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# Studies of the Chemiluminescence of Several Xanthene Dyes. IV. Theoretical Considerations

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On the basis of the results shown in the previous papers, the following reaction scheme was proposed for the chemiluminescent reaction. Hydrogen peroxide decomposes spontaneously to the radical (R·). A portion of the radical then reacts with the dye molecule (E) to produce an energy-rich intermediate ($M_E$*): $E+R\cdot\rightarrow M_E^*$ The energy-transfer takes place from $M_E$* to an unoxidized dye, so the dye is excited electronically: $M_E^*+E\rightarrow E^*+\cdots$ However, most of the $M_E$* is decomposed by non-radiative processes. E* is deactivated by fluorescence emission processes ($E^*\rightarrow E+h\nu$) or quenching processes ($E^*+E\rightarrow E+E$). According to this reaction scheme, most of the experimental results can be explained reasonably. It is expected that further experimental evidence, such as the detection of an intermediate, will verify the theory.

The present authors have investigated the chemiluminescence of several xanthene dyes and obtained the following results:[1]

1) The intensity of the emission versus the time at the decay stage was represented by:

$$I_t = A\exp(-a\,t) \qquad (1)$$

where $I_t$ is the intensity of the emission at time $t$, $a$ (the decay constant) is equal to the rate constant of the decomposition of the dye, and $A$ is a parameter which is proportional to the initial concentration of the dye. This indicates that the decay of the luminescence is first order with respect to the concentration of the dye. The curve was much affected by the concentration of the added sodium hydroxide solution, while it was not affected by the concentration of added hydrogen peroxide when its concentration was high.

2) The energy distribution of the chemiluminescence emission was very similar to that of the fluorescence emission. This indicates that the emitting species are probably excited singlet states of the dye molecule.

3) The luminescent light was enfeebled by the addition of some kind of foreign dye. The enfeebling action seemed to be due to external quenching; it was found in all of the uranine-trypaflavine, uranine-erythrosine and eosine-erythrosine systems.

On the contrary, the intensity of the emission was enhanced when uranine was added to the eosine chemiluminescent system. However, the enhancing action was found only when the concentration of alkali was too low to cause the decomposition of uranine. The enhancing action was due neither to the production of a stable compound which emitted light strongly nor to the increase in the rate of decomposition of the dyes. A possible mechanism may be proposed, in which eosine is oxidized to an energy-rich intermediate and the energy is transferred from the intermediate to unoxidized eosine and uranine, thereby exciting both dyes to emit fluorescence.

## Concideration

**Reaction Scheme.**—On the basis of the results summarized above, the following mechanism seems plausible for the luminescent reaction.

In an alkaline solution, hydrogen peroxide decomposes spontaneously to radicals, R·, such as OH· and $O_2H$·. Some of the radicals react with the dye to produce an energy-rich intermediate. In a uranine-eosine system with a low alkali concentration, the radical reacts only with eosine, E, to produce an energy-rich intermediate, $M^*_E$:

$$E + R\cdot \rightarrow M^*_E \quad \text{[I] reaction rate: } k_1$$

Recently, Hayashi and Maeda[2] have shown that the intermediate in lophin chemiluminescence is a radical. Steele[3] has reported, in his studies of the photo-induced chemiluminescence of riboflavin in water containing hydrogen peroxide, that the emission is mediated by an excited metastable triplet state of riboflavin. White and

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[1] I. Kamiya and R. Iwaki, This Bulletin, **39**, 257, 264, 269 (1966).

[2] T. Hayashi and K. Maeda, ibid., **35**, 2057 (1962).

[3] R. H. Steele, *Biochem.*, **2**, 529 (1963).

Bursey[4] proposed that an excited triplet state of the aminophthalic acid ion was an intermediate in luminol chemiluminescence. However, no information as to $M^*_E$ has yet been obtained.

An energy-transfer takes place from $M^*_E$ to unoxidized eosine and uranine, so the dyes are excited electronically.

$$M^*_E + E \rightarrow E^* + \cdots \qquad [II]: k_2$$
$$M^*_E + U \rightarrow U^* + \cdots \qquad [III]: k_3$$

Since the efficiency of the emission of the luminescent reaction is low, most of $M^*_E$ would be decomposed or deactivated by non-radiative processes. E* and U* are deactivated either by fluorescence emission processes or by quenching processes:

$$M^*_E \rightarrow \cdots \qquad [IV]: k_4$$

E* and U* are deactivated either by fluorescence emission processes or by quenching processes:

$$\begin{array}{lll} E^* & \rightarrow E + h\nu & [V]: k_5 \\ U^* & \rightarrow U + h\nu & [VI]: k_6 \\ E^* + E & \rightarrow E + E & [VII]: k_7 \\ E^* + U & \rightarrow E + U & [VIII]: k_8 \\ U^* + E & \rightarrow U + E & [IX]: k_9 \\ U^* + U & \rightarrow U + U & [X]: k_{10} \end{array}$$

Although further quenching may take place at the initial stage, as has been shown in a previous paper, our discussion will be limited to the decay stage, where the intensity versus time is represented by Eq. 1.

**The Curve of the Intensity versus the Time ((*I-t* Curve) in a Uranine-Eosine System with Low Alkali Concentration.**—According to the mechanism proposed above, the intensity of chemiluminescence in a uranine-eosine system, $I_{UE}$, is given by:

$$I_{UE} = \phi_E[E^*] + \phi_U[U^*] = k_2[M^*_E][E]\phi_E + k_3[M^*_E][U]\phi_U \qquad (2)$$

where $\phi_E$ and $\phi_U$ are the fluorescence efficiencies of eosine and uranine respectively and are given by:

$$\phi_E = k_5/\{k_5+k_7[E]+k_8[U]\}$$
$$\text{and} \quad \phi_U = k_6/\{k_6+k_9[E]+k_{10}[U]\}$$

In luminescent reaction systems, the apparent fluorescence is so weak that

$$\text{and} \quad \left.\begin{array}{l} k_5 \ll k_7[E] + k_8[U] \\ k_6 \ll k_9[E] + k_{10}[U] \end{array}\right\} \qquad (3)$$

are obeyed approximately.

From Eq. 3 and the assumption that $k_7=k_9$ and $k_8=k_{10}$, $I_{UE}$ is reduced to:

$$I_{UE} = [\{k_2k_5[E]+k_3k_6[U]\}/\{k_7[E] + k_8[U]\}][M^*_E] \qquad (4)$$

$d[M^*_E]/dt$ is then given by:

$$d[M^*_E]/dt = k_1[R\cdot][E] - k_2[M^*_E][E] - k_3[M^*_E][U] - k_4[M^*_E] \qquad (5)$$
$$= k_1[R\cdot][E] - k_4[M^*_E]$$

because $k_4 \gg k_2 + k_3$.

It has been found that the decay curve is not affected by the concentration of hydrogen peroxide when its concentration is high and that the intensity of emission is increased by the addition of a dye solution at the final stage of the reaction. These facts indicate that the concentration of R· does not vary during the luminescent reaction, provided the concentration of the added hydrogen peroxide is high (in excess). Furthermore, the experimental results show that the concentrations of eosine and uranine are given by:

$$[E] = [E]_0\exp(-K_Et), \ [U] = [U]_0$$

where $[E]_0$ and $[U]_0$ are the initial concentrations of eosine and uranine respectively, and where $K_E$ is the rate constant of the decomposition of eosine. (The decomposition of uranine can be disregarded.) Therefore, Eq. 5 can be rewritten approximately as:

$$d[M^*_E]/dt = k_1[R\cdot][E]_0\exp(-K_Et) - k_4[M^*_E] \qquad (6)$$

Since $[R\cdot]$ is independent of the time, Eq. 6 can easily be resolved to:

$$[M^*_E] = \{k_1[R\cdot][E]_0/(k_4-K_E)\} \times \{\exp(-K_Et) - \exp(-k_4t)\}$$

It seems likely that $k_4 \gg K_E$, so:

$$[M^*_E] = \{k_1[R\cdot][E]_0/k_4\}\exp(-K_Et) \qquad (7)^{**}$$

From Eqs. 4 and 7, $I_{UE}$ is finally given by:

$$I_{UE} = k_1[R\cdot](k_2k_5[E]^2 + k_3k_6[E][U]_0)/ k_4(k_7[E] + k_8[U]_0) \qquad (8)$$

**The *I-t* Curve for Eosine and Uranine Systems.**—By taking into account reactions I, II, IV and VII, the intensity of the emission of eosine chemiluminescence, $I_E$, at time $t$ can similarly be derived as:

$$I_E = k_1k_2k_5[R\cdot][E]/k_4k_7 = (k_1k_2k_5[R\cdot][E]_0/ k_4k_7)\exp(-K_Et) \qquad (9)$$

Similarly, the intensity of the emission of uranine chemiluminescence, $I_U$, in a solution with a high alkali concentration is given by:

$$I_U = k_1'k_2'k_5'[R\cdot][U]/k_4'k_7' = (k_1'k_2'k_5'[R\cdot][U]_0/k_4'k_7')\exp(-K_Ut) \qquad (10)$$

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[4] E. H. White and M. M. Bursey, *J. Am. Chem. Soc.*, **86**, 941 (1964).

** Equation 7 can also be obtained by the "Steady-State Method," $d[M^*_E]dt = 0$.

The experimental results in a previous paper have shown that $I_E$ and $I_U$ can be represented by Eq. 1. This seems to give support to the present equations of 9 and 10. However, when the concentration of hydrogen peroxide is low, Eqs. 9 and 10 are no more obeyed, because $[R\cdot]$ is no longer constant during the reaction. Moreover, $k_5$ is not ignorable compared to $k_7[E]$ in the final stage, because the concentration of the dye becomes low.

**The Value of $\Delta$ in a Uranine-Eosine System with a Low Alkali Concentration.**—The value of $\Delta$ (the increment of the total emission) observed in a uranine-eosine chemiluminescent system with a low alkali concentration was defined as:

$$\Delta=\int_0^T (I_{UE}-I_U-I_E)\mathrm{d}t \tag{11}$$

where $T$ is the duration of the chemiluminescence of eosine. Using Eqs. 8 and 9, and recalling that $I_U=0$, Eq. 11 becomes:

$$\Delta=(k_1[R\cdot]/k_4)\int_0^T \{(k_2k_5[E]^2+k_3k_6[E][U]_0)/(k_7[E]+k_8[U]_0)-(k_2k_5/k_7)[E]\}\mathrm{d}t$$

or

$$\Delta=(k_1[R\cdot][U]_0/k_4)(k_3k_6-k_2k_5k_8/k_7)\times\int_0^T \{[E]/(k_7[E]+k_8[U]_0)\}\mathrm{d}t \tag{12}$$

On substituting the relation $[E]=[E]_0\exp(-K_Et)$ and $[E]_0\exp(-K_ET)=0$, Eq. 12 is integrated to:

$$\left.\begin{aligned}\Delta&=(k_1[R\cdot]/k_4K_Ek_7)(k_3k_6-k_2k_5k_8/k_7)[U]_0\\&\quad\times\ln(1+k_7[E]_0/k_8[U]_0)\\\text{or}&\\\Delta&=2.30P[U]_0\log(1+Q[E]_0/[U]_0)\end{aligned}\right\} \tag{13}$$

where

$$P=(k_1[R\cdot]/k_4K_Ek_7)(k_3k_6-k_2k_5k_8/k_7)$$
$$Q=k_7/k_8$$

TABLE I. OBSERVED AND CALCULATED $\Delta$-VALUES IN URANINE-EOSINE SYSTEM
($0.3$N NaOHaq., 20% $H_2O_2$aq., 40°C)

| $[U]_0$ ($mu$) | $[E]_0$ ($ne$) | $\int I_E\mathrm{d}t$ (in arbitrary unit) | $\int I_U\mathrm{d}t$ (in arbitrary unit) | $\int I_{UE}\mathrm{d}t$ (in arbitrary unit) | $\Delta$calcd.* (in arbitrary unit) | $\Delta$obs. |
|---|---|---|---|---|---|---|
| 32 | 32 | 35.5 | 1.5 | 80.0 | 44.8 | 43.0 |
| 16 | 32 | 35.5 | 0.8 | 66.0 | 29.3 | 29.7 |
| 8 | 32 | 35.5 | 0.4 | 52.0 | 18.3 | 16.1 |
| 4 | 32 | 35.5 | 0.2 | 46.0 | 11.0 | 10.3 |
| 2 | 32 | 35.5 | 0.0 | 41.8 | 6.5 | 6.3 |
| 1 | 32 | 35.5 | 0.0 | 39.0 | 3.7 | 3.5 |
| 32 | 16 | 17.5 | 1.5 | 50.0 | 32.2 | 31.0 |
| 32 | 8 | 9.1 | 1.5 | 33.0 | 25.1 | 22.4 |
| 32 | 4 | 3.5 | 1.5 | 16.3 | 13.3 | 11.3 |
| 32 | 2 | 1.5 | 1.5 | 9.8 | 7.5 | 6.8 |
| 32 | 1 | 0.6 | 1.5 | 6.0 | 4.1 | 3.9 |

* $\Delta$calcd.$=1.6m\log(1+6.5n/m)$

TABLE II. OBSERVED AND CALCULATED $\Delta$-VALUES IN URANINE-EOSINE SYSTEM
($0.5$N NaOHaq., 20% $H_2O_2$aq., 42°C)

| $[U]_0$ ($mu'$) | $[E]_0$ ($ne'$) | $\int I_E\mathrm{d}t$ (in arbitrary unite) | $\int I_U\mathrm{d}t$ (in arbitrary unite) | $\int I_{UE}\mathrm{d}t$ (in arbitrary unite) | $\Delta$calcd.* (in arbitrary unite) | $\Delta$obs. |
|---|---|---|---|---|---|---|
| 37 | 59 | 28.2 | 10.0 | 63.4 | 25.4 | 25.2 |
| 37 | 37 | 19.1 | 10.0 | 50.2 | 22.1 | 21.1 |
| 37 | 22 | 16.2 | 10.0 | 40.8 | 16.1 | 16.2 |
| 37 | 15 | 13.7 | 10.0 | 33.8 | 13.4 | 13.7 |
| 37 | 11 | 11.9 | 10.0 | 28.2 | 11.3 | 11.9 |

$u'=37u/16$, $e'=37e/16$;
* $\Delta$calcd.$=6.5m\log(1+6.5n/m)$

In a system composed of 3 ml. of an eosine solution (1 g./100 ml.), 3 ml. of a uranine solution (1 g./100 ml.), 2 ml. of a 0.3 N sodium hydroxide solution and 1 ml. of a 20% hydrogen peroxide solution, $[U]_0$ and $[E]_0$ are put, for illustrative convenience, as $32u$ and $32e$ respectively, so $u$ and $e$ are equal to $1.4\times10^{-4}$ and $7.5\times10^{-5}$ mol./l. respectively. The concentration of uranine or eosine is successively reduced by half ($16u$, $8u$, etc.) and the values of $\Delta$ are calculated with Eq. 13, where $2.3\times(Pu)$ is taken to be 1.6 and $(Qe/u)$, 6.5:

$\Delta=1.6\,m\log(1+6.5n/m)$ ($m$ and $n$ are integers). They are shown in Table I, together with the observed values. Table II tabulates the $\Delta$-values observed when the concentration of the added sodium hydroxide solution is slightly higher (0.5 N), so that $I_U$ can no longer be ignored. It also includes values calculated by the following equation:

$$\Delta=6.5m\log(1+6.5n/m) \tag{14}$$

In both cases, the calculated values are in fairly good agreement with the observed values. It is worth noting that, whether $I_U$ is smaller or not, the values of $\Delta$ can be expressed by the same equation. This means that the uranine chemiluminescence is not enhanced by the presence of eosine.

**The Values of $\sigma$.**—The relative increment, $\sigma$, was defined as:

$$\sigma=\Delta\bigg/\int_0^T I_E\mathrm{d}t \tag{15}$$

From Eqs. 9 and 15, we obtain:

$$\int_0^T I_E\mathrm{d}t=k_1[R\cdot]k_2k_5[E]_0/k_4K_Ek_7 \tag{16}$$

since $[E]_0\exp(-K_ET)=0$.

On substituting Eqs. 13 and 16 into Eq. 15, we obtain:

$$\sigma=\{(k_3k_6/k_2k_5)-k_8/k_7\}([U]_0/[E]_0)\times\ln(1+k_7[E]_0/k_8[U]_0) \tag{17}$$

Equation 17 indicates that $\sigma$ is constant when the ratio of $[U]_0$ to $[E]_0$ remains constant (at a constant temperature). The experimental values of $\sigma$ obtained at several different concentrations of uranine and eosine but at a constant ratio of $[U]_0/[E]_0$ are shown in Table III. As is to be expected, the values of $\sigma$ are almost constant except in the systems with low dye concentrations. The value of $\sigma$ probably decreases in a system with a low dye concentration because the energy-transfer from $M^*_E$ to U can no longer take place.

TABLE III. OBSERVED VALUES OF $\sigma$ IN URANINE-EOSINE SYSTEMS WITH THE CONSTANT RATIO OF $[U]_0$ TO $[E]_0$
(0.5N NaOHaq., 20% $H_2O_2$aq., 40°C)

| $[U]_0$ | $32u$ | $16u$ | $10.7u$ | $8u$ | $5.3u$ | $4u$ | $2.7u$ |
|---|---|---|---|---|---|---|---|
| $[E]_0$ | $32e$ | $16e$ | $10.7e$ | $8e$ | $5.3e$ | $4e$ | $2.7e$ |
| $\sigma$ | 1.24 | 1.22 | 1.20 | 1.23 | 0.59 | 0.43 | 0.22 |

**The Effect of the Temperature on the Luminescent Reaction.**—It is reasonable to assume that there are two processes with a heat of activation in the reaction; one is the process of reaction I, reaction between R· and the dye, the other is that of II (III); an energy-transfer from an energy-rich intermediate to an unoxidized dye. Thus, the rate constants of $k_1$ and $k_2$ (and $k_3$) are much more affected by the temperature than any other rate constants in the reaction scheme. On this arguments, we can derive the conclusion that the values of $A$ and $a$ in Eq. 1 and the value of $\Delta$ increase with an increase in the temperature, while $\sigma$ is not affected by the temperature from the consideration that $A$ is second order; $a$ and $\Delta$ are first order, and $\sigma$ is zero-th order with respect to the rate constants of $k_1$ and $k_2$ ($k_3$) since $K_E$ seems to be $k_1$.

The activation energy of reaction I can be estimated from the plot of log $a$ (log $K_E$) against the reciprocal of the absolute temperature. Figure 1 is the plot obtained from the system composed of 6 ml. of a uranine solution, 2 ml. of a sodium hydroxide solution, and 1 ml. of 20% hydrogen peroxide. Figure 2 is a similar plot in eosine chemiluminescence (6 ml. of an eosine solution, 2 ml. of a 0.5 N sodium hydroxide solution, and 1 ml. of 30% hydrogen peroxide). The activation energies of uranine and eosine chemiluminescent reactions are found to be 13 and 11 kcal./mol. respectively. We can see that the activation energy for the reaction in an aqueous system is equal to that in a water-organic solvent system, although the light intensity in the former is lower than that in the later. Moreover, the energy is practically the same, irrespective of the concentration of the added sodium hydroxide solution.

**The Effect of the Added Organic Solvent on the Intensity of Emission.**—In Table IV, the products of $\Delta$ and $K_E$ in methanol-water and ethanol-water systems of different alcohol concentrations are shown. It may be seen that the product is almost constant in each system.

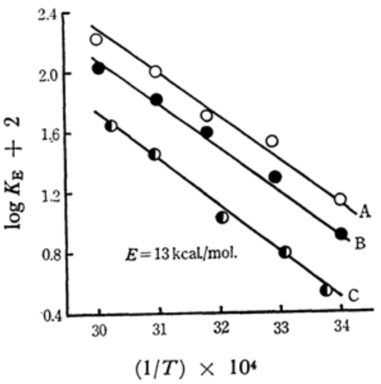

Fig. 1. Log $K_E$ - $1/T$ plots in uranine chemiluminescence.
A: 3N NaOHaq., 20% $H_2O_2$, 1/4 conc. to original solution ($[U]_0$=8u): aq. solution
B: 2N NaOHaq., 20% $H_2O_2$, original solution ($[U]_0=32u$): aq. solution
C: 2.5N NaOHaq., 20% $H_2O_2$, original solution ($[U]_0=32u$): 25% water-methanol solution

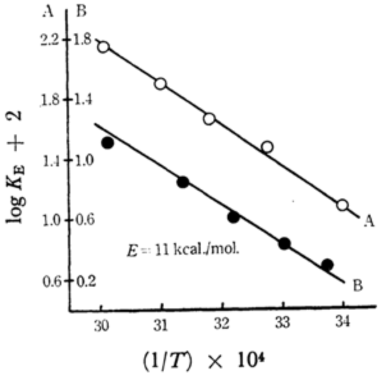

Fig. 2. Log $K_E$ - $1/T$ plots in eosine chemiluminescence.
A: 0.5N NaOHaq., 30% $H_2O_2$, 1/4 conc. to original solution ($[E]_0=8e$): aq. solution
B: 0.5N NaOHaq., 30% $H_2O_2$, 1/2 conc. to original solution ($[E]_0=16e$): 30% water-isopropanol solution

TABLE IV. RELATIONSHIP BETWEEN $\Delta$-VALUE AND $K_E$ (EOSINE)

(1) Ethanol-water systems (at 25°C)

| Ethanol concentration (% in volume) | 10 | 20 | 30 | 40 | 60 |
|---|---|---|---|---|---|
| $K_E$ (min$^{-1}$) | 0.32 | 0.11 | 0.083 | 0.062 | 0.046 |
| $\Delta$ (relative unit) | 8.9 | 26.9 | 34.2 | 46.8 | 51.7 |
| $K_E\Delta$ (×10) | 28.5 | 29.6 | 28.4 | 29.1 | 23.8 |

(2) Methanol-water systems (at 45°C)

| Methanol concentration (% in volume) | 10 | 20 | 40 | 60 |
|---|---|---|---|---|
| $K_E$ (min$^{-1}$) | 0.30 | 0.20 | 0.16 | 0.09 |
| $\Delta$ (relative unit) | 16.0 | 24.6 | 28.0 | 40.4 |
| $K_E\Delta$ | 4.8 | 4.9 | 4.5 | 3.6 |

From Eqs. 13 and 17, $K_E \times \varDelta/\sigma$ is given by the following equation:

$$K_E \times \varDelta/\sigma = k_1k_2k_5[\mathrm{R}\cdot][\mathrm{E}]_0/k_4k_7 = A[\mathrm{E}]_0 \qquad (18)$$

$\sigma$, as has been shown in a previous paper, does not change in the systems; hence, $A$ $(E_E \times \varDelta/\sigma)$ should remain constant. This invariability of $A$ is consistent with the experimental finding that the value of $A$ remains constant upon the addition of organic solvent.

## Conclusion

By the reaction scheme proposed in the present paper, most of the experimental results reported in preceding papers can be explained reasonably. This would indicate that the present theory is plausible. It is expected that further experimental evidence, such as the detection of an intermediate or end product, will verify the theory.