[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND THE PHYSICS DEPARTMENT OF COLUMBIA UNIVERSITY]

The Paramagnetism of the Semiquinone of Phenanthrenequinone-3-sulfonate

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A molecular species on the oxidation level of a quinhydrone may be either a semiquinone radical or a dimeric valence-saturated compound. One method for the decision between these alternatives has been the analysis of the potentiometric titration curve, another method may be based on an investigation into the magnetic properties. A semiquinone radical contains an unpaired electron and would be expected to be paramagnetic. A dimeric, valence-saturated compound, is expected to be diamagnetic, with a susceptibility of a lower order of magnitude, as compared with that of a radical. A restricted number of quinhydrone-like compounds have been measured, but only in the crystalline state. The crystalline quinhydrone of pyocyanine has been found to be paramagnetic by Katz[1] although not to the full extent expected theoretically for a radical. In some other organic compounds known to be radicals, the numerical value of the paramagnetic susceptibility had been found to be in agreement with Van Vleck's theory of paramagnetic susceptibility for molecules with an odd number of electrons especially by Galavics,[2] Müller[3] and by Katz.[1]

No magnetic measurement has been carried out as yet for quinhydrones in the dissolved state. Since all potentiometric experiments give information only for the dissolved state, magnetic measurements should be carried out also on solutions in order to obtain comparable results. Most dyestuffs are not soluble enough for the purpose. A suitable material appeared to be the quinhydrone of phenanthrenequinone-3-sulfonate[4] which is sufficiently soluble, and for this and other reasons has served the purpose especially well for the potentiometric method. This method showed the following result. The intermediate form is in part the semiquinone radical,[5] in part the dimeric, valence-saturated form. In acid solution the semiquinone exists only to a very slight extent, whereas the dimeric form may exist in an appreciable amount provided the total concentration of the dye is great. In alkaline solution the intermediate form is always capable of existence to a large extent. It is almost entirely the radical at very low concentration of the dye, and it is in part a radical, in part a dimer at higher total concentration of the dye.

Usually one important difficulty in the obtaining of reliable magnetic measurements is the elimination of traces of impurities of paramagnetic character and, furthermore, the correction for the diamagnetism of the solvent. Since one is compelled to work in rather dilute solution this correction may be very great and troublesome. These difficulties are eliminated by a method based on the following principle. The dyestuff is dissolved in a solution containing a reducing agent such as would bring about the reduction quite slowly, say within one hour or two. During this period a series of magnetic measurements is carried out. If, during the reduction, a semiquinone radical should arise as intermediate step of the reduction, it should manifest itself by decreasing the original diamagnetism. The diamagnetism of the solution as a whole may be comparatively high. However, during the process of reduction, no change in diamagnetism of any appreciable extent can be expected. If during the reduction the diamagnetic susceptibility should decrease in the first half of the reduction and again rise in the second half, this change can be attributed to the formation and, later on, the disappearance of a paramagnetic molecular species.

On working in alkaline solutions and at room temperature, glucose is a reducing agent of the desired property. It is not so easy to find a reducing agent acting slowly and in homogeneous solution in an acid medium in the way glucose does in an alkaline medium. So we restrict ourselves for the time being to experiments in alkaline solution.

Apparatus and Method.—Because of the small concentration of dye in solution, the diamagnetism of the solvent will greatly outweigh the para-

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(1) H. Katz, *Z. Physik*, **87**, 238 (1933).

(2) I. Galavics, *ibid.*, **6**, 555 (1933).

(3) E. Müller, I. Müller-Rodloff and W. Bunge, *Ann.*, **520**, 235 (1935).

(4) L. Michaelis and M. P. Schubert, *J. Biol. Chem.*, **119**, 133 (1937); L. Michaelis and E. S. Fetcher, THIS JOURNAL, **59**, 2460 (1937).

(5) A semiquinone may be defined as an organic molecule with an odd number of electrons which in the dissolved state is in mobile equilibrium with a molecular species containing one more electron and another with one electron less.

magnetism of the free radical, so the effect can be detected only as a small change of the diamagnetic susceptibility of the solution. This necessitates an extremely sensitive measuring device.

The apparatus used was the manometric balance of Wills and Boeker[6] as modified by Woodbridge.[7] The principle of the apparatus is that of balancing the actions of two magnetic fields on two liquid columns. The strengths of the magnetic fields at the free surfaces or menisci being designated by H and H_0, the condition for balance is

$$\tfrac{1}{2}(K_0 - K_\alpha)H_0^2 = \tfrac{1}{2}(K - K_\alpha)H^2$$

where K_0 is the volume susceptibility of the unknown liquid whose meniscus is in the field H_0, K that of the standard comparison liquid whose meniscus is in the field H, and K_α that of the gas in contact with the liquid surfaces. In this experiment the gas in contact with the menisci was carefully purified hydrogen for which the volume susceptibility is negligible, so that K_α can be disregarded. The expression then reduces to

$$K_0/K = (H/H_0)^2$$

Procedure and Results.—The test solutions were made up by dissolving varying amounts of the dye, suitable amounts of glucose (from 0.5 to 1 g.), and 2 cc. of 1.21 M sodium hydroxide in water to a total volume of 25 cc. This solution was placed immediately in the apparatus. The first magnetic measurement was taken as soon as possible and additional readings were taken frequently during the run until the reaction was complete and the readings became constant in time. The temperature was kept at 21.0°.

The results of three runs are given in Fig. 1, which shows the changes in volume susceptibilities as functions of the time of reaction for three solutions of differing concentrations of the dye. These differences were calculated by subtracting the susceptibility of the solution for which the reduction was completed ($t = \infty$) from the susceptibility of the solution at time t. Duplicate runs confirmed these results within the limits of experimental error.

If we rely on the assumption, confirmed for a number of organic radicals by E. Müller,[3] that such an organic molecule is in what is called the Σ state in which all contributions of electron orbits to paramagnetism vanish and the spin of the odd electron is the only source of paramagnetism, the concentration of the radical can be calculated from the formula derived by Van Vleck[8]

$$\chi_m = \frac{4N\beta^2S(S+1)}{3\,KT} + N_\alpha$$

where χ_m = molar susceptibility
N = Avogadro's number
β = Bohr magneton (0.9174×10^{-20} erg. gauss^{-1})
K = Boltzmann constant
T = absolute temperature
S = spin quantum number, here = $^1/_2$

N_α is a term independent of temperature, very small compared with the first term. It will be neglected here. Then, the numerical value of χ_m at 21° is approximately 0.00126.

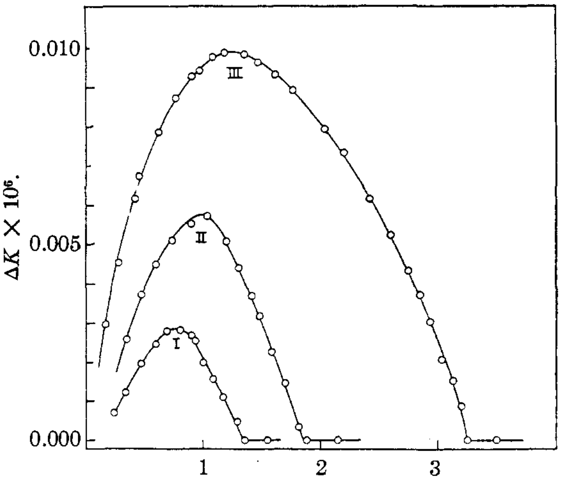

Fig. 1.

For any solution the contribution of a solute to the volume susceptibility will be $\chi_m \times M/1000$ where χ_m is the molar susceptibility and M the molar concentration. Setting this equal to the changes in the volume susceptibilities of our experiments, we obtain the molar concentration of the free radical.

We are particularly interested in the concentration of the free radical corresponding to the maximum change in diamagnetism which, as expected, appears in the half-reduced state. These values, calculated by the above procedure, are given in the fifth column of Table I. The sixth column gives the total concentration of dye, and the seventh column contains the calculated maximal fraction of the dye in the free radical form.

Errors.—The dye, originally yellow, passes on reduction in an alkaline solution through an intense black-brown and then fades out again to

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(6) A. P. Wills and G. F. Boeker, *Phys. Rev.*, **42**, 687 (1932).

(7) D. B. Woodbridge, *Phys. Rev.*, **48**, 672 (1935).

(8) J. H. Van Vleck, "Electric and Magnetic Susceptibilities," Oxford University Press, 1932, p. 274.

TABLE I

Solution	$K_1 \times 10^6$	$K_2 \times 10^6$	$K \times 10^6$	s	a	$(s/a)_{\text{max.}}$	*p*H
1	−0.72330	−0.72042	0.00288	0.00222	0.00503	0.44	11.6
2	− .72584	− .72008	.00576	.00458	.0126	.36	11.57
3	− .72906	− .71914	.00992	.00787	.0252	.31	11.49

K_1 = volume susceptibility at end of reaction
K_2 = volume susceptibility at half reduced state
K = maximum difference in volume susceptibility
s = calculated molar concentration of the semiquinone radical
a = molar concentration of dye added
$(s/a)_{\text{max.}}$ = maximal fraction of the dye in free radical form.

light yellow. Because of the deep color which the dye takes on in the most important stage of the observation, the usual sharp outline of the meniscus became less distinct. The error due to this was minimized by keeping this meniscus in the maximum portion of the field where the magnetic gradient was found to be very small. With this precaution we were able to duplicate any reading to within less than 0.0002×10^{-6}.

In calculating the molar susceptibilities from measurements of volume susceptibility the tacit assumption was made that the density of the solution remained constant during a reaction. Separate experiments showed that density changes are less than 0.01% and therefore do not affect the result. Other errors which enter into an absolute determination of susceptibility are not important for the small differences which we obtained.

*p*H was determined by a parallel experiment. The solution was poured (at 24°) into a glass electrode and *p*H measured during the whole period of reduction. The readings varied very little during this reaction, in experiment 1 not at all within the limits of error. The reading recorded here is the one at one-half the time of complete reduction. These readings are not corrected for liquid junction potentials or the error inherent in the glass electrode at such a high *p*H. The necessary correction may be estimated as about +0.2.

There is a radical formed and its maximum concentration obtains at the time where the reduction is half completed. Furthermore, this maximum ratio of the radical to the total dye decreases with increasing concentration of the dye, showing that the intermediate form is present not only as the radical but also in non-paramagnetic dimeric form. The data are not sufficient for a strictly quantitative comparison of these results with those obtained potentiometrically. A calculation using the constants determined by the potentiometric methods shows a rather satisfactory, though not perfect agreement, but the data are not sufficient to emphasize too much the quantitative side of this agreement at the present stage of the technical development of the method, especially because the working conditions could not yet be made quite equal for the two methods.

We are deeply indebted to Columbia University for the use of its laboratory facilities and especially to the late Professor A. P. Wills for his encouragement and advice during the initial stages of this research.

Summary

The measurement of the changes in the magnetic susceptibility of phenanthrenequinone-3-sulfonate during its reduction by glucose in alkaline solution confirms the result previously obtained with the potentiometric method that there is an intermediate stage of reduction represented in part by a free radical. This is detected by its paramagnetic property which manifests itself by decreasing the diamagnetism of the whole solution. The maximum amount of the radical arises at 50% reduction.

NEW YORK, N. Y.

RECEIVED NOVEMBER 8, 1937