Excited-state Reactions of Coumarins in Aqueous Solutions. II. The Fluorescence Quenching of 7-Ethoxycoumarins by Halide Ions

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The fluorescence yield and the fluorescence lifetime of 7-ethoxycoumarins in aqueous solutions are reduced with the increase in the temperature and in the concentration of the quenching agents. The radiationless deactivation of the excited state in the absence of quenchers is temperature-dependent; its activation energy has been determined to be 32.5—34.8 kJ mol^{-1}. The quenching reaction due to halide ions proceeds *via* both a diffusional and a static process. The rate constant for the diffusional process has a value of (9.7—10.2)$\times 10^9$ mol^{-1} dm^3 s^{-1} in the case of I$^-$ as a quencher. The static quenching component can be approximately described by the $V[Q]$ expression, with the static quenching constant, V, being 1.6—1.8 mol^{-1} dm^3. The quenching efficiencies that represent the probability leading to real quenching reactions by an encounter of the molecule and the halide ion are unity for I$^-$, 0.39—0.42 for Br$^-$, and almost zero for Cl$^-$. The possible physical interpretation of the quenching mechanism is discussed in relation to the electron transfer between colliding species.

Although a considerable volume of data has been accumulated on the luminescence properties of various coumarin derivatives,[1] few works have appeared on the fluorescence-quenching of the molecule in solution by unlike molecules or by inorganic ions. We have already examined in detail the fluorescent behavior of 7-hydroxycoumarins,[2] and our study has revealed that the molecules have several molecular species in the optically excited state, depending on the pH of the solution. This situation has made it very difficult to investigate the fluorescence-quenching effects of these molecules because of the complexity of the fluorescence spectrum itself in the absence of quenchers. If the hydroxyl group of 7-hydroxycoumarins is replaced by the ethoxyl group, then the neutral species becomes the only species existing in the excited state as well as in the ground state, and the fluorescence spectrum becomes a very simple one over a wide pH range in aqueous solutions, in contrast to the case of 7-hydroxy-substituted coumarins. Accordingly, 7-ethoxy-substituted coumarins are a convenient group for a fundamental study of fluorescence-quenching reactions of coumarins.

The quantum yield of molecules in solution is known to be affected by a variety of factors, such as the temperature, the viscosity, and the concentration of the quenching agents. In this paper, halide ions have been chosen as quenchers, and the fluorescence intensities of 7-ethoxycoumarin (**1**) and 7-ethoxy-4-methylcoumarin (**2**) in aqueous solutions have been measured as a function of the concentration of the quencher and the temperature of the solution. The reaction-rate constant for fluorescence quenching by halide ions and the several activation energies related to it will be derived by means of the obtained data, and the quenching mechanism will be discussed in detail.

Experimental

Materials. The high-purity-grade reagents of 7-ethoxycoumarin (**1**) and 7-ethoxy-4-methylcoumarin (**2**) were obtained from Molecular Probes, Inc., and used as purchased. Their melting points were determined distinctly by means of differential thermal analysis (DTA); the purity was revealed to be very high by the measured DTA curves:[3] **1**, mp 84.8 °C **2**, mp 115.5 °C. The sodium chloride, sodium bromide, sodium iodide, and sodium thiosulfate were pure and were obtained from Wako Pure Chemical Ind. The water was permeated and distilled.

Methods. The UV absorption spectra at the ambient temperature were obtained on a Hitachi 323 spectrophotometer. The fluorescence-intensity measurements were done on a Hitachi MPF 4 spectrofluorometer with an S-5 type photomultiplier tube. For the temperature-dependence studies, we used a hollow cell holder, through which water from a constant-temperature bath was circulated. The temperature in the cuvette could be controlled to within ±0.5° between 10 and 50 °C, and was monitored directly by means of a thermocouple immediately after the fluorescence measurement.

The wavelengths of the excitation light were chosen to be 324 nm for **1** and 321 nm for **2**, which corresponded to the absorption maxima. The concentrations of the fluorescent molecules in the aqueous solution were $\approx 5\times 10^{-5}$ mol dm^{-3} for **1** and $\approx 4\times 10^{-5}$ mol dm^{-3} for **2**, with which the reliable and stable recording of the fluorescence spectra was ensured.[4] Since the shape and position of the emission spectrum were not noticeably altered by either the change in the temperature or by the addition of a quencher, the fluorescence intensity was usually determined by the measurement of the relative intensity at the emission maxima, which were found at 390 nm and 382 nm for **1** and **2** respectively.

In the quenching experiment, the fluorescence of a series of samples, containing a constant amount of a fluorescent compound and a graduated amount of a quencher, was measured. The stocked solutions of quenchers used to vary the concentration of the halide ions were 4M NaCl, 2M NaBr, and 0.2M NaI (containing $\approx 10^{-4}$ M $Na_2S_2O_3$ to prevent I_3^- formation; 1 M=1 mol dm^{-3}). The quenchers had no disturbing absorption in the wavelength region of interest.

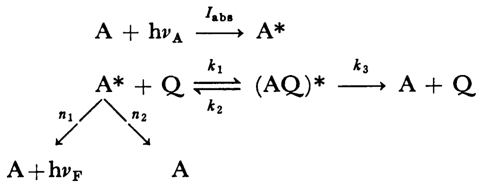
The fluorescence lifetime measurements were performed by using a time-correlated single-photon-counting fluorometer similar to the one described in Ref. 5. Because the determined lifetime becomes less accurate for lifetimes shorter than 2 ns when our apparatus was used, the supplemental relationship:

$$\tau_0(t) = \tau_0^{20}\frac{I_0(t)}{I_0^{20}}, \tag{1}$$

was used to obtain the temperature dependence of the lifetime at higher temperatures, where τ_0^{20} and $\tau_0(t)$, I_0^{20} and $I_0(t)$ are the lifetimes and the emission intensities at 20 °C and t °C in the absence of a quencher. The viscosity of water was taken from the literature.

Results and Discussion

Temperature Dependence of the Fluorescence Lifetime. A frequently postulated kinetic scheme for the fluorescence-quenching process is as follows:



Here, A, Q, and hν represent the fluorophore, the quencher, and the light quantum; A* and (AQ)* are the excited states of the fluorophore and of the encounter complex between A and Q respectively; the *k*'s are the reaction-rate constants, and the *n*'s are the transition probabilities. When the quencher is absent, the lifetime, τ_0, and the quantum yield, ϕ_0, of the excited molecule at t °C are given by:

$$\tau_0(t) = \frac{1}{n_1 + n_2(t)} \text{ and } \phi_0(t) = \frac{n_1}{n_1 + n_2(t)}, \tag{2}$$

where n_1 and n_2 are the radiative and radiationless transition probabilities respectively. The temperature dependence of the lifetime or the quantum yield of **1** and **2** in an aqueous solution is quite large, as may be seen from Fig. 1. Because of the effect of the slight broadening of the emission spectra at higher temperatures, the integrated area of the spectrum was used to estimate the rigorous quantum yield. No shift in the wavelength of the emission maximum was observed upon raising the temperature, suggesting that the fluorescing state itself is not altered at all.

If the reasonable assumptions are made that n_1 is independent of the temperature (at least in the region between 10 to 50 °C) and that only one radiationless deactivation process is significant, then the temperature dependence of the lifetime may be expressed by:

$$\frac{1}{\tau_0(t)} - n_1 = n_2(t) = n_2^0 \exp\left(-\frac{E_2}{RT}\right), \tag{3}$$

where n_2^0 is the frequency factor for the deactivation process and involves the entropic component of the corresponding free energy of activation, E_2 is the activation energy for the deactivation process, R is the gas constant, and T is the absolute temperature. By means of the numerical fitting of Eq. 3 with the data in Fig. 1, the value of n_1 can be determined; Fig. 2 shows the logarithmic plots of $(1/\tau_0) - n_1$ *vs.* $1/T$, from the slope of which we have obtained the E_2 of Eq. 3. The actual lifetime, τ_0, at 20 °C, the radiative lifetime, $\tau_1 (= 1/n_1)$, and the activation energy, E_2, are listed in Table 1. It is known from the obtained values that the radiative and radiationless transition probabilities are of the same order at around 20 °C. The activation energies of 32.5—34.8 kJ mol^{-1} are similar to those for tryptophan derivatives in water;[6] this similarity may be related to the activation of a "tunneling" process from the excited to the ground state or, alternatively, of an electron-ejecting process of the molecule to the solvent. It may be noted here that the assumption of only one deactivation process is an expedient one because the examined temperature range is not so wide as to exclude completely the existence of the temperature-independent term or other types of temperature-dependent terms. In contrast to the case of an organic solvent,[7] the quenching by dissolved dioxygen in an aqueous solution has been shown by the lifetime measurement to be unimportant.

Fluorescence Quenching by Halide Ions. In the quenching reaction between an excited state of a fluoro-

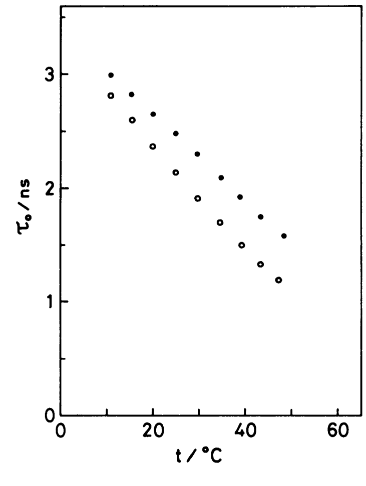


Fig. 1. Temperature dependence of the fluorescence lifetimes of 7-ethoxycoumarins in aqueous solution. ○, 7-ethoxycoumarin (**1**); ●, 7-ethoxy-4-methylcoumarin (**2**).

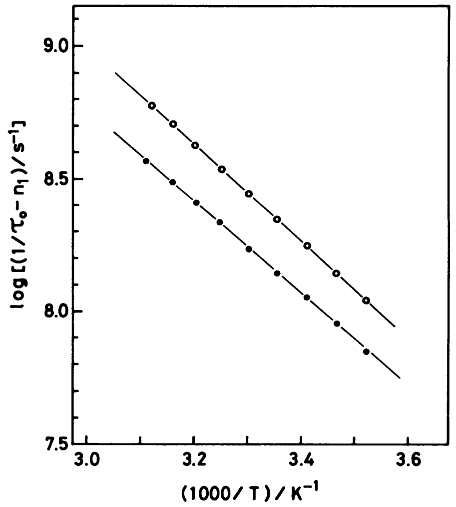


Fig. 2. Logarithmic plots of $(1/\tau_0) - n_1$ *vs.* $1/T$. n_1 is chosen to be 2.92×10^8 s^{-1} for **1** and 3.11×10^8 s^{-1} for **2**: ○, **1**; ●, **2**.

TABLE 1. ACTUAL AND RADIATIVE LIFETIMES, AND ACTIVATION ENERGIES FOR THE RADIATIONLESS TRANSITION PROCESS

Substance	τ_0/ns$^{a)}$	n_1^{-1}/ns	E_2/kJ mol^{-1}
1	2.0_0	3.4_3	34.8
2	2.2_5	3.2_2	32.5

a) The values at 20 °C.

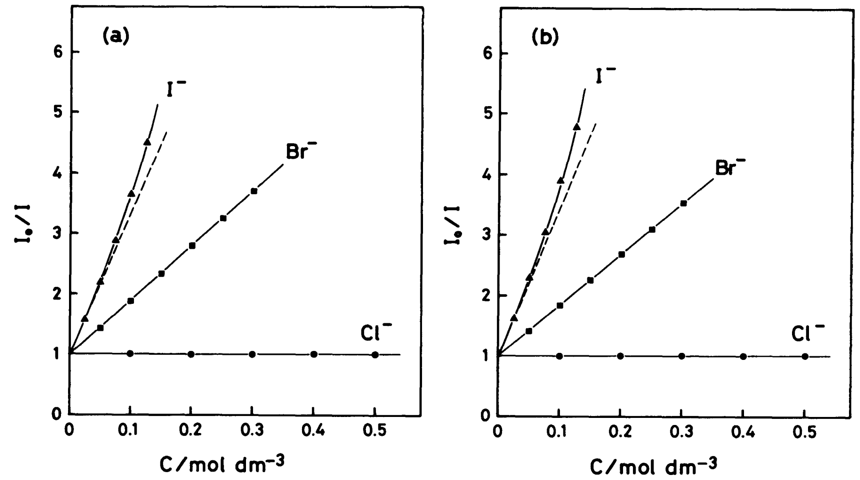


Fig. 3. Stern-Volmer plots for the fluorescence quenching by I^- (▲), Br^- (■), and Cl^- (●) in aqueous solution at 20°C. (a), 7-ethoxycoumarin (**1**); (b), 7-ethoxy-4-methylcoumarin (**2**).

phore, A^*, and a quencher, Q, the encounter complex, $(AQ)^*$, that is formed with a diffusion-controlled rate constant, k_1, reacts to dissipate the electronic energy by some internal-mechanism rate constant, k_3. For such a case, the Stern-Volmer relationship under steady-state conditions is described by:[8]

$$\frac{\phi_0}{\phi} = 1 + K_{sv}[Q] = \frac{I_0}{I}, \tag{4}$$

where ϕ_0 and ϕ, I_0 and I, are the quantum yields and the emission intensities in the absence and in the presence of Q, respectively. K_{sv} is the collisional quenching constant, which is equal to $k_q\tau_0$; k_q is the apparent rate constant for the collisional quenching process; it is given by:

$$k_q = \gamma k_1 \text{ and } \gamma = \frac{k_3}{k_2+k_3}. \tag{5}$$

Here, γ represents the efficiency of the quenching reaction, which approaches unity if $k_2 \ll k_3$. When not every encounter between Q and A^* results in quenching, the efficiency of the reaction becomes less than unity, and k_q is approximately equal to k_1k_3/k_2 if $k_2 \gg k_3$.

The data in Figs. 3(a) and 3(b) are the Stern-Volmer plots obtained when Cl^-, Br^-, and I^- were used as quenchers. Cl^- had no effect on the fluorescence intensity when $[Cl^-] \lesssim 0.5$ mol dm^{-3}. Br^- and I^- drastically quenched the fluorescence of both **1** and **2**. When I^- is used as a quencher, it may be seen from Fig. 3 that the plots are apparently curved upwards in a fashion similar to those found in many other detailed quenching studies.[9] This type of deviation from the expected linear plot can be explained by the fact that only a certain fraction, W, of the excited state is actually quenched by the collisional mechanism. Some molecules in the excited state, the fraction of which is $1-W$, are deactivated almost instantaneously after being formed because a quencher happens to be randomly positioned in the proximity at the time the molecules are excited and interacts very strongly with them. Several models can be employed to describe this instantaneous or "static" quenching process, all leading to the following modified form of the Stern-Volmer equation:

$$\frac{\phi_0}{\phi} = \frac{1+K_{sv}[Q]}{W} = \frac{I_0}{I}. \tag{6}$$

Model 1. The complete Smolchowski equation for diffusion-controlled reactions contains an often neglected transient term:[10,11]

$$\begin{aligned} k_1 &= 4\pi N'RD + 4R^2(\pi D)^{1/2}t^{-1/2} \\ &= \text{(steady state term)} + \text{(transient term)} \end{aligned} \tag{7}$$

where N′ is Avogadro's number per millimole, R is the encounter distance between the reactants, D is the sum of the individual diffusion coefficients of the reactants, and t is the time. The retention of the latter term leads to an additional factor, W, in the Stern-Volmer equation which is approximately equal to $\exp(-V[Q])$, where V is the static quenching constant.[10]

Model 2. Frank and Vavilov[12] have suggested that instantaneous quenching results at those instances in a randomly distributed system when a quencher happens to reside within a "sphere of action" with a volume of V/N' and a radius of r, *i.e.*, $V/N' = 4\pi r^3/3$, surrounding a fluorophore at the time of excitation. In this case, W is also given by this expression: $\exp(-V[Q])$.

Model 3. In the model of Moon *et al.*,[13] instantaneous quenching follows the excitation of the ground-state complex, (AQ). Such a situation can be described by adding the following reactions to the primary scheme:

$$\begin{tikzcd} A + h\nu_A \arrow[r, "I_{abs}\delta"] \arrow[d, leftrightarrow, "K^0"'] & A^* \\ (AQ) + h\nu_A \arrow[r, "I_{abs}(1-\delta)"] & (AQ) \end{tikzcd}$$

where:

$$\delta = \frac{1}{1+(\varepsilon_{AQ}/\varepsilon_A)K^0[Q]} \text{ and } K^0 = \frac{[AQ]}{[A][Q]}, \tag{8}$$

ε_{AQ} and ε_A being the molar extinction coefficients of (AQ) and A respectively at the excitation wavelength. Then the fraction, W, is given by:

$$W = \frac{\delta + k_2/k_3}{1+k_2/k_3} = \gamma\left(\delta + \frac{k_2}{k_3}\right). \tag{9}$$

If the dark complex, (AQ), is really formed in the solution, δ decreases from unity and, therefore, W decreases, leading to the decrease in the quantum yield.

Because the fraction, W, depends on the concentration of Q in all the cases mentioned above, the Stern-Volmer plots for a quencher with high quenching ability generally deviate from a linear plot. In order to extract more tractable information from these models, it is sensible to calculate the value of $\{1-(I/I_0)\}/[Q]$, that is:

$$\frac{1-(I/I_0)}{[Q]} = K_{SV}\frac{I}{I_0} + \frac{1-W}{[Q]}. \tag{10}$$

For Models 1 and 2, when $V[Q] \ll 1$ is satisfied, $W \approx 1-V[Q]$ and Eq. 10 is written as:

$$\frac{1-(I/I_0)}{[Q]} = K_{SV}\frac{I}{I_0} + V. \tag{11}$$

For Model 3, when $(\varepsilon_{AQ}/\varepsilon_A)K^0[Q] \ll 1$ is satisfied, $W \approx 1-\gamma(\varepsilon_{AQ}/\varepsilon_A)K^0[Q]$ and Eq. 10 is written as:

$$\frac{1-(I/I_0)}{[Q]} = K_{SV}\frac{I}{I_0} + \gamma\frac{\varepsilon_{AQ}}{\varepsilon_A}K^0. \tag{12}$$

In any case, $\{1-(I/I_0)\}/[Q]$ is described by the form of $a(I/I_0)+b$ when the concentration of quenchers is relatively low. Naturally, the second term, b, of Eq. 10 becomes strictly zero if the linear condition, *i.e.*, $W=1$, is satisfied. The plot of $\{1-(I/I_0)\}/[Q]$ *vs.* I/I_0, therefore, becomes linear in this approximation, the slope, a, of which is K_{SV}, and the intercept, b, of which at $I/I_0 \rightarrow 0$ becomes V or $\gamma(\varepsilon_{AQ}/\varepsilon_A)K^0$, depending upon the model being used.

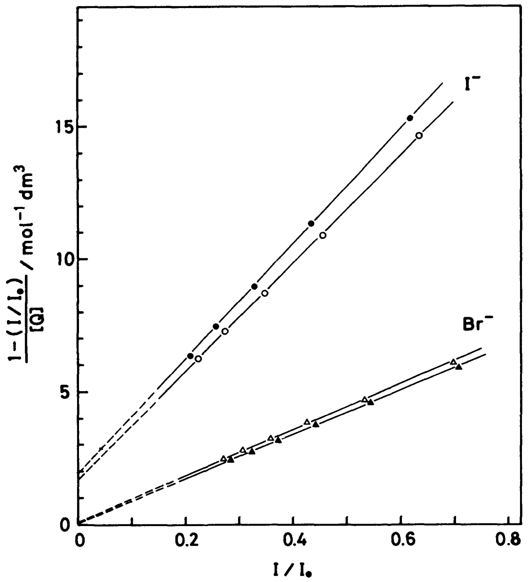


Fig. 4. Plots of $[1-(I/I_0)]/[Q]$ *vs.* I/I_0 at 20°C. I⁻ as a quencher: ○, **1**; ●, **2**. Br⁻ as a quencher: △, **1**; ▲, **2**.

Fig. 4 shows the plots of $\{1-(I/I_0)\}/[Q]$ *vs.* I/I_0 for Compounds **1** and **2** in an aqueous solution at 20 °C. The quenching effects by two halide ions, *i.e.*, Br⁻ and I⁻, have been examined. As can readily be seen from Fig. 4, the effect of varying the fluorescent molecule on the slope and intercept is rather small, but that of varying the quenching ion from Br⁻ to I⁻ is large; the values of K_{SV} $(=k_q\tau_0)$ and b at 20 °C are listed in Table 2. As for the case of Br⁻, the deviation from the linear Stern-Volmer relation is small, because b is very small; this is also known from the plots in Fig. 3. On the other hand, b is much larger for I⁻, and the deviation becomes quite large. It may be noted that, in spite of such a deviation from the Stern-Volmer plot, the approximations made in Eqs. 11 and 12 are fully appropriate, as the expansion inaccuracy is less than 4% over the concentration range of the quencher used. Accordingly, the newly derived relation of Eqs. 11 and 12 turns out to be a more powerful equation for the analysis of strongly quenching processes than the usual Stern-Volmer equation.

Table 2. Values of the quenching constant K_{SV} and the constant b at 20 °C

Substance	Quencher	K_{SV}/mol^{-1} dm^3	b/mol^{-1} dm^3 a)
1	Br⁻	8.5_9	0.1
	I⁻	20.3_0	1.7
2	Br⁻	8.4_6	0.0_4
	I⁻	21.7_2	1.9

a) b is the value for the second term of Eq. 11 or 12.

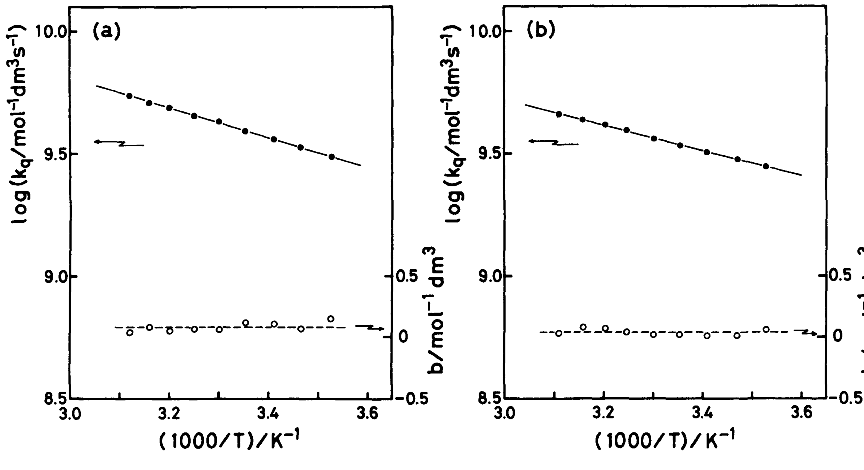


Fig. 5. Arrhenius plots of the rate constants for the collisional quenching, and plots of the static quenching constants *vs.* $1/T$. Br⁻ as a quencher: (a), **1**; (b), **2**.

Temperature Dependence of the Rate Constant. The collisional quenching constant, K_{SV}, depends, of course, on the temperature through the temperature dependence of the quenching-rate constant, k_q, and the lifetime, τ_0. The values of K_{SV} and τ_0 at temperatures between 10 and 50 °C could be known by independent measurements, so that the temperature dependence of k_q was readily estimated from these values; the logarithm of k_q is plotted against the inverse of the absolute temperature in Figs. 5 and 6. It is clear that the plots of log k_q *vs.* $1/T$ are linear in the temperature range measured. Assuming $k_q \propto \exp(-E_q/RT)$, the activation energy, E_q, for the collisional quenching process was obtained from the slope of this plot; it is listed in Table 3. It seems that E_q is dominated mainly by the species of quenchers, since Compounds **1** and **2** have very similar values of E_q for Br^- and I^- respectively.

Concerning the case of I^-, E_q has been found to be 16.3 and 15.6 kJ mol^{-1} for **1** and **2** respectively; these values are very similar to the value obtained for indole derivatives in water, *i.e.*, 15.5 kJ mol^{-1}.[14] As will be mentioned later, these values are also very near the activation energy for the viscosity of water; this suggests that the rate of reaction is limited only by the diffusion of the reactants through the aqueous solution. E_q for Br^- is somewhat less than these values; the reason for such a difference will be discussed below.

The plots of b in Figs. 5 and 6 show no indication of the temperature dependence in any case. Model 3 predicts that b is temperature-dependent, for the equilibrium constant, K^0, contained in the expression of b varies with the temperature. This fact would contradict the experimental result, although the small possibility that K^0, as well as b, varies very slowly with the temperature cannot be denied. Furthermore, no trace of the ground-state complex, (AQ), could be found by means of the absorption measurement, even if the concentration of a halide ion was set extremely high. Therefore, the deviation from the linear Stern-Volmer relation is not due to the process described by Model 3. Model 1 also predicts the dependece of b on the temperature through the temperature dependence of the diffusion coefficient, which is contained in the transient term of Eq. 7.

On the other hand, Model 2 uses only the statistical nature of the quenchers distributed around the fluorescent molecule, so that no dependence on the temperature is predicted in accordance with the experimental result. For these reasons, it seems most reasonable, in the case of the coumarin derivatives in an aqueous solution, to discuss the quenching phenomena in terms of Model 2, *i.e.*, the sphere-of-action model. By equating b with V, the values of V averaged over temperatures shown in Table 3 are obtained. Using the relation: $V/N' = 4\pi r^3/3$, the radius of the sphere of action for the quenching by I^- is estimated to be 8.6 Å for **1** and 9.0 Å for **2**. These radii are slightly larger than the value, 6—7 Å, which can be estimated as the sum of the radii of the coumarin and the iodide ion. This clearly shows that the instantaneous quenching can occur if the quencher is very near to, or in contact with, the fluorescent molecule at the exact moment that it becomes excited. In the case of Br^-, the active volume element,

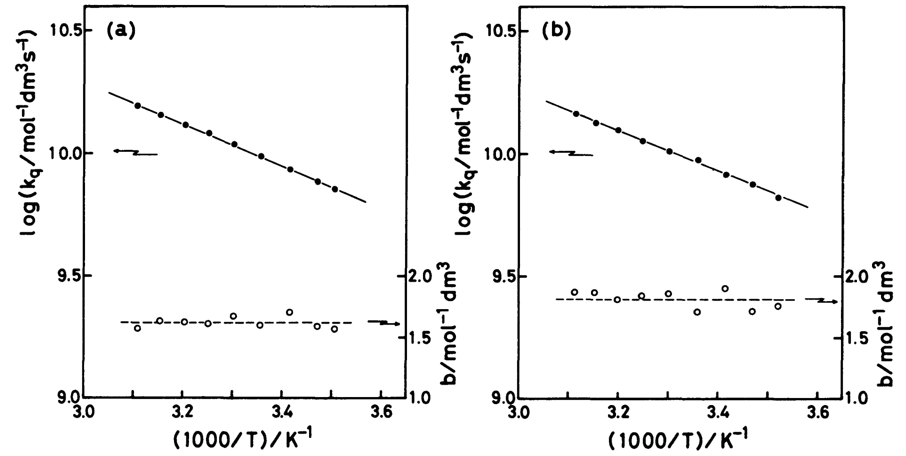


Fig. 6. Arrhenius plots of the rate constants for the collisional quenching, and plots of the static quenching constants *vs.* $1/T$. I^- as a quencher: (a), **1**; (b), **2**.

TABLE 3. RATE CONSTANTS FOR THE COLLISIONAL QUENCHING PROCESS AT 20 °C, ACTIVATION ENERGIES FOR THE COLLISIONAL QUENCHING, STATIC QUENCHING CONSTANTS, AND RADII OF THE SPHERE OF ACTION

Substance	Quencher	$k_q \times 10^{-9}$ / mol^{-1} dm^3 s^{-1}	E_q / kJ mol^{-1}	V / mol^{-1} dm^3	r / Å
1	Br^-	4.3_0	11.7	0.0_8	3.2
	I^-	10.1_5	16.3	1.6_2	8.6
2	Br^-	3.7_6	9.9	0.0_4	2.5
	I^-	9.6_5	15.6	1.8_1	9.0

V/N', is much smaller and r is less than 4 Å (see Table 3). This is attributable to the weakness in the quenching ability of Br^-, in comparison with that of I^-, because the contact of the quencher with the fluorophore does not always lead to subsequent fluorescence quenching.

Quenching Efficiency. If the values of R and D are reasonably assigned as 7 Å and $1.5\times10^{-5}\,cm^2\,s^{-1}$, based on the molecular and ionic volumes and the Stokes-Einstein relation,[8] the diffusion-limited value of the quenching-rate constant, k_1, can be calculated to be $7.9\times10^9\,mol^{-1}\,dm^3\,s^{-1}$ by using the steady-state portion of Eq. 7, *i.e.*, $k_1=4\pi N'RD$. It was previously mentioned that the actual quenching rate, k_q, is given by $k_q=\gamma k_1$, where γ is the efficiency of the quenching reaction. Although the value obtained above is only a rough estimation, it is close to the experimental values for I^- which are shown in Table 3; the quenching-rate constants for Br^- are somewhat smaller. This indicates that the efficiency of the quenching reaction by I^- is almost unity, while that of the reaction by Br^- is less than unity. The diffusion coefficients of Br^- and I^- in water do not differ very much; therefore, assuming $\gamma=1$ for I^-, the value of γ for Br^- is estimated by means of Eq. 5 to be 0.42 for **1** and 0.39 for **2**, which means more than half of the encounter events between the coumarin and Br^- do not result in the fluorescence quenching. In other words, the encounter distance, R, is shortened effectively to γR for inefficient quenchers such as Br^-.

It has become clear that the efficiency of the collisional deactivation depends on the strength of the quenching ability of each quencher, which is represented by the value "γ". For an efficient quencher like I^-, the efficiency of the quenching reaction is unity, and the magnitude of k_q can be interpreted as the frequency of the collisional encounter between A* and Q. In the case of $\gamma=1$, the temperature behavior of k_q coincides with that of the diffusion-controlled rate constant, k_1 $(=4\pi N'RD)$; the temperature dependence of k_1 is due to that of the diffusion coefficient, D. When a relatively narrow region of temperature is considered, D is approximately written as:[15]

$$D = D'\exp(-E_d/RT), \tag{13}$$

where E_d is the activation energy of diffusion and D' is the value at $T=\infty$. Therefore, the obtained value of E_q is equivalent to the activation energy of diffusion.

If γ happens to be less than unity, as in the case of Br^-, the expression for k_q becomes more complicated; in particular, the magnitudes of k_2 and k_3, which may depend on the temperature, become significant. Nevertheless, if the dependence of γ on the temperature is roughly represented by an activation energy, E_γ, *i.e.*, $\gamma\propto\exp(E_\gamma/RT)$, the discussion proceeds parallel to that for the case of $\gamma=1$. The activation energy of k_q for Br^- is smaller than that for I^- by 4.6—5.7 kJ mol^{-1}, as may be seen in Table 3. Considering $k_q\propto\gamma D$, the activation energy of k_q for Br^- may be said to correspond with E_d-E_γ. If such an idea of "efficiency" is also applicable to the radius of the sphere of action, and if r is replaced by γr, then the value of V for Br^- becomes smaller by an order of magnitude than that for I^- due to the factor of γ^3. This prediction agrees well with the experimental results shown in Table 3.

Relation between the Rate Constant and the Viscosity. As has been mentioned previously, $k_1\propto D$, and, as a first approximation, D is obtained by the Stokes-Einstein relation, $D=kT/6\pi R\eta$, where η is the viscosity of water; the plot of k_q *vs.* T/η should be linear if $\gamma=1$. Fig. 7 shows such plots for Br^- and I^-. In reality, k_q for I^- is linearly dependent on T/η, as is to be expected from a diffusion-controlled reaction; in addition, the plot for Br^- is also linear, but the slope is more gentle than that for I^-. Although linear dependence is obtained for both cases, a curious point remains that k_q at a low T/η does not go exactly to zero, which contradicts the simple Stokes-Einstein relation. The deviation from this relationship is due to the inapplicability of the Stokes coefficients of viscous friction, which are introduced by the assumption that the solute molecules are moving in a solvent continuum. Taking proper account of the relative size of the solute and solvent molecules, the expression for the diffusion coefficient sufficient to describe experiments is given by:[8]

$$D = A\frac{T}{\eta} + b\eta^x\frac{T}{\eta}, \tag{14}$$

where A, b and x are constants and the rate constant of diffusional quenching is written as:

$$\begin{aligned} k_q &= \gamma k_1 = \gamma\cdot 4\pi N'RD \\ &= A'\gamma\frac{T}{\eta} + b'\gamma\eta^x\frac{T}{\eta}. \end{aligned} \tag{15}$$

By using the activation energy of the quenching efficiency and of the fluidity, γ and η can be described by:

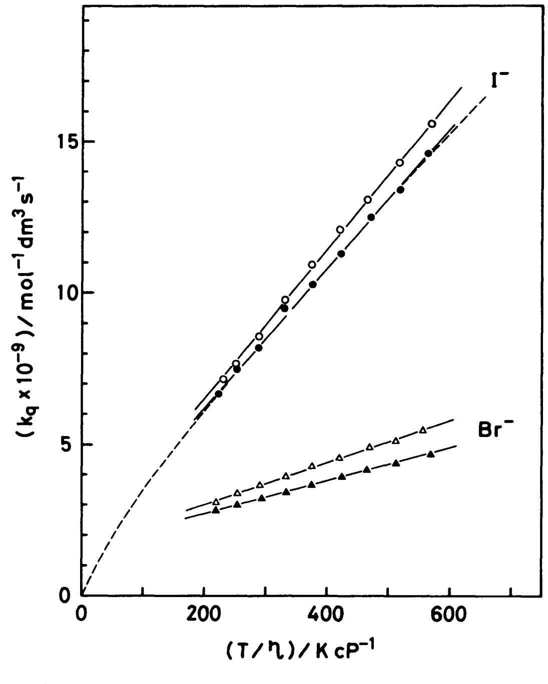


Fig. 7. Dependence of the rate constants for the collisional quenching upon T/η. I^- as a quencher: ○, **1**; ●, **2**. Br^- as a quencher: △, **1**; ▲, **2**. The dashed curve indicates the variation of k_q according to Eq. 17 without any approximation.

Table 4. Parameters obtained for the modified Stokes-Einstein relation and for the quenching efficiency

Substance	Quencher	$A'\times 10^{-7}$ / mol^{-1} dm^3 s^{-1} cP K^{-1}	$B'\times 10^{-4}$ / mol^{-1} dm^3 s^{-1} cP K^{-1}	E_γ / kJ mol^{-1}	$\gamma'\times 10^2$
1	Br$^-$	2.6_0	8.5_4	4.6_0	6.6_8
	I$^-$			0	1
2	Br$^-$	2.3_9	8.4_9	5.7_3	3.8_4
	I$^-$			0	1

$$\gamma = \gamma'\exp(E_\gamma/RT) \text{ and } \eta = \eta'\exp(E_\eta/RT), \qquad (16)$$

in the temperature range of 10—50 °C used; in the case of water, $\eta'\approx 1.2\times 10^{-3}$ cP and $E_\eta\approx 16.4$ kJ mol^{-1} in the neighborhood of 30 °C, the mean temperature of the measured region; the values of E_γ are already known as $E_\gamma\approx 0$ ($\gamma'=1$) in the case of I$^-$ and 4.60 and 5.73 kJ mol^{-1} for **1** and **2** respectively in the case of Br$^-$. The value of x was presumed to be 0.7 by plotting $k_q\eta/T$ *vs.* η^x in the case of I$^-$ for various values of x and by searching for a linear dependency. Then, Eq. 15 is rewritten as:

$$k_q = A'\gamma'\exp(E_\gamma/RT)\frac{T}{\eta} + B'\gamma'\exp\{(E_\gamma + xE_\eta)/RT\}\frac{T}{\eta}, \qquad (17)$$

where A', B' and γ' are constants. Regarding $\exp(E_\gamma/RT)$ and $\exp\{(E_\gamma+xE_\eta)/RT\}$ as functions of η/T, and by expanding them around $(\eta/T)_{30}$, the value of η/T at 30 °C, we obtain approximately this relation:

$$\begin{aligned} k_q = {} & \gamma'\exp(E_\gamma/RT_0)\left\{A'\frac{E_\eta+RT_0-E_\gamma}{E_\eta+RT_0}\right. \\ & \left. + B'\exp(xE_\eta/RT_0)\frac{E_\eta+RT_0-(E_\gamma+xE_\eta)}{E_\eta+RT_0}\right\}\frac{T}{\eta} \\ & + \gamma'\exp(E_\gamma/RT_0)\left(\frac{T}{\eta}\right)_{30}\left\{A'\frac{E_\gamma}{E_\eta+RT_0}\right. \\ & \left. + B'\exp(xE_\eta/RT_0)\frac{E_\gamma+xE_\eta}{E_\eta+RT_0}\right\}, \end{aligned} \qquad (18)$$

where T_0=303.15 K. Eq. 18 is valid in the neighborhood of 30 °C; this explains very well the experimental relationship of $k_q=a(T/\eta)+b$. By substituting all known parameters and by using the data in Fig. 7, the unknown constants, A', B', and γ', can be obtained as listed in Table 4. It can be recognized from Fig. 7 that the k_q values for both Br$^-$ and I$^-$ have a linear dependence on T/η in the measured region, as is to be expected from Eq. 18. The difference in the slopes for Br$^-$ and I$^-$ is due to the lower quenching efficiency of Br$^-$; it is interesting that the intercepts at T/η=0 are unexpectedly nearly equal in all cases.

In conclusion, the quenching-rate constant of the coumarin derivatives by halide ions is reasonably well described by means of the diffusion-controlled mechanism of the quenching reaction. I$^-$ has been shown to be an "efficient" quencher; Br$^-$ is rather an "inefficient" quencher; Cl$^-$ is a very weak quencher and has no effect on the fluorescence under the conditions examined. Such an order of quenching ability is parallel with the order of the ionization energies of halide anions.[16] Therefore, the initiation of the quenching process is perhaps related with the electron transfer from the ion to the fluorescent molecule in the excited state.[18] Fig. 8 shows a schematic drawing of the energy levels of the interacting species; it takes account of all the results discussed so far. At an infinite distance, the energy differences between the singlet ground and excited states of 7-ethoxycoumarins are 338—343 kJ mol^{-1}, and the ionization energies of Cl$^-$, Br$^-$, and I$^-$ in an aqueous solution are 678, 623, and 544 kJ mol^{-1}.[16] If the crossing of the energy curves of ^{1}A*+X$^-$ and A$^{\bar{\cdot}}$+X$^{\cdot}$ resides near the encounter distance, the electron transfer from a halide anion to an excited molecule will easily occur, which results in an efficient conversion of the singlet state of the encounter complex, ^{1}(AX$^-$)*, to its triplet state or, alternatively, to the charge-transferred singlet state, ^{1}(A$^{\bar{\cdot}}$X$^{\cdot}$). Because the stability of the charge-transferred complex, (A$^{\bar{\cdot}}$X$^{\cdot}$), is altered by the magnitude of the ionization energy of halide anions, and because its energy position goes lower as the ionization energy decreases, the above-mentioned conversion induced by the electron-transfer process may become a main quenching pathway for I$^-$ and Br$^-$, but not for Cl$^-$. However, from a consideration of only the information obtained here, the so-called heavy-atom effect of halide anions, which directly converts the singlet state of the encounter complex to its triplet state, *i.e.*, ^{1}(AX$^-$)*$\rightarrow$^{3}(AX$^-$)*, cannot be excluded completely. This point is dis-

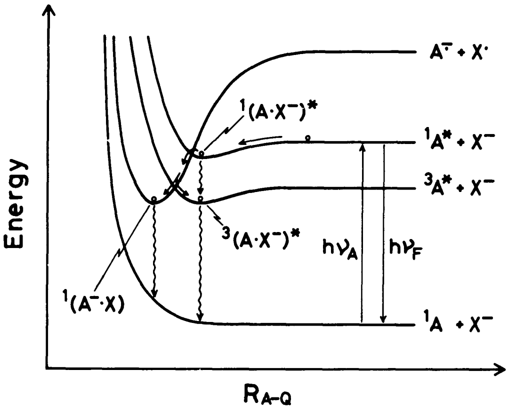


Fig. 8. Schematic potential energy curves of the colliding species and presumable pathways for the fluorescence quenching. R_{A-Q} is the distance between the fluorescent molecule and the halide anion. ^{1}A* and ^{3}A* are the singlet and triplet state of the excited molecule, and A$^{\bar{\cdot}}$ is the anion radical generated by the electron transfer from the halide anion, X$^-$. The upward and downward arrows describe the radiative transition, and the downward wavy arrows represent the radiationless transition caused by the collisional reaction.

cussed fully in Refs. 17 and 18, where it is concluded that the main pathway for the fluorescence quenching by diamagnetic inorganic anions is strongly related to the electron-transfer process. It is also revealed that the Stokes-Einstein relation must be somewhat modified in order to explain the dependence of the quenching-rate constants on the viscosity and temperature of the solution.

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References

1) O. S. Wolfbeis, E. Lippert, and H. Schwarz, *Ber. Bunsenges. Phys. Chem.*, **84**, 1115 (1980), and the references cited therein.

2) T. Moriya, *Bull. Chem. Soc. Jpn.*, **56**, 6 (1983).

3) T. Moriya and H. Anzai, *Bull. Electrotech. Lab.*, **46**, 431 (1982).

4) T. Moriya, *Bull. Electrotech. Lab.*, **47**, 81 (1983).

5) C. Lewis, W. R. Ware, L. J. Doemeny, and T. L. Nemzek, *Rev. Sci. Instrum.*, **44**, 107 (1973).

6) E. P. Kirby and R. F. Steiner, *J. Phys. Chem.*, **74**, 4480 (1970).

7) W. R. Ware, *J. Phys. Chem.*, **66**, 455 (1962).

8) A. H. Alwattar, M. D. Lumb, and J. B. Birks, "Organic Molecular Photophysics," Vol. 1, ed by J. B. Birks, Wiley, New York (1973), p. 403.

9) T. L. Nemzek and W. R. Ware, *J. Chem. Phys.*, **62**, 477 (1975).

10) A. Weller, *Z. Phys. Chem. N. F.*, **13**, 335 (1957).

11) W. R. Ware and J. S. Novros, *J. Phys. Chem.*, **70**, 3246 (1966).

12) J. M. Frank and S. J. Wawilow, *Z. Phys.*, **69**, 100 (1931).

13) A. Y. Moon, D. C. Poland, and H. A. Scheraga, *J. Phys. Chem.*, **69**, 2960 (1965).

14) M. R. Eftink and C. A. Ghiron, *J. Phys. Chem.*, **80**, 486 (1976).

15) S. G. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill, New York and London (1941).

16) A. Treinin and E. Hayon, *J. Am. Chem. Soc.*, **98**, 3884 (1976).

17) A. R. Watkins, *J. Phys. Chem.*, **78**, 2555 (1974).

18) R. Beer, K. M. C. Davis, and R. Hodgson, *J. Chem. Soc., Chem. Commun.*, **1970**, 840.