The Competition of Unimolecular and Bimolecular Processes with Special Applications to the Quenching of Fluorescence in Solution

By RICHARD M. NOYES

The probability per unit of time that an electronically excited molecule will fluoresce is independent of its age, but the probability per unit of time for a bimolecular quenching reaction decreases with increasing time after excitation. Since in an illuminated solution the average age of excited molecules is decreased by increasing the concentration of quencher, the apparent rate constant for the quenching reaction should increase. The equations for this effect are developed in detail, and it is shown that the predicted concentration dependence of the quenching constant is experimentally accessible if the rate of the quenching reaction is determined by the diffusion of reactants together. Data from the previous literature covering a wide range of rate constants indicate a concentration dependence of the quenching constants that is uniformly about twice that predicted by the theory. Measurable effects depend only on macroscopic diffusion coefficients independent of the magnitudes of individual displacements. Therefore, detailed information about diffusive motion is not accessible from the time dependence of the reactivity of molecules that have been produced singly.

Introduction

If a reference molecule has existed for a finite time in a solution containing molecules capable of reacting with it, there is less chance of finding a potential reactant near the reference molecule than near a random position in the solution, and the probability of reaction of the reference molecule per unit of time is less than the a priori probability at the time it was formed.

In a previous paper,[1] we have shown how the time-dependence of the reactivity of a molecule can be described in terms of the behavior of an isolated pair of molecules in the same solvent. The present paper applies to the competition for a reference molecule between a time-independent unimolecular process and a bimolecular process (whose probability is necessarily a function of time). Quenching of fluorescence is probably the best example of such a situation, and our equations are derived for this case. However, the results are applicable without modification for any situation in which uni- and bi-molecular processes compete to determine the fates of reactive molecules.

The study was undertaken in the expectation that by measuring the concentration dependence of the quenching constant one could use the excited molecule as a probe to study the magnitudes of diffusive displacements in its immediate neighborhood. In the last section, we discuss the failure to obtain microscopic information from such measurements.

Mathematical Development

Formulation of Problem.—If a solution containing absorbing molecules, A, and quencher molecules, Q, is illuminated, the fluorescent intensity is proportional to the instantaneous concentration of activated molecules, A*. If the quenching process does not involve a chemical reaction or if the concentrations of A and Q are great enough that they do not change significantly during an experiment, a steady state is set up for the following processes

Process	Rate
A + $h\nu \longrightarrow$ A*	
A* $\longrightarrow$ A + $h\nu'$	$k_f[A^*]$
Q + A* $\longrightarrow$ quenching	$k_q(av)[Q][A^*]$
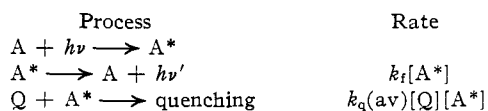
(1) R. M. Noyes, J. Chem. Phys., **22**, 1349 (1954).

The rate constant for quenching, k_q, depends on the age of an excited molecule, and $k_q(av)$ has been averaged over all the excited molecules present in the solution at any instant.

If f is the fluorescent intensity observed from the solution and if a *following* superscript 0 denotes a value at zero concentration of quencher, it is customary to define a quenching constant, k_{ex}, having dimensions of reciprocal concentration (but not time) and defined by the expression

$$k_{ex} = \frac{f^0/f - 1}{[Q]} = k_q(av)/k_f \qquad (1)$$

According to conventional kinetics, k_q is independent of the age of an excited molecule and k_{ex} is independent of concentration of quencher. However, as the concentration of quencher increases, the average age of the excited molecules in the solution will go down, and our previous treatment[1] suggests that $k_q(av)$ and k_{ex} should increase.

In order to treat the anticipated concentration dependence, let S be the probability that an A molecule that was excited at time zero is still excited at time t.

$$-\frac{dS}{dt} = k_f S + k_q[Q]S \qquad (2)$$

As indicated above, k_f is independent of time but k_q may not be. If the concentration of quencher is constant during an experiment, we can integrate to

$$S = e^{-\left\{ k_f t + [Q] \int_0^t k \ dt' \right\}} \qquad (3)$$

where t' is a time between zero and t.

If a solution has been illuminated long enough to establish a steady state

$$f/f^0 = \frac{\int_0^\infty k_f S \ dt}{\int_0^\infty k_f e^{-k_f t} dt} = \int_0^\infty k_f S \ dt \qquad (4)$$

where the integrals are taken over past time and involve the probability of fluorescence at the present of molecules excited at time t previously. If k_q is independent of time, equation 4 rearranges easily to equation 1.

The time dependence of k_q is given by[1]

$$k_q = {}^0k_q \left(1 - \int_0^t h(t') \ dt' \right) \qquad (5)$$

where a *preceding* superscript 0 denotes a value at zero time. Here $h(t)$ dt is the probability that an isolated pair consisting of a quencher molecule and an excited molecule (that does not fluoresce) initially separated as though they had undergone a non-quenching encounter at time zero will undergo a quenching reaction between t and $t + $ dt. The integral of $h(t)$ over all times is less than unity, and we have used the definition

$$\beta' = \int_0^\infty h(t) \, dt \tag{6}$$

It is also convenient to define the function

$$Z(t) = \int_0^t \int_0^{t'} h(t'') \, dt'' dt' \tag{7}$$

where t'' is a time between zero and t'. Then equation 3 can be rewritten

$$S = e^{-[k_f + {}^0k_q[Q]]t} e^{{}^0k_q[Q] Z(t)} \tag{8}$$

The solution of these equations depends upon the form of $h(t)$. At long times, it is inversely proportional to $t^{3/2}$, but this form cannot be valid at very short times. In the following sections, the problem is treated by two approximation formulas for $h(t)$ which have been discussed elsewhere.[2] The discontinuous function permits an almost exact mathematical solution, while numerical methods are necessary for the more plausible continuous approximation.

Solution by Discontinuous Approximation.—The discontinuous function

$$\begin{aligned} h(t) &= 0 & t < 4a^2/\beta'^2 \\ &= a/t^{3/2} & t > 4a^2/\beta'^2 \end{aligned} \tag{9}$$

is the simplest approximation for $h(t)$ that fits the long-time behavior and also satisfies equation 6. In this equation, a is a constant having dimensions sec.$^{1/2}$; its dependence on more fundamental parameters has been discussed previously.[2]

For this function

$$\begin{aligned} Z_d(t) &= 0 & t < 4a^2/\beta'^2 \\ &= \beta' t - 4at^{1/2} + 4a^2/\beta' & t > 4a^2/\beta'^2 \end{aligned} \tag{10}$$

where the subscript d indicates the discontinuous approximation.

Application to equations 8 and 4 gives

$$f/f^0 = k_f e^{4a^2 \, {}^0k_q[Q]/\beta'} \int_0^\infty e^{-[k_f + {}^0k_q(1 - \beta')[Q]]t - 4a{}^0k_q[Q]t^{1/2}}$$
$$dt + k_f \int_0^{4a^2/\beta'^2} e^{-[k_f + {}^0k_q[Q]]t} [1 -$$
$$e^{\beta'{}^0k_q[Q]t - 4a{}^0k_q[Q]t^{1/2} + 4a^2{}^0k_q[Q]/\beta'}] \, dt \tag{11}$$

The second integral is not exactly soluble but can be expanded in powers of $k_f a^2/\beta'^2$ and ${}^0k_q[Q]a^2/\beta'^2$. We shall see later that these quantities are less than unity. Such a solution of the second integral involves only terms in a^4 and higher orders. Since the continuous and discontinuous approximations differ in the a^2 terms, the second integral in equation 11 is without significance and can be totally neglected.

The first integral is exactly soluble. Let

$$b = \frac{4a{}^0k_q[Q]}{\{k_f + {}^0k_q(1 - \beta')[Q]\}^{1/2}} \tag{12}$$

(2) R. M. Noyes, This Journal, **78**, 5486 (1956).

Then integration of equation 11 gives

$$f/f^0 = \frac{k_f e^{b^2/4} + 4a{}^{20}k_q[Q]/\beta'}{k_f + {}^0k_q(1 - \beta')[Q]} (1 - \sqrt{\pi}b/2) \tag{13}$$

If quenching is an activation controlled process, terms in a and β' become vanishingly small and equation 13 reduces to the conventional kinetic expression for quenching of fluorescence.

Solution by Continuous Approximation.—The theory of random flights[3] predicts that the probability of finding a particle near its initial position at time t later is given by $ce^{-c'/t}/t^{3/2}$ where c and c' are constants. If $h(t)$ fits this form and satisfies equation 6, we can write the continuous function

$$h(t) = \frac{ae^{-\pi a^2/\beta'^2 t}}{t^{3/2}} \tag{14}$$

where a is the same constant as in equation 9.

If we let the subscript c denote this continuous approximation

$$z_c(t) = \beta't - \beta' \int_0^t I_x \, dt' \tag{15}$$

where the probability integral

$$I_x = (2/\sqrt{\pi}) \int_0^x e^{-x^2} \, dx \tag{16}$$

and

$$x = \frac{a}{\beta'} \sqrt{\frac{\pi}{t'}} \tag{17}$$

We have not developed an exact solution for f/f^0 in terms of this function, but we have been able to treat the difference between the two approximations. $Z_c - Z_d$ is positive at all times and approaches a limiting value of $2.283a^2/\beta'$ at long times. If we assume that the time to approach this value is short compared to the average life-time of an excited molecule, S in equation 11 will be increased by a constant factor of $e^{{}^0k_q[Q][Z_c(\infty) - Z_d(\infty)]}$ and equation 13 becomes

$$f/f^0 = \frac{k_f e^{b^2/4} + 6.28a^{20}k_q[Q]/\beta'}{k_f + {}^0k_q(1 - \beta')[Q]} (1 - \sqrt{\pi} \, b/2) \tag{18}$$

The true situation should be intermediate between equations 13 and 18 and probably considerably closer to (18). We shall see below that the difference is a second order effect and probably without experimental significance.

Concentration Dependence of Quenching Constant.—Equation 18 can be written in terms of the following three parameters, all of which have dimensions of reciprocal concentration.

$$J = {}^0k_q(1 - \beta')/k_f \tag{19}$$

$$K = a{}^0k_q/k_f{}^{1/2} \tag{20}$$

$$L = a^{20}k_q/\beta' \tag{21}$$

The parameter J describes the competition between quenching and fluorescence of excited molecules so "old" that the concentration gradient of potential quenchers is independent of time. Here, ${}^0k_q(1 - \beta')$ is the "long-time" rate constant for quenching. If the reaction is diffusion controlled, J is concerned with times so long that it is permissible to apply the Fick's first law treatment for steady-state diffusion into a sink.

(3) S. Chandrasekhar. Rev. Modern Phys., **15**, 1 (1943).

The parameter K relates to competition of fluorescence with the rate of establishment of the steady-state concentration gradient; it is concerned with times during which Fick's second law must be used to describe changes in concentration near the excited molecule.

Fick's laws, and similar treatments in which diffusion is treated as a continuous process, predict an infinite initial flux when a diffusion sink (such as a reactive molecule) is suddenly created in a solution of uniform concentration. The parameter L is contained in transient correction terms dealing with the period of a few molecular displacements before it is permissible to describe the situation even with Fick's second law.

ing equations, all of which also have been discussed previously.[1,2]

$$\beta' = \frac{\alpha\beta}{1 - \beta + \alpha\beta} \tag{23}$$

$$\beta = 1 - \frac{2}{1 + 3\rho/\sigma} \tag{24}$$

$$a = \frac{3\alpha\rho^2(1 - \beta')^2}{4\sigma\sqrt{\pi D}} \tag{25}$$

$$^0k_q = \frac{6\pi\alpha\rho^2 DN}{1000\sigma} \tag{26}$$

The rate of the quenching reaction may be controlled either by the diffusion together of reactive molecules, or by the passage over a free energy po-

TABLE I

QUANTITIES EXPRESSED IN TERMS OF FUNDAMENTAL PARAMETERS

Type of control	Diffusion	Diffusion	Activation	Activation	Intermediate
Magnitudes of displacements	Large	Small	Large	Small	Intermediate
Mathematical description of conditions	$\alpha \approx 1$ $\sigma \approx \rho$	$\alpha >> \sigma/\rho$ $\sigma << \rho$	$\alpha << 1$ $\sigma \approx \rho$	$\alpha << \sigma/\rho$ $\sigma << \rho$	$0 < \alpha < 1$ $\sigma < \rho$
a/β'	$\dfrac{3\rho^2\sigma}{\sqrt{\pi D}(9\rho^2 - \sigma^2)}$	$\dfrac{\sigma}{3\alpha\sqrt{\pi D}}$	$\dfrac{3\rho^2}{2\sqrt{\pi D}(3\rho - \sigma)}$	$\dfrac{\rho}{2\sqrt{\pi D}}$	$\dfrac{3\rho^2\sigma}{\sqrt{\pi D}(3\alpha\rho + 2\sigma - \alpha\sigma)(3\rho - \sigma)}$
$a/(1 - \beta')$	$\dfrac{3\rho^2}{2\sqrt{\pi D}(3\rho + \sigma)}$	$\dfrac{\rho}{2\sqrt{\pi D}}$	$\dfrac{3\alpha\rho^2}{4\sqrt{\pi D}\sigma}$	$\dfrac{3\alpha\rho^2}{4\sqrt{\pi D}\sigma}$	$\dfrac{3\alpha\rho^2}{2\sqrt{\pi D}(3\alpha\rho + 2\sigma - \alpha\sigma)}$
$k_t J = {}^0k_q(1 - \beta'$	$\dfrac{12\pi\rho^2 DN}{1000(3\rho + \sigma)}$	$\dfrac{4\pi\rho DN}{1000}$	$\dfrac{6\pi\alpha\rho^2 DN}{1000\sigma}$	$\dfrac{6\pi\alpha\rho^2 DN}{1000\sigma}$	$\dfrac{12\pi\alpha\rho^2 DN}{1000(3\alpha\rho + 2\sigma - \alpha\sigma)}$
$K/J^{1/2} = \dfrac{a\sqrt{{}^0k_q(1 - \beta')}}{1 - \beta'}$	$\left(\dfrac{3\rho^2}{3\rho + \sigma}\right)^{1/2}\sqrt{N/1000}$	$\rho^{1/2}\sqrt{N/1000}$	$\left(\dfrac{3\alpha\rho^2}{2\sigma}\right)^{1/2}\sqrt{N/1000}$	$\left(\dfrac{3\alpha\rho^2}{2\sigma}\right)^{1/2}\sqrt{N/1000}$	$\left(\dfrac{3\alpha\rho^2}{3\alpha\rho + 2\sigma - \alpha\sigma}\right)^{1/2}\sqrt{N/1000}$
$L = a^2 {}^0k_q/\beta'$	$\dfrac{54\rho^6\sigma N}{1000(3\rho + \sigma)^3(3\rho - \sigma)}$	$\dfrac{2\rho^2\sigma N}{3000\alpha}$	$\dfrac{27\alpha^2\rho^6 N}{4000\sigma^2(3\rho - \sigma)}$	$\dfrac{9\alpha^2\rho^5 N}{4000\sigma^2}$	$\dfrac{54\alpha^2\rho^6\sigma N}{1000(3\alpha\rho + 2\sigma - \alpha\sigma)^3(3\rho - \sigma)}$

If these parameters are employed when substituting equation 18 into equation 1, we obtain

$$k_{ex}[Q] = \frac{1 + J[Q]}{\left(1 - \dfrac{2\sqrt{\pi}K[Q]}{1 + J[Q]}\right)e^{\frac{4K^2[Q]^2}{1 + J[Q]} + 6.28L[Q]}} - 1 \tag{22}$$

Unless the quenching reaction is so efficient that its rate is determined chiefly by the rate at which reactants can diffuse together, the parameters K and L will be vanishingly small and this rather terrifying expression reduces to the conventional $k_{ex} = J$.

Discussion

Magnitudes of Parameters.—Equation 22 is a refinement of conventional kinetic expressions and predicts that the quenching constant determined by experiment should be a function of the concentration of quencher even if the solution is behaving ideally in a thermodynamic sense. However, it is necessary to examine the probable magnitudes of the parameters to determine whether the anticipated concentration dependence of the quenching constant is apt to be of experimental significance.

In addition to the symbols defined above, we shall use the following symbols, all of which have been discussed in more detail in previous papers.[1,2]

ρ = encounter diameter
σ = root-mean-square diffusive displacement distance
ν = frequency of relative diffusive displacements
D = $\nu\sigma^2/6$ = coefficient for relative diffusion
α = probability of reaction during an encounter

The various quantities are related by the follow-

tential barrier (activation control). Also, it has not yet been established[4] whether diffusive displacements in liquids are large (of the order of a molecular diameter) or much smaller. Table I extends a table in a previous publication[2] to provide expressions under different types of rate control for the quantities of importance in the present problem.

If an approximately linear response is received from the device used to measure fluorescent intensity, then f/f^0 should lie roughly between 0.1 and 0.9 to obtain the best accuracy in k_{ex}. This means that $0.1 < k_{ex}[Q] < 10$ must be approximately fulfilled. If we arbitrarily set one mole per liter as the maximum concentration of quencher satisfactory for tests of equation 22, k_{ex} should not be less than 0.1 but may be greater. Data reported in the literature indicate values of k_{ex} and hence of J in the range 10 to 150 liter/mole.

If the quenching reaction is diffusion controlled and if diffusive displacements are small, Table I indicates that $K = 0.274J^{1/2}$ liter/mole for the situation $\rho = 5 \times 10^{-8}$ cm. This value depends very little on the magnitude of diffusive displacements. Substitution of these values of J and K into equation 22 indicates that k_{ex} should vary significantly with concentration of quencher even for values well below 0.1 mole/liter, so the proposed equation is susceptible to experimental test.

The parameter L will have its maximum value for diffusion control with large displacements. For $\alpha = 1$ and $\rho = \sigma = 5 \times 10^{-8}$ cm., $L = 0.0318$ liter/mole. For quencher at 1 mole/liter, the

(4) R. M. Noyes, J. Chem. Phys., 23, 1982 (1955).

term $e^{6.28L[Q]}$ could not contribute more than 20% to k_{ex}, and effects even at this concentration would be hard to separate from those of activity coefficients different from unity. Also, if diffusive displacements are less than a molecular diameter, L will be correspondingly smaller. Therefore, although terms in K are significant, it appears that at least for the present the term in L in equation 22 is without experimental significance.

Comparison with Experiment.—We are aware of two sets of quenching experiments in which k_{ex} was measured as a function of concentration. Bowen and Metcalf[5] studied the quenching of anthracene by carbon tetrabromide in a series of hydrocarbon solvents varying over 200-fold in viscosity. Values of k_{ex} varied more than 10-fold with change of solvent but always tended to increase with increasing concentration of quencher. The authors ascribed this effect to a complex between anthracene and carbon tetrabromide, but application of this explanation to data with other quenchers required the implausible assumption that the degree of association *increased* with increasing temperature.

Williamson and La Mer[6] observed a similar effect in the quenching of uranin by aniline and ascribed the variation semi-quantitatively to the same type of cause discussed here.

Data from both of these studies were fitted by least squares to an equation of the form

$$k_{ex} = k_{ex}^0 + U[Q] \qquad (27)$$

If equation 22 is expanded as a power series in $[Q]$ neglecting all terms of higher order and also neglecting the term in L, one obtains

$$k_{ex}^0 = J + 2\sqrt{\pi}K \qquad (28)$$

$$\left(\frac{dk_{ex}}{d[Q]}\right)^0 = \sqrt{\pi}KJ + 4(\pi - 1)K^2 \qquad (29)$$

The use of linear equation 27 is not strictly justified for the larger values of $J[Q]$ in some experiments, and experimental values of U from least squares are somewhat less than values of $(dk_{ex}/d[Q])^0$ to be anticipated from a more refined treatment. However, the difference is not important for the type of comparison undertaken here.

The results are presented in Table II. The quantities k_{ex}^0 and U_{obsd} come from the least squares fit of the data to equation 27. Values of U_{calcd} are obtained from values of k_{ex}^0 on the assumptions that the quenching reaction is diffusion controlled, that diffusive displacements are small, and that the encounter diameter is 5 Å. As is shown above, these assumptions lead to $K = 0.274 \ J^{1/2}$, and a value of k_{ex}^0 is sufficient to calculate J, K and $(dk_{ex}/d[Q])^0 = U_{calcd}$.

The data in Table II cover a range of 11-fold in k_{ex}^0 and of 25-fold in U obtained by two independent sets of investigators for two different quenching reactions. The treatment developed here successfully describes *relative* changes in U over the entire range but consistently predicts values about half of those observed experimentally. It might appear that more quantitative agree-

(5) E. J. Bowen and W. S. Metcalf, *Proc. Roy. Soc. (London)*, **206A**, 437 (1951).

(6) B. Williamson and V. K. La Mer, THIS JOURNAL, **70**, 717 (1948).

ment could be obtained by assuming a larger value for the encounter diameter. However, application of experimental values of k_{ex}^0 and U to equations 28 and 29 frequently leads to imaginary values for J and K. We have not been able to find any algebraic error in the derivations, but we find the fit of relative U values is so impressive that we believe the ideas developed here deserve further consideration.

TABLE II

COMPARISON WITH EXPERIMENT

k_{ex}^0, l./mole	U_{obsd}, l.²/mole²	U_{calcd}, l.²/mole²	U_{calcd}/U_{obsd}
	Bowen and Metcalf[5] data		
124.6	953	671	0.70
110.2	785	556	.71
93.0	853	431	.51
76.4	468	320	.68
49.4	355	166	.47
35.34	202	100	.49
22.05	157	48.7	.31
21.30	133	46.2	.35
11.64	38.4	18.4	.48
16.16	60.7	30.3	.50
	Williamson and La Mer[6] data		
23.12	86.7	52.4	0.60

Application to Theory of Liquids.—This study was undertaken in the hope that an electronically excited molecule could be used as a probe to test a small region of solution for a short time and thereby to obtain evidence concerning the nature of diffusive motions in liquids. Only hindsight reveals the futility of this expectation. At times sufficient for more than a very few molecular motions, equation 5 can be written

$$k_q = {}^0k_q(1 - \beta' + 2a/t^{1/2}) \qquad (30)$$

At long times, ${}^0k_q(1 - \beta')$ for a diffusion controlled reaction can be related to the rate of diffusion into a sink; its value is determined essentially by the encounter diameter and the macroscopic diffusion coefficients of the species. The parameter J is concerned with quenching of molecules that are old enough for this situation to apply.

Since the parameter a depends directly upon the size of molecular displacements,[2] it seemed that the time dependence of reactivity would also depend on this quantity. However, an examination of Table I will show that ${}^0k_q a$ is virtually independent of the size of diffusive displacements. The parameter K is concerned with changing reactivity at times when equation 30 is applicable to the rate of establishment of the steady-state gradient in concentration. For a diffusion controlled reaction, the value of K depends on exactly the same quantities that determine J. This dependence explains the failure of the original expectation, and it also explains why for a specified encounter diameter only one disposable parameter is sufficient to define the value of k_{ex} at any value of $[Q]$.

The parameter L does depend directly on the magnitudes of diffusive displacements, but it is concerned with times of the order of those for individual displacements and describes deviations from equation 30. We have already seen that even

the order of magnitude of L seems beyond experimental accessibility.

Because $^0k_q(1 - \beta')$ and ^0k_qa are both virtually independent of σ, it does not appear that any information about detailed microscopic behavior in liquids is obtainable from kinetic measurements on reactive molecules that are produced singly. The best hope for obtaining such information continues to lie with situations in which reactive species are produced in pairs.[2,7]

Acknowledgment.—This problem was conceived and solved in principle at Columbia University,

(7) R. M. Noyes, THIS JOURNAL, **77**, 2042 (1955).

and the work was supported in part by the U. S. Atomic Energy Commission under Contract AT-(30-1)-1314. The application of the continuous approximation and several algebraic refinements were developed at the University of Leeds while the author held a fellowship from the John Simon Guggenheim Memorial Foundation.

The author is indebted to Dr. W. S. Metcalf of Canterbury University College, Christchurch, New Zealand, for helpful correspondence and for providing the numerical data used for the least squares calculations in Table II.

NEW YORK, N. Y.
LEEDS, ENGLAND

The Equilibrium Distribution of Light and Heavy Waters in a Freezing Mixture

BY JOHN C. POSEY AND HILTON A. SMITH

The equilibrium distribution of the deuterium content of a mixture of light and heavy water between the solid and liquid phases has been determined. The deuterium concentrates in the ice phase. The separation factor, α, in solutions containing approximately 18 mole % of the total hydrogen as deuterium was found to be 1.0211 ± 0.0007.

Since the freezing point of pure heavy water is $3.80°$ higher than that of ordinary water,[1,2] it might be expected that a partial separation of the two isotopic hydrogen species could be accomplished by freezing. This observation has led to several investigations, with varying results. Bruni,[3] Chang,[4] Dezelic,[5] and La Mer, Eichelberger and Urey[6] report that no measurable separation occurs. Gilfillan[7] reported enrichment of deuterium in the ice phase when natural water was partly frozen. Teis and Florensky[8] reported enrichment of deuterium in the liquid phase under similar circumstances. However Teis[9] later stated that these results were in error, and reported that when snow melts, the deuterium is concentrated in the ice phase.

La Mer and Baker[10] and Eucken and Schaefer[1,2] calculated the equilibrium concentrations of deuterium in the ice and water phases. The values of the separation factor calculated from the data of those investigators at deuterium concentrations near those of this work (approximately 20%) were 1.040 and 1.023, respectively. Both of the groups of investigators used indirect methods of experimental verification. These calculations have apparently never been verified by direct measure-

ment of the isotopic compositions of the two phases.

The very slow rate of diffusion of water molecules in the liquid phase causes true equilibrium separation to be difficult to obtain. According to Wang, Robinson and Idelman,[11] the diffusion coefficient of HDO in water is only 1.57×10^{-5} cm.^{2} sec.$^{-1}$ at $10°$, and decreases as the temperature is lowered. As ice crystals grow in a freezing mixture, the water adjacent to their surface becomes depleted in deuterium as compared to the bulk of the liquid, and the concentration of deuterium in the ice formed will correspond to this reduced concentration. Therefore, the observed separation, as based on the bulk composition of the phases, will be less than the true value. This effect can be reduced by the use of vigorous agitation and very slow freezing rates.

Even with vigorous agitation a layer of water will remain motionless at the ice–water interface. As the distance from the solid increases the motion of the liquid increases so that this layer has no definite outer boundary. However, for purposes of mathematical analysis the film may be considered to be equivalent to a film of a definite thickness, B. This assumption is often made in engineering calculations.[12]

The true separation factor α_0, and the observed separation factor, α, are defined by the equations

$$\alpha_0 = \frac{y(1 - x^1)}{x^1(1 - y)} \tag{1}$$

and

$$\alpha = \frac{y(1 - x)}{x(1 - y)} \tag{2}$$

(1) A. Eucken and K. Schaefer, Z. anorg. allgem. Chem., **225**, 319 (1935).

(2) A. Eucken and K. Schaefer, Nachr. Ges. Wiss. Gottingen, Math.-physik. Kl., Fachgruppe III, **I**, 109 (1935).

(3) G. Bruni, THIS JOURNAL, **56**, 2013 (1934).

(4) T. L. Chang, Ber., **69B**, 1302 (1936).

(5) M. Dezelic, Bull. soc. chim. roy. Yougoslav., **7**, 65 (1936).

(6) V. K. La Mer, W. C. Eichelberger and H. C. Urey, THIS JOURNAL, **56**, 248 (1934).

(7) E. S. Gilfillan, ibid., **56**, 2201 (1934).

(8) R. V. Teis and K. P. Florensky, Compt. rend. acad. sci. U.R.S.S., **32**, 199 (1941).

(9) R. V. Teis, ibid., **53**, 529 (1946).

(10) V. K. La Mer and W. N. Baker, THIS JOURNAL, **56**, 2641 (1934).

(11) J. H. Wang, C. V. Robinson and I. S. Idelman, ibid., **75**, 446 (1953).

(12) W. L. Badger and W. L. McCabe, "Elements of Chemical Engineering," McGraw-Hill Book Co., Inc., New York, N. Y., 1936, p. 246.