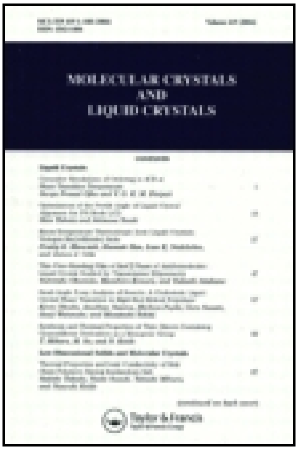

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The Validity of Perrin's Equation in Solute Quenching

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The Validity of Perrin's Equation in Solute Quenching

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Abstract—Perrin's equation for solute quenching is redefined. Multicomponent concentration quenching curves are obtained by plotting log (I/c) vs. c. Addition of quencher to the solvent-solute system and use of inefficient solvent cause the quenching curves to become linear. Based on the shift of maximum activating wavelength during photo-activation, formation of excimers or molecular aggregates of similar nature is postulated to explain the observed phenomenon.

The relationship between the fluorescence power, Q, of a dye and its concentration, c, was introduced by Perrin 1 as early as 1924 in the form of the equation

$$Q = Q_0 \exp(-c/c_m) \tag{1}$$

where Q_0 is the value which Q tends to as the concentration c tends to zero and c_m is the concentration at which maximum fluorescence of the dye solution occurs. The validity of this equation has been disputed.[2, 3, 4]

In the Perrin equation for concentration quenching, the concept of a critical transfer concentration is implicitly present. This concept is in accord with Förster's resonance transfer mechanism between donor and acceptor molecules assuming a dipole-dipole interaction.[5] Förster's equation for the quantum yield of the acceptor fluorescence can be simplified by assuming a critical transfer distance with instantaneous excitation transfer for shorter distances and no transfer for longer distances; consequently, the experimental results on the efficiency of energy transfer can be represented by a Stern-Volmer type equation or by a simple exponential form, $\exp(-\alpha c/c_0)$, where α is a constant, c, the concentration, and c_0, the critical concentration from which the critical transfer distance can be calculated. In systems where the energy transfer between triplet states occurs predominately by the exchange mechanism as in sensitized phosphorescence, the donor luminescence yield can also be approximated by an exponential form, exp $(-\alpha c)$, where α is a positive constant.[6,7] Inokuti and Hirayama[8] pointed out the constancy of α in the expression for the Perrin model of energy transfer by the exchange mechanism. In sensitized fluorescence systems employed in liquid scintillation counting, quenching of fluorescence depends upon an exponential function of the concentration of the quencher and an empirical equation was derived which is identical in form to Eq. 1.[9,10] Thus, by inference, the exponential factor in Perrin's equation for concentration quenching should be generally valid.

The similarity in shape between the radioactivity concentration quenching curve of a ^{14}C-, ^{35}S-, or ^{3}H-labeled quencher in a liquid scintillation system[9,10] and the fluorescence intensity concentration quenching curve of a solvent-solute system[11] is marked. The former when replotted using log (apparent specific radioactivity) vs. concentration of the quencher yielded a linear relationship. Since liquid scintillator is a sensitized solvent-solute or donor-acceptor system, it is likely that the fluorescence quenching by an added labeled compound and the fluorescence self-quenching by a solute may involve some common mechanisms. This report presents data and gives evidence on the validity of Eq. 1 in solute quenching if Q is redefined as apparent specific fluorescence intensity, namely,the observed intensity per unit concentration of solution and Q_0 as the specific fluorescence intensity in the absence of concentration quenching.

1. Experimental

The organic scintillators, 2,5-diphenyloxazole (PPO) and 2-(1-naphthyl)-5-phenyloxazole (αNPO), purchased from Pilot Chemicals, Inc., 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene (dimethyl-POPOP) and p-terphenyl from Packard Instrument Company, Inc., naphthalene as "Baker Analyzed" reagent and anthracene (blue-violet fluorescence) from Eastman Organic Chemicals, were used without further purification. White-labeled carbazole from Eastman, 1,2-benzanthracene, fluoranthene and 1,2,3,4,5,6,7,8-octahydroanthracene from Aldrich Chemical Company, Inc. were purified by adsorption with alumina and silica gel according to the procedure of Sangster and Irvine.[12] The scintillation solvents were toluene from Eastman, redistilled before use and n-hexane, pure grade, 99 mol per cent minimum from Phillips Petroleum Company, used as supplied. Carbon tetrachloride and phenyl isothiocyanate both from Eastman were used as quenchers.

Tritiated forms of the above organic scintillators were prepared by Wilzbach labeling technique by exposure of each compound to 4 curies of tritium gas for 3 to 4 days. The labeled products were repeatedly recrystallized from appropriate solvents and the specific radioactivity was determined as previously described.[13] The tritiated scintillators with a specific radioactivity ranging from 0.01 to 0.02 μc/g were used for concentration quenching studies and were prepared by dilution with nonradioactive scintillators followed by recrystallization from solvent.

In general, eight to fifteen homogeneous samples of varying concentrations of each labeled scintillator were employed to obtain the concentration quenching curves. The less soluble scintillators tended to crystallize out from solution on standing at the low temperature of the counting assembly. All the samples were air-saturated.

The integral count rates of the samples were measured in duplicate in 10-ml volumes in Wheaton low-potassium glass counting vials, using a liquid scintillation spectrometer, Model 3003, manufactured by Packard Instrument Company with a ^{226}Ra source for external standardization. The baseline discriminator was set at 0050 of a 1000-division dial; other instrument settings were adjusted for optimum counting of tritium.

Excitation of the scintillator solutions by β-radiation from carbon-14 and from tritium was achieved with ^{14}C- and ^{3}H-labeled toluene, and by γ radiation with the external standard ^{226}Ra. Excitation by light was carried out in a Aminco-Bowmann spectrophotofluorometer, manufactured by American Instrument Company. The activating wavelength and the fluorescent wavelength were adjusted for each sample to give the maximum meter reading.

2. Results and Discussion

The validity of the redefined Perrin's equation can be verified with the extensive data of Furst and Kallmann[11] on relative scintillation efficiencies of a range of organic scintillators in solutions. The fluorescence-intensity concentration quenching curves of these authors, described by the equation

$$I = \frac{Pc}{(Q + c)\ (R + c)} \tag{2}$$

where I is the intensity, c, the concentration and P, Q, and R are the constants, can be replotted using $\log(I/c)$ vs. c. The resulting curves show in general an exponential decrease of apparent specific fluorescence intensity, I/c, with increasing solute concentration, c. From the difference in the assigned values of P, Q, and R, given in Table 1 for the selected few compounds, it is not apparent that the replotted quenching curves obtained by α-ray excitation and by γ-ray excitation should be identical in form as shown in Fig. 1. The displacement of the quenching curve to lower intensity values in the case of α-ray excitation is due to ionization quenching. Because of the multicomponent nature of the replotted quenching curves with its significance to be discussed later and an uncertainty in the intensity values at low solute concentration as limited by the nature of the above equation, the "relative" critical transfer concentration which can be obtained from the replotted quenching curves was therefore not calculated.

TABLE 1 Assigned Constants for Equation 2.[11]

Solute	Excitation	P	Q	R
p-Terphenyl 1	γ	5680	0.72	33.3
	α	7110	1.30	57
p-Terphenyl 2	γ	6050	0.85	36.0
	α	7980	1.35	62.7
Phenyl-α-naphthylamine	γ	1810	0.49	20.9
	α	2105	0.83	42.2
Methylanthranilic acid	γ	866	1.15	16.0
		577	1.75	15.5

The concentration quenching curves of diphenylhexatriene in various solvents excited by α- or by γ-rays were replotted as shown in Figs. 2 and 3. Upon extrapolation of these curves to zero concentration of solute, the intercepts in a majority of cases were found to be identical or correspondent in value to the P/R values given.[11] The curves obtained by α-ray excitation are practically all superimposable upon one another with the exception of that of p-dioxane solution; those by γ-ray excitation behave similarly except for the systems involving solvents p-cymene and p-dioxane.

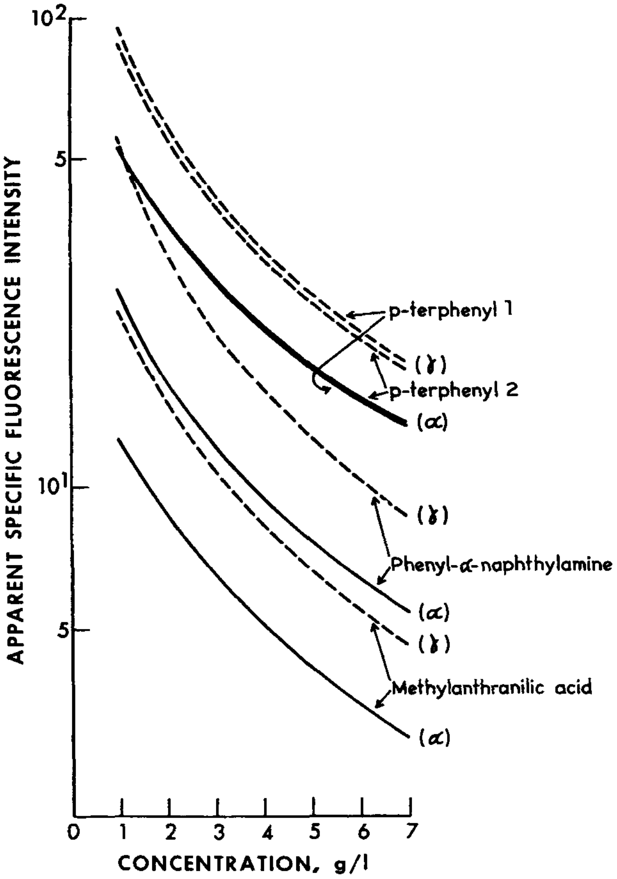

Figure 1. Exponential plots of quenching curves by α- and by γ-excitation. Calculated from reported P, Q, and R values by Eq. 2.

These exceptions may be interpreted on the basis of a difference in transfer and emission efficiencies as compared to other systems or may be attributable, as shown later, to the presence of trace amount of quenching substances which tend to diminish fluorescence intensity to a greater extent at low than at high solute concentrations. The reported P/R values for the p-dioxane system excited by α- and by γ-rays are approximately twice as large as the visually extrapolated values from the exponential plot to the quenching curve. If the P/R value and the I/c value at 7.0 g/l concentration are assumed to be both correct, a hypothetical quenching curve connecting these two points will have

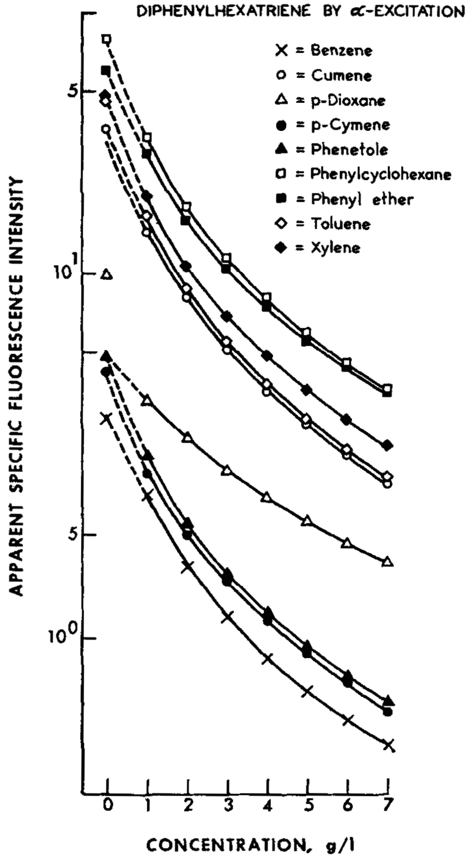

Figure 2. Exponential plots of quenching curves calculated from reported *P*, *Q* and *R* values by Eq. 2. Intercepts are by visual extrapolation. Points at intercepts are *P*/*R* values by Furst and Kallmann.[11]

approximately identical slopes as other quenching curves. Since the quenching curves for the solute in p-dioxane as shown in Figs. 2 and 3, give lower fluorescence intensity at low solute concentrations it may also be interpreted as indications of interference by quenching agents. Considering the ease with which peroxides are formed in p-dioxane on standing, such an interpretation is not implausible.

It should be noted that for diphenylhexatriene-solvent systems, the reported *P*/*R* values show good agreement with the extrapolated value, i.e., the specific fluorescence intensity in the absence of concentration quenching, only when the assigned *Q* values of Eq. 2 fall between 0.55

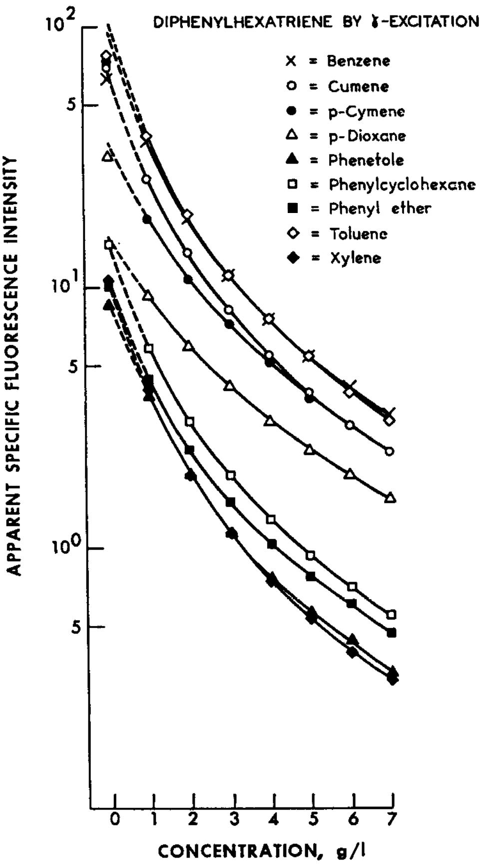

Figure 3. Exponential plots of quenching curves calculated reported P, Q and R values by Eq. 2. Intercepts are by visual extrapolation. Points at intercept are P/R values by Furst and Kallmann.[11]

and 1.3. For exact agreement, a Q value of 1.00 is required. To lend credence to our interpretation above, it should be pointed out that Furst and Kallmann had stressed the unusually large values of Q for cumene, p-cymene and especially for p-dioxane as compared to those for other solvents. Because of the dependence of τ_i/τ_e upon the P/R value, the exponential plot of the quenching curve may also be useful for comparison of the processes of light emission and internal quenching in different solvents at low solute concentrations.

The validity of the redefined Perrin's equation was further verified using tritium-labeled organic scintillators. In this case, as the concentration of the labeled scintillator is increased, the fluorescence of the solution increases as a result not only of the concentration of the solute but also of the increased strength in the source of excitation from tritium label in solution. It has been pointed out that in liquid scintillation counting of non-radioactive quenchers, the observed count rate decreases exponentially with increasing concentration of the quencher, whereas of radioactive quenchers, the observed count rate needs to be divided by the concentration of the quencher before the exponential relationship is obeyed.[9] If the same analogy applies to organic scintillators in concentration quenching studies it would require that the fluorescence intensity observed as count rate be expressed on a unit concentration basis, and that for labeled scintillators the observed count rate be divided twice by the solute concentration before their concentration quenching curves can be compared. In other words, the observed radioactivity in association with the quenching curves of the labeled scintillators in solution will increase with increasing solute concentration and will be greater by a factor of *c* than that of the corresponding non-labeled scintillator containing a given source of the same label. Experimental verification of this assumption is that the quenching curves obtained from samples containing varying concentrations of the labeled organic scintillators (^{3}H-anthracene, ^{3}H-1,2-benzanthracene, ^{3}H-carbazole, ^{3}H-1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene, ^{3}H-2,5 diphenyl-oxazole, ^{3}H-fluoranthene, ^{3}H-naphthalene, ^{14}C-naphthalene, ^{3}H-2-(1-naphthyl)-5-phenyloxazole, ^{3}H-1,2,3,4,5,6,7,8-octahydroanthracene and ^{3}H-p-terphenyl) and their non-labeled forms are identical in shape. Figure 4 illustrates the identity of the quenching curves obtained from ^{3}H-PPO and from PPO plus ^{3}H-toluene in toluene as solvent with and without carbon tetrachloride as a quencher. It may also be pointed out that the relative fluorescence intensity of these organic scintillators can be obtained from a comparison of their counting efficiences for tritium label.†

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† The relative fluorescence intensity is expressed as per cent ratio of the counting efficiency of a 0.001*M* solution of the scintillator in n-hexane or in toluene to that of a commonly used liquid scintillator (13). The values given are for toluene solution: ^{3}H-anthracene, 13.56, ^{3}H-1,2-benzanthracene, 5.07 (0.18 in n-hexane), ^{3}H-carbazole, 22.86, ^{3}H-1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene, 115.14, ^{3}H-2,5-diphenyloxazole, 108.9 (17.9 in n-hexane), ^{3}H-fluoranthene, 13.68, ^{3}H-naphthalene 2.10 (0.80 in n-hexane), ^{3}H-2-(1-naphthyl)-5-phenyloxazole, 111.36 and ^{3}H-p-terphenyl, 103.6.

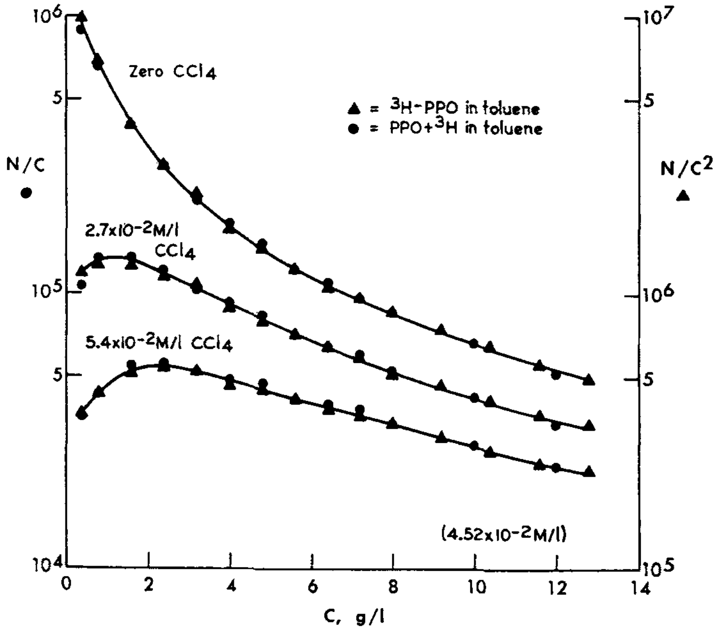

Figure 4. Exponential plots of concentration quenching curves of ^{3}H-PPO and PPO + ^{3}H in toluene. Concentration of PPO at 10 g/l is equivalent to 4.52 × 10^{-2} M/l.

Since the shape of the concentration quenching curve is an expression of the efficiency of excitation transfer in a given system, it can be used in the form of an exponential plot to compare the influence of the source of irradiation, the excitation transfer by solvents and the effect of added quenchers on the fluorescence efficiency of a solvent-solute system. Figs. 5 and 6 represent the quenching curves of organic scintillators excited by γ-rays from ^{226}Ra and by β-rays from tritium, respectively. In ^{226}Ra excitation, dimethyl-POPOP, PPO, α-NPO and p-terphenyl as well as anthracene and carbazole have quenching curves identical in shape that are superimposable to one another. The less efficient scintillators. 1,2-benzanthracene, fluoranthene, naphthalene and octahydroanthracene gave slightly different quenching curves. In tritium β-excitation, the scintillators yielded dissimilar but more linear quenching curves than those obtained under γ-ray excitation. The highly efficient scintillators, dimethyl-POPOP, PPO, αNPO and p-terphenyl, gave slightly but distinctly different quenching curves under these conditions. In the

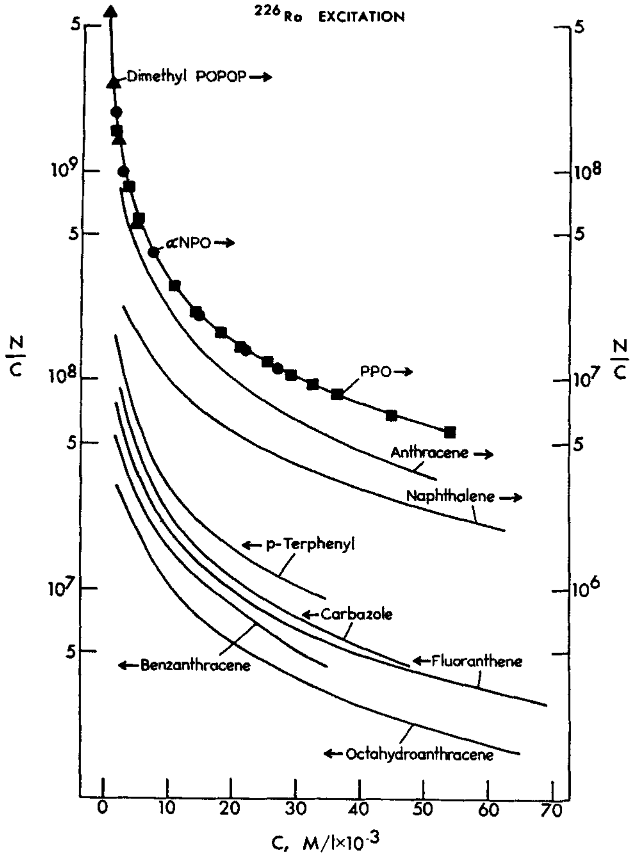

Figure 5. Exponential plots of concentration quenching curves of various organic scintillators by γ-ray excitation.

case of PPO, excitation by β-radiation from ^{14}C or by light affords essentially identical quenching curves as by ^{226}Ra excitation.

Figures 7, 8, and 9 show the effects of solvents and added quenchers on the shape of the quenching curve. The data presented were obtained with samples containing PPO in toluene and in n-hexane by β-excitation from ^{14}C and ^{3}H sources and varying amount of carbon tetrachloride or phenyl isothiocyanate as the quencher. The results show that the presence of the quencher causes a decrease of the transfer efficiency between solvent and solute molecules which lowers the fluorescence efficiency of the sample. Concomittantly, it affects the shape of the quenching curve chiefly by

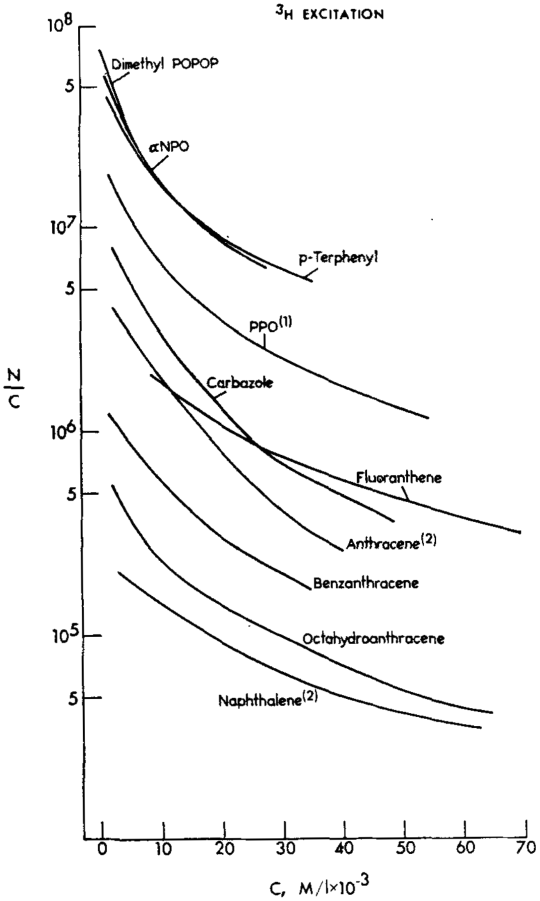

Figure 6. Exponential plots of concentration quenching curves of various organic scintillators by ^{3}H-β-excitation. The scintillator samples PPO(1) and Anthracene(2) together with Naphthalene(2) contained different strengths of ^{3}H as excitation source. The geometrical position of their quenching curves relative to that of others in the figure cannot be interpreted as an indication of their relative fluorescence intensity such as that given in footnote 1.

diminishing the fluorescence intensity at low solute concentrations. This dual effect of a quencher is more pronounced with inefficient solvent n-hexane than with efficient solvent toluene. As the quencher concentration is increased, the quenching curve at high solute concentrations becomes progressively more linear. In the case of ^{3}H-PPO in n-hexane as shown in Fig. 9, the presence of phenyl isothiocyanate, which is a

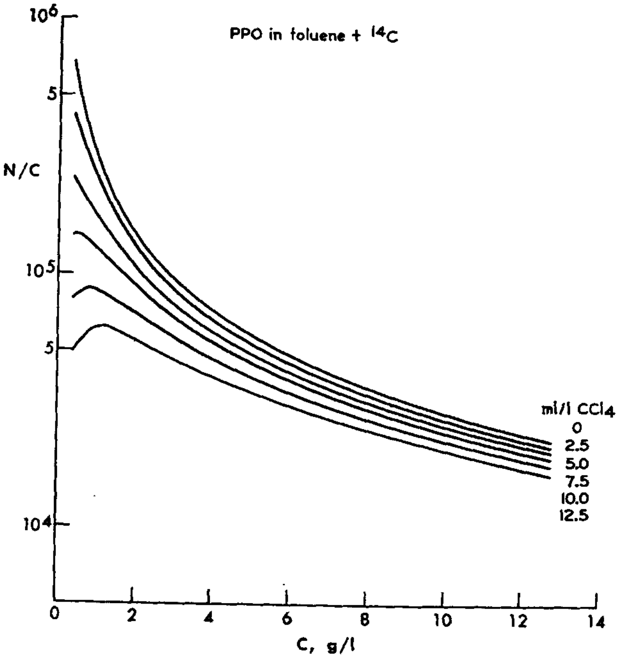

Figure 7. Effect of added quencher on concentration quenching curves of PPO-toluene systems by ^{14}C-β-excitation.

more severe quencher than carbon tetrachloride, reduced the apparent specific fluorescence intensity observed as count rate, practically to a constant value for all solute concentrations. The maximum which appeared in the low solute concentration end of the quenching curve in the presence of an increasing concentration of the quencher tended to change from a sharp to a broad peak and to shift from low to high solute concentrations. The cause of the appearance of the maximum in the curve may be attributable to a diminution of the height of weak light pulses at low solute concentrations below the acceptance level set by the baseline discriminator of the spectrometer and lost as counts, since increasing the gain of the spectrometer can offset the effect of quenching under these conditions.

Dependent upon the source of irradiation, the influence of inefficient solvent can render the quenching curve more linear in shape than that obtained using efficient solvent for a given solute. The quenching curves

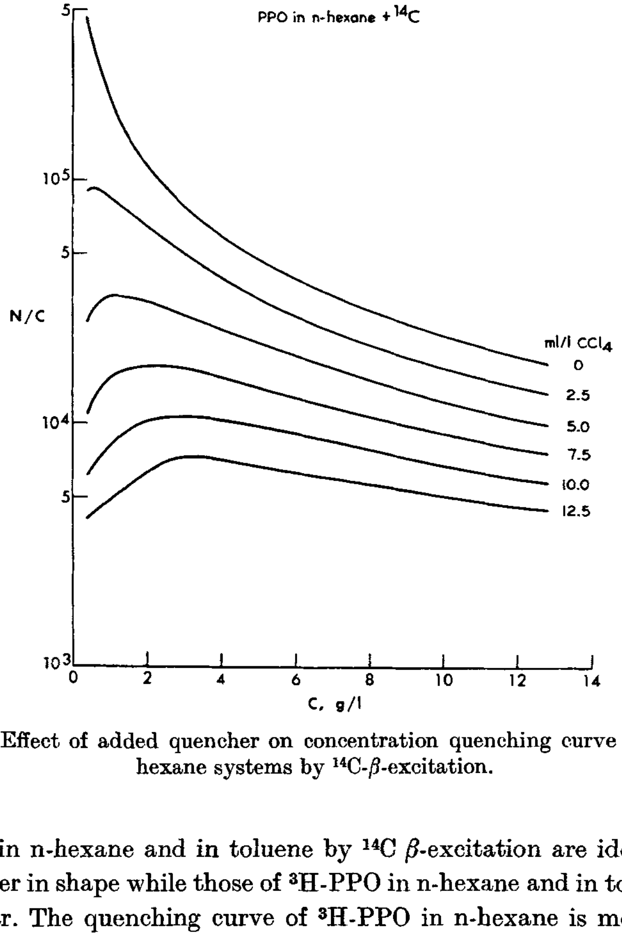

Figure 8. Effect of added quencher on concentration quenching curve of PPO-n-hexane systems by ^{14}C-β-excitation.

of PPO in n-hexane and in toluene by ^{14}C β-excitation are identical to each other in shape while those of ^{3}H-PPO in n-hexane and in toluene are dissimilar. The quenching curve of ^{3}H-PPO in n-hexane is more linear in appearance. This tendency to shift to linearity in the shape of the quenching curve is characteristic of systems of low transfer efficiency.

The concentration quenching curves obtained from unquenched samples of the organic scintillators are of multicomponent nature and can be fitted by an equation of the general form

$$Q = \sum Q_{0i} \exp\left(-c/c_{mi}\right), \; i = 1, 2, \ldots \qquad (3)$$

where i represents the number of components. This equation can be analytically resolved.[14] It may be postulated that in concentrated solutions, the solute molecules may aggregate under the influence of radiation and form "excimers"[15] of various aggregation which facilitate

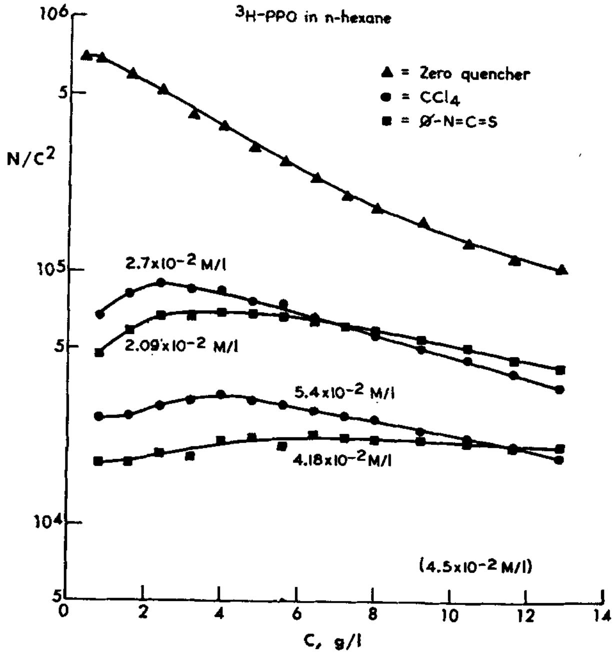

Figure 9. Effect of added quencher on the quenching curve of ^{3}H-PPO-n-hexane system.

excitation transfer and/or fluorescence emission. The "excimers" may have different quenching properties and their formation may depend upon the energy of the source of irradiation.

This formation of excimers or molecular aggregates of a similar nature in a solvent-solute system is not contrary to Förster's theory of resonance transfer of energy involving a whole ensemble of molecules with varying distributions of distance from the excited molecule. In the presence of a quencher, photo-association of the solute molecules may be hampered which may affect the formation of excimers of certain aggregation. The fact that the multicomponent quenching curve of an efficient solvent-solute system tends to be linear upon the addition of a quencher and that the linearity of the quenching curve of an inefficient solvent-solute system is more pronounced than that of an efficient system may be interpreted on the basis of the above hypothesis.

Experimental evidence which may indicate photo-association or aggregation of solute molecules is the bathochromic shift of the maximum activating wavelength for PPO-n-hexane and PPO-toluene systems as the solute concentration is increased, and the hypsochromic shift of the maximum activating wavelength for PPO-n-hexane systems and the quenched systems when compared with that for PPO-toluene system. Table 2 lists the results of two extreme concentrations of PPO studied.

TABLE 2 Influence of solute concentration, solvent and quencher on the maximum activating and maximum fluorescence wavelengths

Concentration of 2,5-diphenyl-oxazole	Solvent	Quencher concentration	Maximum activating wavelength	Maximum fluorescent wavelength
$M/l \times 10^{-3}$		$M/l \times 10^{-1}$	mμ	mμ
1.81	n-hexane	—	360	380
57.80	n-hexane	—	371	380
1.81	n-hexane	1.30	357	375
57.80	n-hexane	1.30	368	385
1.81	Toluene	—	367	385
57.80	Toluene	—	378	390
1.81	Toluene	1.30	362	385
57.80	Toluene	1.30	373	390

Addition of increasing amounts of carbon tetrachloride to given concentrations of PPO in n-hexane and in toluene causes no shift of maximum activating or maximum fluorescent wavelengths. The fluorescence intensity concentration quenching curves obtained by light excitation for the efficient PPO-toluene systems are usually of the multicomponent type while those for the inefficient PPO-n-hexane system at the high solute concentrations are straight lines. Therefore, in quenched and unquenched systems were only inefficient excitation transfer occurs, adherence to the redefined Perrin's law is usually obeyed.

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