Studies of the ESR Spectra of Semiquinone Anions of Xanthene Dyes. Variation in the Linewidth of the Phloxine Semiquinone Anion with the Temperature and the Viscosity in Protic Solvents

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The ESR linewidths of semiquinone anions of xanthene dyes in alkaline protic solvents were studied as functions of the temperature and the viscosity. It was found that the linewidths of phloxine semiquinone, the ESR spectra of which show three hyperfine lines due to two equivalent protons, decrease with a decrease in the temperature or an increase in the viscosity of the solvents, and vary unimolecularly. Such a variation can not be explained either by a usual "chemical exchange" process between different states or by intramolecular motion. We propose that the linewidths may be due to the modulation of the hyperfine coupling constants of H atoms; the modulation results from the dissociation and/or distortion of the hydrogen bonds.

We previously reported the formation of stable semiquinone anions of xanthene dyes by the γ-irradiation of degassed alkaline alcoholic solutions, and the photo-dehalogenation reaction of the halogenated ones.[1]

In the course of ESR studies of these semiquinone anions, we have found that the linewidths of the ESR spectra of some of them, *e. g.*, phloxine semiquinone (X=Br and Y=Cl, in Fig. 1), decrease without any further splitting of their three lines with a decrease in the temperature or an increase in the viscosity of solutions, and that the process of sharpening is pseudo-unimolecular.

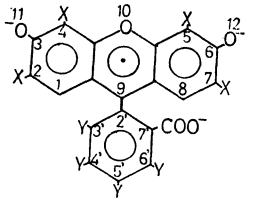

Fig. 1. Structure of xanthene-dye semiquinone anion.

The linewidth variation can not be explained in terms of a "chemical exchange" process between different states or the relaxation matrix theory developed by Freed *et al.*[2]

The present results seem to suggest that hydrogen bonding between semiquinone anions and solvent molecules plays an important role in the variation of linewidths. This paper will describe the results obtained with phloxine semiquinone and the discussion leading to the conclusion put forward above.

Experimental

Materials. The xanthene dyes were recrystallized three times from slightly alkaline methanolic solutions. The alcohols and water were purified by distillation as has been described previously.[1] The glycerol was distilled *in vacuo*. Extra pure lithium, sodium, and potassium hydroxides were used without further purification.

Preparation of Semiquinone Solutions. Xanthene semiquinones (S) were prepared by the ^{60}Co γ-irradiation of degassed alkaline solutions of xanthene dyes (D) in alcohols or in water containing small amounts of methanol at room temperature. The reduction of D proceeds by these reactions:

$$RCH_2OH \xleftarrow{\gamma} e^-_{sol},\ \dot{H},\ \dot{R}CHOH$$

$$D + e^-_{sol} \longrightarrow S$$

$$D + \dot{H} \xrightarrow{OH^-} S + H_2O$$

$$D + \dot{R}CHOH \xrightarrow{OH^-} S + RCHO + H_2O$$

This method gives S in a higher yield and with fewer by-products than other methods, such as chemical or photochemical reduction. The concentration of S produced was adjustable by controlling the initial concentration of the dye and the γ-ray dose, and was determined spectrophotometrically. The semiquinone solutions were so stable in the dark that no detectable change in the concentration was found during ESR measurements.

The ESR spectra were recorded by using a JEOL JES-X spectrometer at various temperatures between -100 and 60 °C with an accuracy of $\pm$0.5 °C. The temperature of the cavity was carefully kept constant by circulating thermostated water through a pipe around the cavity. The magnetic field was calibrated by means of proton resonance.

Results

The Lineshape and Temperature Dependence of the Linewidth. Phloxine semiquinone gives three well-resolved hyperfine lines with an intensity ratio of 1 : 2 : 1 in the temperature range of -80—$+60$ °C. They have the Lorentzian shape and equal linewidths, independent of the total nuclear spin quantum number, M_1, in the temperature range of -50—$+5$ °C. Below -60 °C, the lines were broadened heterogeneously and were dependent on M_1, indicating "anisotropic broadening."

On the other hand, as is shown in Fig. 2, the linewidth of 1.02$\pm$0.02 gauss at 23 °C in methanolic solutions (T/η=510 K/cp) decreases with a decrease in the temperature to attain a minimum of 0.4$\pm$0.02 gauss at -58 °C (T/η=80 K/cp). No such a variation in the linewidth has been previously reported, and so detailed experiments were focused on this point.

Effects of Solutes. If alkali metal ions play an important role in some such a manner as ion-pair formation or any "chemical exchanges," the concentration and the kind of metal ion should affect the linewidth variation with the temperature. However, no

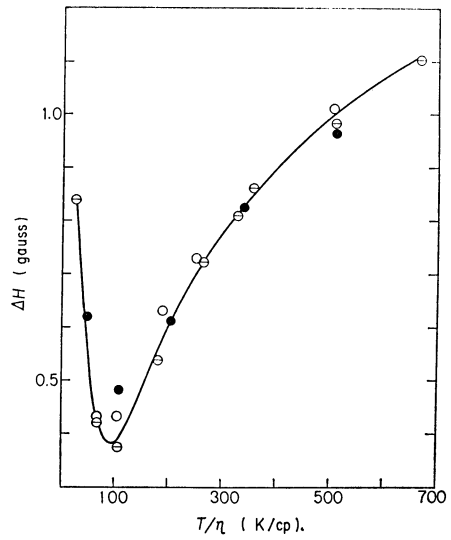

Fig. 2. Temperature dependence of the linewidth of phloxine semiquinone anion in alkaline methanolic solutions: Concentrations of the semiquinone and unreduced phloxine are: ⊖ [2.9×10^{-4}, 6.4×10^{-5} M; ○ 4.2×10^{-5}, 1.4×10^{-5} M; ● 1.3×10^{-3}, 5×10^{-3} M. [KOH], 0.1 M.

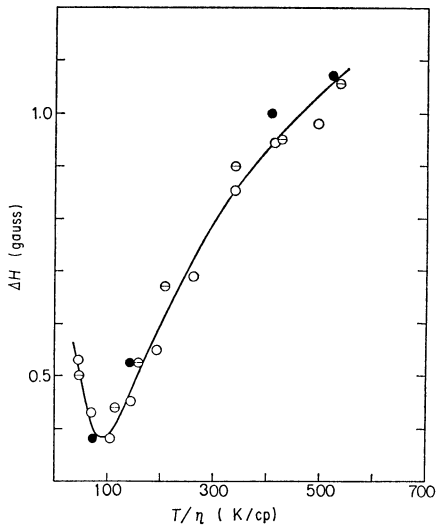

Fig. 3. Variation of linewidth with T/η at various concentrations of KOH in methanol: ○ 0.2 M, ⊖ 0.04 M, ● 0.005 M. Concentration of the semiquinone is about 1.5×10^{-4} M.

essential change in the curve was observed over a range of KOH concentrations (Fig. 3). The variation in the linewidth with T/η was compared for LiOH, KOH, and NaOH solutions, but no detectable differences were observed among these systems, as is shown in Fig. 4. Therefore, it may be concluded that the effect of the metal ions on the linewidth is negligible in the present case; any effect on the linewidths by protons is also ruled out.

Neither the dependence of the semiquinone concentration on the linewidth up to 1.3×10^{-3} M, nor the effects of unreduced parent phloxide $D(5\times10^{-5}$—5×10^{-3} M) on the linewidth of the semiquinone (10^{-4} M), were observed between 35 and −75 °C. (Fig. 2).

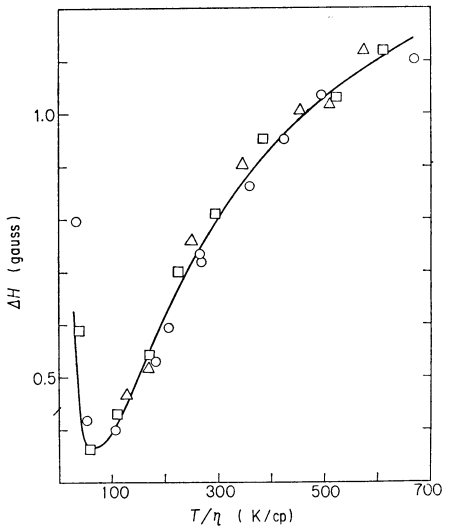

Fig. 4. Variation of linewidth with T/η in the 0.05 M solutions of KOH (○), NaOH (□), and LiOH (△).

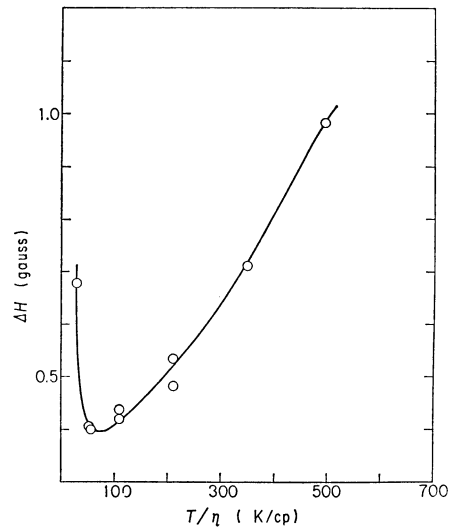

Fig. 5. Dependence of linewidth on solvent viscosity at 22°C. The viscosity was varied by changing the concentration of glycerol (10, 20, 30, 40, and 50 vol%) in methanolic solutions.

It is apparent, therefore, that the variation in the linewidth proceeds unimolecularly.

Dependence of the Linewidth on the Viscosity of a Solvent. Since the viscosity of the solution varies with the temperature, measurements were made of the net viscosity effect on the linewidth, using solutions of various compositions of glycerol in methanol at room temperature. The linewidths at 22 °C are shown in Fig. 5 as a function of T/η for mixtures containing 10, 20, 30, 40, and 50 vol% glycerol in methanol, where the concentration of the semiquinone was kept constant in the range of 7.2×10^{-5}—1.42×10^{-4} M. Figure 5 shows that the increase in viscosity caused a decrease in the linewidth of about 0.6 G, which is the same amount of variation as when the temperature was lowered; it also shows that the linewidth has a minimum value of

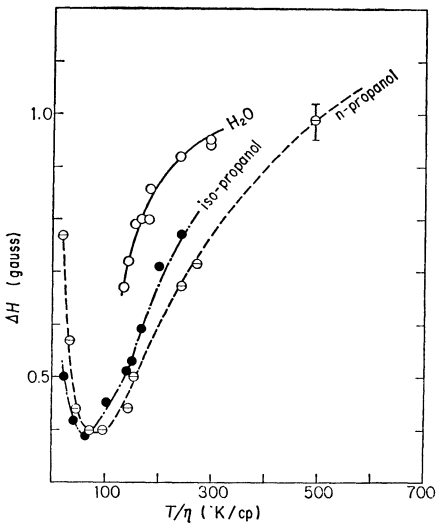

Fig. 6. Variation of linewidth with T/η in 1-propanol (⊖), 2-propanol (●), and aqueous solutions. (○).

about 0.4 Gauss at $T/\eta=80$ K/cp. These results indicate that the linewidth is strongly dependent on the viscosity of the solvent, or that the linewidth variation is due to the motion of the solvent molecules. The temperature dependence of the linewidth mentioned above may also be explained as being due to the motion of solvent molecules rather than to an intramolecular motion of the semiquinone.

Effect of Solvents. The ESR spectra of the phloxine semiquinone were also taken in other protic solvents: 1-propanol, 2-propanol, and water. The linewidths in three solvents were 0.46, 0.51, and 0.96 Gauss respectively at room temperature. However, the plots of the linewidth against T/η are similar to that in the methanolic solution except for slight differences in curvatures, as is shown in Fig. 6. Since semiquinones were found to be very unstable in aprotic solvents or even in alcoholic solutions containing only a few tens vol% of aprotic solvents, no experiment has been carried out.

Dependence on the Temperature of Hyperfine Coupling Constants of Two Protons in Xanthyl Ring. In Fig. 7 is shown the temperature dependence of a sum of the hyperfine coupling constants of two protons (2a in Fig. 7) in a methanolic solution. For the other solvents mentioned above, a similar dependence was also obtained within the range of experimental errors.

Measurements at the Q-band were also carried out at room temperature, but no difference from the above results at X-band was observed in either the linewidth or the line-shape. Therefore, it may be concluded that the linewidth is not dependent on the static magnetic field, H_0.

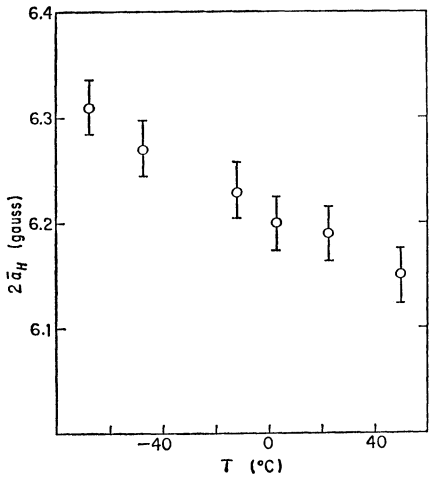

Fig. 7. Temperature dependence of hyperfine coupling constants of protons. The $2a_H$ means the sum of the coupling constants of two equivalent protons.

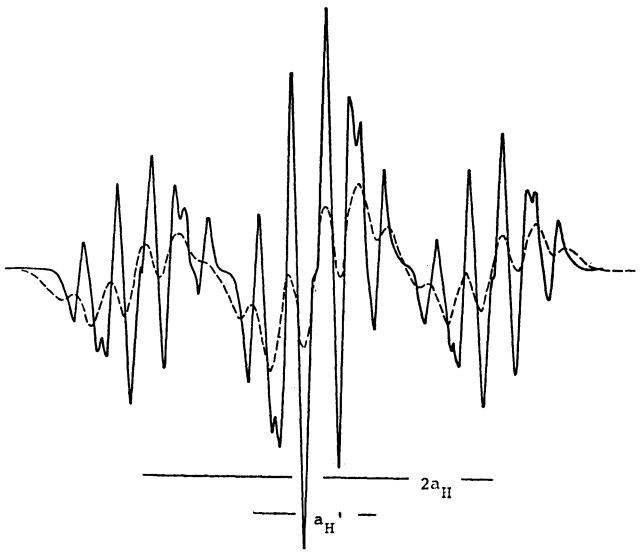

Fig. 8. ESR spectra of eosine semiquinone anion: ——, 24 °C; ----, −54 °C.

ESR Spectra of Eosine Semiquinone. The linewidths of eosine semiquinone (X=Br and Y=H) increase with a decrease in the temperature, while the total hyperfine coupling constants of the four hydrogen atoms on the 9-phenyl ring increase slightly (Fig. 8 and Table 1). From the spectral simulation, it was found that large three groups with an intensity ratio of 1 : 2 : 1 are ascribed to two equivalent protons at the 1- and 8-positions on the xanthyl ring. Each group is further split into lines with an intensity ratio of 1 : 1 : 3 : 3 : 3 : 3 : 1 : 1 by three nearly-equivalent protons with hyperfine coupling constants of 0.3 and by one proton

TABLE 1. TEMPERATURE DEPENDENCE OF HYPERFINE COUPLING CONSTANTS OF EOSINE SEMIQUINONE

Temp. (°C)	24.0	4.0	−17.0	−31.0	−43.3	−54.4
a_H' (G)	2.3_0	2.3_0	2.3_4	2.3_7	2.4_0	2.4_9
$2a_H$ (G)	6.4_4	6.4_4	6.5_2	6.6_0	6.4_7	6.5_2

of 0.2 G on the 9-phenyl ring.[1)]

However, the MO calculation shows that the spin densities at the 3′- and 5′-positions on the 9-phenyl ring are much larger than those at the 4′- and 6′-positions, and that the latter two are nearly equivalent. These discrepancies must be due to the calculation being made for a fixed molecular structure. Therefore, the observed coupling constants are thought to be the averaged ones under the hindered rotation of the 9-phenyl ring.

Discussion

The experimental results on the linewidths of phloxine semiquinone may be summarized as follows. The linewidth decreases with a decrease in the temperature or an increase in the solvent viscosity, while no further splitting of the lines is observed and their Lorentzian shapes are kept. The variation in the linewidth is unimolecular, strongly dependent on the motion of the solvent molecules and independent of the H_0.

The linewidth variation has mostly been explained by a usual "chemical exchange" process. However, this process can not be applied to the present results as will be described below. According to the simple exchange process between two different states, A and B, when the exchanging rate is high, the lines of a Lorentzian shape should appear between each pair of A and B. As the rate is slowed down, however, the lines are broadened, deviate from Lorentzian, and finally separate into Lorentzian lines intrinsic to A and B, the linewidths of which decrease. Similar behavior may be expected when there are three or four different states.[3)]

Other processes which are worth further consideration may be (1) Orbach effects,[4)] (2) the spin-rotational coupling,[5)] (3) the collapsing of hyperfine lines of Cl atoms, and (4) the modulation of the hyperfine coupling constants of H atoms by interaction with the solvent molecules.

For the (1) process to be appreciable, the phloxine semiquinone should have energy levels adjacent to and above the ground Zeeman levels. However, its electronic absorption band at 415 nm is sharp, indicating that the (1) process does not apply.

The (2) process does not apply either, because the linewidth calculated by Kivelson's equations[5)] is only about 5×10^{-6} gauss.[6)]

According to the (3) process, the linewidth should be dependent on the spin density on the 9-phenyl ring. It may be plausible that, with an increase in the temperature, the xanthyl and the 9-phenyl rings of phloxine semiquinone may approach a coplanar structure and that, therefore, the spin density on the latter ring may increase. This might not be inconsistent with the linewidth variation in the present results. However, the roughly estimated linewidth of the collapsed hyperfine lines of four Cl atoms is about 0.18 gauss at room temperature,[7)] six times smaller than the observed value, indicating that the (3) process is not appropriate for explaining the results. It is also noted that the linewidth caused by the quadrupole effects of Cl atoms is generally smaller in a liquid state, and that the effects of the temperature and the viscosity are opposite to the present results.[9)]

Now, therefore, we should like tentatively to propose the (4) process as the most plausible explanation for the present results. The semiquinone anion of xanthene dye must form hydrogen bonds at the anionic oxygen atoms ($-O^-$) of the 11- and 12-positions. This induces the variation in the spin densities, especially at the 1- and 8-positions, which has been demonstrated experimentally[10)] and from the MO calculation.[11)] Therefore, in view of the fact that the linewidth is strongly dependent on the motion of the solvent molecules, it seems plausible that the modulation of hydrogen bonds is a source of the linewidth.

As the temperature increases, the frequency of (a) the dissociation and/or (b) the ligand exchange of hydrogen bonds will increase. Suppose that, in the case of (a), the hyperfine coupling constants of the hydrogen-bonded form are modulated by its dissociation that the dissociated (anionic) form has no sufficient lifetime to be observable in ESR. Then the transverse relaxation time, $T_{2,\mathrm{obs}}$, may be given as below, intuitively by analogy with the lifetime broadening or by solving the modified Bloch equations by assuming that the dissociated form has a short lifetime:

$$T_{2,\mathrm{obs}}^{-1} = T_2^{-1} + \tau^{-1} \qquad (1)$$

where τ is the lifetime of the hydrogen-bonded semiquinone and where T_2 is the transverse relaxation time when no dissociation of hydrogen bonds occurs. If, on the other hand, the lifetime of the dissociated form is long, the process may be regarded as a usual "chemical exchange" process, which has already been ruled out.

In the case of (b), a simple exchange of solvent molecules in the ligand causes neither line broadening nor sharpening, since no difference in the spin state is expected before and after the exchange. Therefore, we assume that, in the ligand exchange of the hydrogen-bonded form, there is an exchange intermediate which coordinates with excess solvent molecules. Such an intermediate may have a very short transverse relaxation time, since it is easily and frequently distorted by the collisions of solvent molecules. Therefore, the relaxation time in the hydrogen-bonded form is given by the same equation as has been given above from the modified Bloch equations. This equation is based on a similar one reported by McConnell[12)] and Johnson, Jr.[13)] for the linewidth of the broad-line NMR of diamagnetic ions undergoing electron-exchange reactions with their paramagnetic ions.

The temperature effect of the linewidth being sharpened with a decrease in the temperature without any further splitting can be explained by Eq. (1).

The decrease in the linewidths with a decrease in the temperature, which does not affect the Lorentzian shape and which proceeds unimolecularly, implies that the relaxation time can be represented by this equation:

$$\Delta H = \hbar(\sqrt{3}g\beta T_2)^{-1} \propto \tau^{-1}$$

The τ^{-1} may be given by $\tau^{-1}=A\exp(\Delta E/RT)$; therefore, as is obvious from Eq. (1), $T_{2,\mathrm{obs}}^{-1}$ may also be given by a similar equation when T_2 can be neglected. In fact, the plots of $\ln T_{2,\mathrm{obs}}^{-1}$ *vs.* $1/T$ are linear, from which

activation energies of 2.1, 3.1, and 2.4 kcal/mol were obtained for methanol, 2-propanol, and aqueous solutions. Each of these values is very close to the activation energy of the self-diffusion of each solvent, suggesting a strong dependence of the linewidth upon the viscosity of the solvent.

The lack of nuclear-spin dependence of the linewidth may be explained on the basis of the fact that the formation of hydrogen bonds at the two $-O^-$ sites should be at random, so the spin densities on the 1- and 8-positions are occasionally in "uncorrelated conditions".[2] (*Cf.* Ref. 11)

On the other hand, it is noted that, in the eosine semiquinone, the 9-phenyl ring undergoes a hindered rotation, and the resulting modulation of the hyperfine coupling constants of the H atoms on the 9-phenyl ring is perhaps a principal source of the linewidth, for the modulation must be much larger than in the cases of (a) and (b).

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6) In these calculations, the following values were used: moments of inertia, $I_{XX}=1.05\times10^{-36}$, $I_{YY}=2.22\times10^{-36}$, $I_{ZZ}=2.60\times10^{-36}$ g cm^2. The deviation of the average g value from that of the free electron, $\Delta g=-6\times10^{-4}$, was used for the deviation of the principal values of the g tensor. Another piece of evidence is that the value of Δg ($=0.03$) necessary to give the observed linewidth is too large to be acceptable in a π-radical of an aromatic molecule.
7) The linewidth of the *p*-chloranil anion is known to be about 1 G at room temperature.[8] The sum of the spin densities on the four carbon atoms with Cl atoms was assumed to be 0.38, which is the observed value for the *p*-benzosemiquinone anion. Similarly, the corresponding sum of the 9-phenyl ring of the phloxine semiquinone anion was assumed from the data of the eosine semiquinone anion to be 0.09. Therefore, $1\times0.09/0.38=0.2$
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11) According to the ASMO SCF CI calculation of the uranine semiquinone (X=Y=H in Fig. 1) protonated at the 12-position, the spin density increases by 0.04 at the 8-position and decreases by 0.02 at the 1-position. The uranine semiquinone protonated at the 11-position shows the opposite change. In the case of the diprotonated one, the spin densities at the 1- and 8-positions increase by about 0.01.
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