmittance at $\mathrm{m}\mu = 560$. Table I gives the results of a typical titration and Table II gives data sufficient to establish the relation of E'_0 to P_{H} in acid solutions. Titrations in buffer solutions of P_{H} values higher than 4.2 were not satisfactory because of the very low solubility of the reductant.

Isorosindulines Nos. 1, 2 and 3.—These three dyes are very similar to neutral blue in structure and in behavior. The very limited solubility of each of the reductants precludes accurate measurements in solutions of P_{H} value greater than 4.5 or 5.0. The effects of substituents would have to be considered in connection with dissociation constants which could not be measured because of this limited solubility. Therefore it suffices to state the data of Table III which shows the slight over-all effect of substitution. The concentration effect was not studied.

TABLE I

NEUTRAL BLUE

Titration with chromous acetate. 30°. P_H 2.946. Approximate composition of buffer: 100 ml. 1 *M* citric acid + 50 ml. 1 *M* NaOH diluted to 1 liter. Solution titrated: 75 ml. buffer + 5 ml. 0.0005 *M* dye. Reference P_H (that of 75 ml. buffer + 5 ml. water) 2.946

y	$y - d$	Reduction, %	0.03006 log $[S_R]/[S_O]$	E_h corrected volt	E'_0	Deviation from average
1.0	0.5	6.84	−0.0341	0.0269	−0.0072	0.0000
2.0	1.5	20.52	− .0177	.0106	− .0071	+ .0001
3.0	2.5	34.20	− .0085	.0012	− .0073	− .0001
4.0	3.5	47.88	− .0011	− .0062	− .0073	− .0001
5.0	4.5	61.56	+ .0061	− .0133	− .0072	.0000
6.0	5.5	75.24	.0145	− .0221	(− .0076)	− .0004
7.0	6.5	88.92	.0272	− .0388	(− .0116)	− .0044
	7.31	100.00		Average	− .0072	
				By R. and B. method	− .0073	

TABLE II

NEUTRAL BLUE (ROWE 832)

Relation of E'_0 to P_H (acid solution only) at 30°. Determined by separate titrations with chromous acetate. Total concentration of dye 3 × 10^{-5} *M*. Values used in calculations: $E_0 = +0.1697$; $K'_{r1} = 1 \times 10^{-5}$ (app.) ($pK'_{r1} = 5.0$); $E'_0 = E_0 + 0.03006 \log [K'_{r1}(H^+) + (H^+)^2]$

Buffer	P_H	E'_0 found	E'_0 calcd.	Found-calcd.
HCl	1.072	0.1051	+0.1053	−0.0002
Citrate	2.946	− .0072	− .0072	.0000
Citrate	3.494	− .0396	− .0399	+ .0003
Acetate	4.219	− .0822	− .0820	− .0002

TABLE III

RELATION BETWEEN SYSTEMS I–IV

Values of E'_0 determined by individual titrations with chromous acetate at 30°. E_0 calculated by: $E_0 = E'_0 + 0.06011\ P_H$

	Dye	Buffer	P_H	E_0	E_0
I	Neutral blue (Rowe 832)	Citrate	2.946	−0.0072	+0.1699
II	Isorosinduline No. 1	Citrate	3.514	− .0124	+ .1988
III	Isorosinduline No. 2	Citrate	3.509	− .0162	+ .1947
IV	Isorosinduline No. 3	Citrate	3.503	− .0090	+ .2015

Induline Scarlet (Rowe 827).—The commercial product was purified as follows. It was precipitated from hot aqueous solution by salting it out with potassium chloride. The material was then dissolved in glacial acetic acid, from which it precipitated on addition of ether. Finally, after being salted out of the aqueous solution a second and a third time with minimal quantities of potassium chloride, beautiful, clean crystals were obtained. These were washed cautiously with water.

Anal. (Kjeldahl) Calcd. for $C_{19}H_{18}N_3Cl$: N, 12.98. Found: N, 13.03, 12.82.

In dilute, aqueous acetate buffer the minimal transmittance was at mμ = 500. No concentration effect was observed by means of the spectrophotometer in concentrations as high as 1 × 10^{-3} molar.

Titrations with chromous acetate in hydrochloric acid and acid phosphate buffers were unsatisfactory because of drifting potentials. In acid citrate buffers and in neutral and alkaline buffers satisfactory data were obtained with deviations from the theoretical comparable with those of Table I. Table IV summarizes the data.

TABLE IV

INDULINE SCARLET (ROWE 827)

Relation of E_0' to P_H at 30°. Determined by separate titrations with chromous acetate. Values used in calculations $E_0 = 0.0472$. $K_r' = 3.13 \times 10^{-5}$ ($pK_r' = 4.51$) $E_0' = E_0 + 0.03006 \log [K_r'(H^+) + (H^+)^2]$. Total concentration of dye: 3×10^{-5} *M* (approx.)

Buffer	P_H	E_0' found	E_0' calculated	Found–calcd.
Citrate	2.946	−0.1300	−0.1295	−0.0005
Citrate	3.448	− .1588	− .1590	+ .0002
Acetate	4.645	− .2195	− .2197	+ .0002
Phosphate	6.488	− .2834	− .2831	− .0003
Borate	8.596	− .3465	− .3465	.0000

Sulfonated Rosindone.[5]—The dye was purified as follows. The ampholyte was precipitated from the aqueous solution of the sodium salt by cautious addition of dilute acetic acid. After a day and a half the material had become crystalline. These crystals were filtered and were repeatedly washed with large amounts of distilled water.

Anal. Calcd. for $C_{27}H_{25}O_4N_3S$: N, 8.62; S, 6.58. Calcd. for $C_{27}H_{25}O_4N_3S \cdot H_2O$: N, 8.32; S, 6.35; N/S, 3.00. Found: N, 8.37, 8.45; S, 6.39, 6.38; N/S, 3.01.

The spectrophotometric examination of this dye showed two absorption bands in water, in aqueous buffer solutions, in ethyl alcohol and in amyl alcohol. In the non-aqueous solvents the bands were narrow, the peaks sharp and there was no shift of the wave length at these peaks as the concentration of the dye was changed. In water and in aqueous buffer solutions, on the other hand, the bands were comparatively broad and both the extinction coefficients at one wave length and the wave lengths of the peaks

TABLE V

SULFONATED ROSINDONE, SPECTROPHOTOMETRIC ANALYSIS

Solvent	Concn. mole/liter	Length of soln., mm.	Band peak, mμ	Mol. extn. coeff.[a]	Band peak, mμ	Mol. extn. coeff.[a]
Ethyl alcohol	0.001	0.35[c]	518	4.3×10^4	554	5.7×10^4
	.00001	37.0	518	4.1×10^4	554	5.5×10^4
Amyl alcohol	.001[b]	0.5[c]	518	3.0×10^4	554	4.0×10^4
	.00001[b]	47.0	518	3.0×10^4	554	4.0×10^4
Aqueous borate-KCl buffer, ionic strength = 0.05	.01	0.1[c]	505	2.0×10^4	585	0.1×10^4
	.001	0.55	518	3.8×10^4	572	0.3×10^4
	.0001	5.0	524	4.0×10^4	570[d]	0.5×10^4
	.00001	45.0	527[d]	4.4×10^4	563[d]	1.4×10^4
	.000001	200.0	525[d]	3.0×10^4	562[d]	4.0×10^4

[a] Molecular extinction coefficient = $1/lm \log 1/T$ where l is length of solution in centimeters, m is concentration in mole/liter, and T is transmittance. [b] The actual concentration is slightly less than this value since the dyestuff did not dissolve completely in the original stock solution. The 0.00001 *M* solution was prepared by diluting the 0.001 *M* solution 100 fold. [c] These values may be in error since they are at the limit of the precision of the instrument. [d] These values are estimated since the absorption bands overlap.

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(5) Dr. Carleton tells us that the position of the sulfonic acid group is unknown but that it may be in the position indicated in formula VI.

varied with the concentration of the dye. In all these solvents the absorption bands changed but little with moderate alteration of the salt concentration. Table V summarizes some typical results.

These results suggest that the species present in the alcohol solutions do not change their nature with change in concentration of the dye and that in aqueous solutions such a change does occur.

To test the assumption that association occurs in aqueous solution, a series of experiments upon the distribution of the dye between two solvents was made. Amyl alcohol was the only solvent found which possesses properties satisfactory for the purpose. The results of one of the distribution experiments are given in Table VI where calculations made with equation (4) are summarized. The value of k' was determined from two sets of data in which K' could be eliminated. The agreement in the calculated values of the constant is somewhat misleading since the errors in determining the concentration of the dye in the more dilute solutions would not permit the suggested accuracy. Nevertheless the demand of equation (4) seems to have been met. Visually, the effect is striking. With very small concentrations of the dye, most of the dye is seen in the alcohol layer, while with large concentration the relation is reversed.

TABLE VI

SULFONATED ROSINDONE

Distribution of dye between amyl alcohol and water at room temperature (22° approx.).
$k' = 10$

C_{alc} mole/liter	C_{water} mole/liter	K'	C_{alc} mole/liter	C_{water} mole/liter	K'
0.0003	0.0007	2.6×10^{-6}	0.00008	0.00007	2.0×10^{-6}
.00022	.00048	2.2×10^{-6}	.000065	.000035	2.8×10^{-6}
.00018	.00032	2.2×10^{-6}	.00005	.00002	2.8×10^{-6}
.00014	.00016	2.6×10^{-6}	.00003	.000007	4.4×10^{-6}
.00011	.00009	3.0×10^{-6}	.000015	.000003	3.0×10^{-6}

A somewhat different distribution coefficient would obviously obtain were the aqueous phase a buffer solution. An experiment was made on the distribution of the dye between amyl alcohol and an aqueous solution buffered with the borate–boric acid–potassium chloride system. This gave an association constant approximately the same as that found by the potentiometric measurements with the same buffer solution.

According to equation (5), which involves the apparent polymeric dissociation constant, K', the "titration curve" relating electrode potential to percentage reduction *at constant* (H^+) should be asymmetric. This was found to be the case. However, accurate evaluations of the characteristics of the curve were difficult for the following reason.

Since an appreciable amount[6] of reducing agent, d, is used by the slight impurities of the buffer solution, it is necessary to correct y, the ml. of

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(6) Equivalent to about 2×10^{-6} normal.

reducing agent used at any given stage, by applying this "*d*-correction" before further calculations are made. Ordinarily this "*d*-correction" as well as the total amount, *A*, of reducing agent can be found by the method of Reed and Berkson.[7] In the present instance the asymmetry of the titration curve prevents the application of this method. Therefore estimates of the "*d*" and "*A*" values had to be made by a graphic method which leaves a small element of judgment.

With $[S_O]$ and the ratio $[S_R]/[S_O]$ thus established, K' could be estimated from any two sets of data. In Table VII the value of K' so estimated is rounded to 1×10^{-5}. Its application leads to a fairly satisfactory agreement in the calculated values of E'_0, the potential at 50% reduction. This is the more satisfactory when it is considered that a titration of the dye at a concentration one-tenth that recorded in Table VII gave a value of E'_0 agreeing within 0.1 millivolt. A titration of the dye at ten times the concentration recorded in Table VII gave a value of E'_0 0.9 millivolt more positive. However, the corrections are here the more uncertain and the concentration of the dye is close to saturation in borate–potassium chloride buffers.

There remains the possibility that the dye is not homogeneous, that it consists of two or more monosulfonates and that the dissymmetry of a titration curve is of an origin comparable with that found by Sullivan, Cohen and Clark[8] in their titration of *mixtures* of the sulfonates of indigo. An asymmetry of only 4 millivolts in the titration curve was found in the most dilute solution measured. If this asymmetry be ascribed entirely to a mixture of two or more monosulfonates, then their E_0 values would be at the most 4 millivolts apart. The agreement in the value of the association constant calculated from the distribution experiment and from the potentiometric measurements indicates that the greater part of the asymmetry, if not all, is due to association. Consequently if there is present a mixture of monosulfonates, they have the same, or nearly the same, E_0 values and for our purposes we can neglect the possibility of mixtures and treat the oxidant as a homogeneous compound.

Calculations with the measurements of the rosindone in phosphate buffers yielded a value of K' considerably higher than the value for borate buffers; 5×10^{-5} for phosphate buffers instead of 1×10^{-5} for borate buffers. Accordingly the calculations of Table VIII, which summarize the results, are made with these distinct constants. In this table the apparent acid dissociation constants of the reductant, K'_{r_1} and K'_{r_2} are those estimated by the usual methods (see previous papers of this series).

Part of the difference between the values for K' in the two buffers may be ascribed to a difference in ionic strengths which affect the activities.

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[7] Reed and Berkson, *J. Phys. Chem.*, **33**, 760 (1929); see also Clark and Perkins, THIS JOURNAL, **54**, 1228 (1932).

[8] Sullivan, Cohen and Clark, *Public Health Reports*, **38**, 1669 (1923).

TABLE VII

SULFONATED ROSINDONE

Titration with chromous acetate at 30°. P_H: 8.852. Approximate composition of buffer: 500 ml. 0.2 M boric acid + 40 ml. 1 M NaOH + 60 ml. 1 M KCl; diluted to 1 liter. Solution titrated: 50 ml. buffer + 5 ml. 0.0005 M dye. Reference P_H (that of 50 ml. buffer + 5 ml. water): 8.852. $K' = 1.00 \times 10^{-5}$

y ml.	$y - d$ ml.	Reduction, %	$[S_O]$ moles/liter	$0.030055 \log \frac{[S_R]}{[S_O]}\left(\frac{K' + \sqrt{K'^2 + 8K'[S_O]}}{2K'}\right)$	E_h corr	E_0	Deviation from av.
0.5	0.22	2.953	4.372×10^{-5}	−0.02928	−0.47412	(−0.50340)	−0.00017
1.0	0.72	9.66	4.033×10^{-5}	− .01331	− .48981	(− .50312)	+ .00011
1.5	1.22	16.38	3.700×10^{-5}	− .00587	− .49737	− .50324	− .00001
2.0	1.72	23.09	3.373×10^{-5}	− .00079	− .50248	− .50327	− .00004
2.5	2.22	29.80	3.052×10^{-5}	+ .00320	− .50643	− .50323	.00000
3.0	2.72	36.51	2.736×10^{-5}	.00660	− .50983	− .50323	.00000
3.5	3.22	43.22	2.427×10^{-5}	.00965	− .51289	− .50324	− .00001
4.0	3.72	49.93	2.121×10^{-5}	.01249	− .51573	− .50324	− .00001
4.5	4.22	56.65	1.822×10^{-5}	.01527	− .51849	− .50322	+ .00001
5.0	4.72	63.36	1.527×10^{-5}	.01809	− .52128	− .50319	+ .00004
5.5	5.22	70.07	1.237×10^{-5}	.02108	− .52432	− .50324	− .00001
6.0	5.72	76.78	0.952×10^{-5}	.02442	− .52780	(− .50338)	− .00015
6.5	6.22	83.49	0.671×10^{-5}	.02851	− .53210	(− .50359)	− .00036
7.0	6.72	90.20	0.395×10^{-5}	.03441	− .53859	(− .50418)	− .00095
7.5	7.22	96.91	0.124×10^{-5}	.04740	− .55158	(− .50418)	− .00095
	7.45	100.00			Average	−0.50323	

TABLE VIII

SULFONATED ROSINDONE

Relation of E'_0 to P_H at 30°. Determined by separate titrations with chromous acetate. Values used in calculations: $E_0 = +0.2430$ with phosphate buffers; $+0.2513$ with borate buffers. $K' = 5 \times 10^{-5}$ with phosphate buffers; 1×10^{-5} with borate buffers. $K'_{r_1} = 3.16 \times 10^{-8}$ ($pK'_{r_1} = 7.5$). $K'_{r_2} = 3.16 \times 10^{-10}$ ($pK'_{r_2} = 9.5$). E_0 (corr.) $= E'_0$(found) $+ 0.03006 \log [K' + \sqrt{K'^2 + 8K'[S_O]}]/2K'$. E'_0(calcd.) $= E_0 + 0.03006 \log [K'_{r_1}K'_{r_2}(H^+) + K'_{r_1}(H^+)^2 + (H^+)^3]$

Buffer	P_H	[So] at 50% reduction	E'_0 Found	E'_0 Corr.	E'_0 Calcd.	Corr.-calcd.
Phosphate	6.082	5.5×10^{-6}	−0.3067	−0.3045	−0.3050	+0.0005
Phosphate	6.083	15.2×10^{-6}	− .3119	− .3073	− .3050	− .0023
Phosphate	6.489	15.1×10^{-6}	− .3441	− .3395	− .3410	+ .0015
Phosphate	6.801	14.7×10^{-6}	− .3719	− .3674	− .3679	+ .0005
Phosphate	7.103	15.1×10^{-6}	− .3977	− .3931	− .3931	.0000
Phosphate	7.313	28.7×10^{-6}	− .4150	− .4082	− .4099	+ .0017
Phosphate	7.632	15.1×10^{-6}	− .4395	− .4349	− .4340	− .0009
Phosphate	7.674	21.0×10^{-6}		− .4374	− .4370	− .0004*
Phosphate	7.830	21.3×10^{-6}		− .4480	− .4480	.0000*
Phosphate	7.861	14.7×10^{-6}	− .4531	− .4486	− .4501	+ .0015
Borate	8.172	15.1×10^{-6}	− .4712	− .4603	− .4624	+ .0021
Borate	8.267	5.8×10^{-6}	− .4726	− .4658	− .4685	+ .0027
Borate	8.352	14.5×10^{-6}	− .4790	− .4682	− .4738	+ .0056
Borate	8.852	21.1×10^{-6}		− .5032	− .5032	.0000*
Borate	9.304	14.8×10^{-6}	− .5393	− .5285	− .5270	− .0015
Borate	9.467	14.9×10^{-6}	− .5461	− .5352	− .5347	− .0005
Borate	9.735	14.9×10^{-6}	− .5570	− .5461	− .5463	+ .0002
Borate	9.810	21.1×10^{-6}		− .5495	− .5494	− .0001*
Phosphate	10.689	14.9×10^{-6}	− .5911	− .5865	− .5885	+ .0020

* The starred values were calculated by equation (5) giving E'_0 (corr.) directly. The other values were estimated graphically and the E'_0 (Found) was then corrected for [So], consequently not as much weight was placed on these values. The error introduced by graphical estimation was not sufficient to warrant the more laborious calculations.

However, in this, as in the previous investigation, our preliminary measurements indicate that moderate variation of ionic strength causes a second order effect. We believe that the salt effect is largely specific and comparable to that noted by Sullivan, Cohen and Clark[8] in the cases of the sulfonates of indigo which behaved differently in borate and in phosphate buffers.

Since association of the dye is doubtless a first step toward the formation of a precipitable aggregate, it is important to note that our approximate measurements of the solubilities of the dye in solutions containing various buffer and neutral salts indicate that the potentiometric measurements were conducted with solutions of the dye too dilute for precipitation.

Discussion

In Fig. 1 are shown the curves relating E'_0 to P_H for each of the systems studied. E'_0 is the potential at 50% reduction. In the figure only the experimental data for complete titrations are shown. Less accurate supplementary data, as, for instance, those obtained by the method of mixtures, indicate that the curves are properly extrapolated, provided no resolution of the titration curve comparable to that found by Michaelis[9] in the case of rosinduline 2G in very acid solutions appears. For purposes of orientation the curves for several other systems are shown in Fig. 1. Curve 2G is for rosinduline 2G (Rowe 830), drawn according to the first data of Michaelis.[10] Rosinduline 2G has a structure very similar to VI except that it has no substituted amino group.

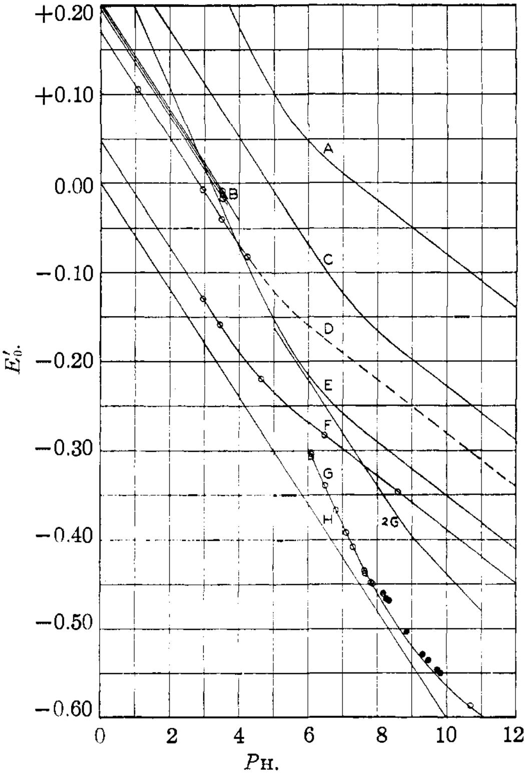

Fig. 1.—Relation of E_0' to *P*H (E_0'; potential in volts, referred to the hydrogen electrode standard, when dye is 50% reduced): A, methylene blue; B, the isorosindulines Nos. 1, 2 and 3; C, indigo disulfonate; D, neutral blue; E, dimethylphenosafranine; F, induline scarlet; G, sulfonated rosindone; O, in phosphate buffers; ●, in borate buffers; 2G, rosinduline 2G (after Michaelis); H, hydrogen electrode, 1 atm. hydrogen.

The system of induline scarlet and that of the rosindone nearly supplement one another as oxidation–reduction indicators with characteristic potentials near that of the hydrogen electrode under one atmosphere of hydrogen. In the case of the sulfonated rosindone at a concentration of total dye of $3 \times 10^{-5}M$, 90% reduction, and *P*H 7.0 the potential is that of the hydrogen electrode under one atmosphere of hydrogen. We are thus encouraged to believe that these dyes will be useful in certain biochemical studies which have been delayed for lack of such indicators; but we must add the caution that in practical applications the several peculiar properties of these systems will have to be considered.

Since the reductants of the isorosindulines, I–IV, are practically insoluble in buffers of *P*H values greater than about 4.5 or 5.0, these systems

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(9) Michaelis, *J. Biol. Chem.*, **92**, 211 (1931).

(10) Michaelis, *ibid.*, **91**, 369 (1931).

are of less biochemical interest. The effects of substitution are slight but appreciable. An analysis of such effects would require knowledge of the dissociation constants.

With regard to the postulate of a reversible association of the sulfonated rosindone it may be said that there is now a first order agreement between the results of the potentiometric measurements and the results of the study of the distribution coefficient between aqueous solutions and amyl alcohol. The purely qualitative spectrophotometric data admit the same explanation. Undoubtedly the following steps of aggregation should be considered: $2\mathrm{O_X} \rightleftharpoons \mathrm{O_{X_2}}$; $\mathrm{O_X} + \mathrm{O_{X_2}} \rightleftharpoons \mathrm{O_{X_3}}$, etc. But obviously the resulting equations would be complex and would involve too many constants for a practical test. Therefore only the first order approximation can be sought with the assumptions used. The calculations suggest that if the number of molecules in the predominant aggregate present in the alcohol is X, the numbers present in the predominant aggregates of the aqueous phase are X and $2X$, the proportion depending upon the concentration. If X were other than unity the equation involving the electrode potential would be so seriously altered that it is doubtful whether the application of equation (5) would have led to the agreement found. Therefore the "internal evidence" seems to justify the assumptions made.

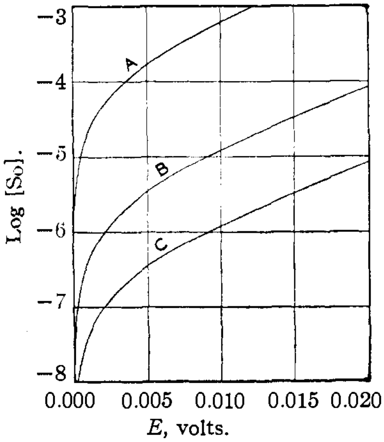

Fig. 2.—Effect of association of oxidant upon potential, K', the apparent polymeric dissociation constant: A, for $K' = 5 \times 10^{-4}$; B, for $K' = 1 \times 10^{-5}$; C, for $K' = 1 \times 10^{-6}$.

In the previous paper Stiehler, Chen and Clark[2] reported several very careful titrations of safranines. Their titration curves were analyzed by the method of Reed and Berkson and failed to show the dissymmetry predicted from the evidence of aggregation found by separate titrations at different concentrations. They noted, however, that the titrations which showed no asymmetry might have been made at unsuitable concentrations.

An examination of equation (5) will show that when the contribution of aggregation to the observed potential is plotted against log $[\mathrm{S_O}]$ there is obtained a family of curves with K' as a parameter (see Fig. 2). Curve A approximately represents the case of dimethylphenosafranine.[11] If, during a titration of this dye, the value of log $[\mathrm{S_O}]$ falls from -4.22 to -6.22, the contribution of declining association to the observed potentials should be about 2 millivolts. The resulting dissymmetry of the

(11) See Stiehler, Chen and Clark, Ref. 2, p. 897.

titration curve would escape visual inspection unless the value of the "*d*" correction were known. When such a curve is treated as if symmetrical by the method of Reed and Berkson, the "*d*" and "*A*" values are automatically adjusted so that the calculated values of E_0' appear constant within experimental error. In the earlier work on methylene blue[12] and on Nile blue[3] the element of judgment remained in the selection of "*A*" values and the slight dissymmetry of a titration curve was obscured.

Therefore the more reliable test of the "concentration effect" is that obtained when a fixed ratio of total oxidant and total reductant is used at different concentrations of the sum. Unfortunately there are limitations to the exploration of the entire course illustrated in Fig. 2. A test at dilutions great enough to bring about complete dissociation would involve measurements at concentrations of the order of millionth molar or less. This involves large experimental errors. Use of relatively high concentrations is precluded in some instances by limited solubilities and in all cases involves some uncertainty of correction for the effect of the oxidation–reduction system upon the buffer system. Therefore the empirical linear relation $\tilde{E} = E_0 + A \log C$ used in the previous paper[2] was legitimate as a first approximation applicable within narrow limits of concentration.[13] The better analysis in the case of the rosindone is made possible by the fortunate circumstance that the greatest change in association takes place within a range of concentration adapted to the potentiometric measurements (see curve B, Fig. 2).

Summary

The equilibrium potentials of six oxidation–reduction systems in which aposafranines are the oxidants have been determined at 30°.

Neutral blue (Rowe 832) and three other closely related isorosindulines have the following "normal potentials," E_0: Neutral blue, 0.170; Isorosinduline No. 1, 0.199; Isorosinduline No. 2, 0.195; Isorosinduline No. 3, 0.202. In acid solution $\Delta E_h/\Delta P_H = -0.0601$. The low solubilities of the free bases prevented measurements in neutral and alkaline solutions. A rough estimate of the dissociation constant of the neutral blue cation, treated as an acid, is 1×10^{-5}.

The system of which induline scarlet (Rowe 827) is the oxidant gave electrode potentials which may be described by the equation

$$E_h = 0.047 - 0.03006 \log [S_R]/[S_O] + 0.03006 \log [K'_{r1}(H^+) + (H^+)^2]$$

K'_{r_1}, the apparent first dissociation constant of the cation of the reductant is 3.1×10^{-5} ($pK'_{r_1} = 4.5$). At $P_H = 7.0$ and $[S_R]/[S_O] = 1$, E_h, $=$ -0.299.

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(12) Clark, Cohen and Gibbs, *Public Health Reports*, **40,** 1131 (1925).

(13) The first value of $\tilde{E}_0$ in Table III of the previous communication is brought nearer to the average by the full correction.

The system of which the sulfonated rosindone is the oxidant gave electrode potentials which may be described by the equation

$$E_h = E_0 - 0.03006 \log [S_R]/[S_O] - 0.03006 \log \left[K' + \sqrt{K_2' + 8K'[S_O]}\right]/2K' + 0.03006 \log [K'_{r_1}K'_{r_2}(H^+) + K'_{r_1}(H^+)^2 + (H^+)^3]$$

$K'_{r_1} = 3.2 \times 10^{-8}$ ($pK'_{r_1} = 7.5$); $K'_{r_2} = 3.2 \times 10^{-10}$ ($pK'_{r_2} = 9.5$). K' is the apparent dissociation constant for an equilibrium between uni- and bimolecular species. K' is approximately 5×10^{-5} when the dye is in phosphate buffers and 1×10^{-5} when the dye is in borate buffers. This specific salt effect is also reflected in the extrapolated values of E_0, 0.243 for phosphate and 0.251 for borate buffers.

Other evidences of a reversible association of the rosindone in aqueous or aqueous buffer solutions are: (1) the qualitative analysis of the changes in the spectrophotometric absorption curves which follow dilution; (2) the analysis of quantitative studies on the distribution of the dye between water and amyl alcohol. The values of K', estimated by the distribution studies, are in substantial agreement with those estimated by the potentiometric measurements.

When the sulfonated rosindone is 90% reduced, $P\text{H} = 7.0$ (phosphate buffer) and total dye concentration is 3×10^{-5} M, the electrode potential is that of the hydrogen electrode under 1 atmosphere of hydrogen. This system gives potentials nearer that of the hydrogen electrode than any system previously reported in this series of papers on oxidation-reduction indicators.

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The Homogeneous Thermal Polymerization of Isoprene

BY WILLIAM E. VAUGHAN[1]

In an earlier publication[2] there has been reported an investigation of the homogeneous thermal polymerization of 1,3-butadiene, and in that paper there has been given a brief review of the several similar reactions hitherto studied, together with a cursory survey of some of the theoretical aspects of the general problem of association processes. There is thus no need to repeat that material. Suffice it to say that Kassel's contention[3] that the formation of polyatomic molecules by a second-order association process is possible at a relatively large fraction of all collisions, is well supported by the experimental evidence thus far presented. It may also be mentioned that the data for the three reactions considered, namely,

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(1) National Research Fellow in Chemistry.

(2) Vaughan, THIS JOURNAL, **54**, 3863 (1932).

(3) Kassel, "Kinetics of Homogeneous Gas Reactions," Chemical Catalog Co., Inc., N. Y., 1932, pp. 44-47; also THIS JOURNAL, **53**, 2143 (1931).