(12) R. C. Rowell and R. S. Stein, *J. Chem. Phys.*, **47**, 2985 (1967).

(13) K. S. Pitzer, *Adv. Chem. Phys.*, **2**, 79 (1959).

(14) C. P. Smyth, "Dielectric Behavior and Structure", McGraw-Hill, New York, N.Y., 1955, Chapter VII.

(15) M. Aroney, R. J. W. Le Fèvre, and G. M. Parkins, *J. Chem. Soc.*, 2890 (1960).

(16) A. Kotera, K. Suzuki, K. Matsumara, T. Nakano, T. Oyama, and U. Kumbayuchi, *Bull. Chem. Soc. Jpn.*, **35**, 797 (1962).

(17) C. Rossi and C. Cuniberti, *J. Polym. Sci., Part B*, **2**, 681 (1964).

(18) Although several other solvents (acetone, water, dioxane, and chloroform) also showed[17] $[\eta] = KM^{0.5}$ behavior at 25 °C, only in benzene was the observed $K = 1.29 \times 10^{-3}$ within experimental error of the unperturbed or theta temperature value $K_\theta = (1.15 \pm 0.15) \times 10^{-3}$ reported by Bailey and Callard.[19]

(19) F. E. Bailey, Jr., and R. W. Callard, *J. Appl. Polym. Sci.*, **1**, 56 (1959).

(20) J. Marchal and H. Benoit, *J. Chim. Phys. Phys. Chim. Biol.*, **52**, 818 (1955); *J. Polym. Sci.*, **23**, 233 (1957).

(21) W. H. Stockmayer, *Pure Appl. Chem.*, **15**, 539 (1967).

(22) K. Nagai and T. Ishikawa, *Polym. J.*, **2**, 416 (1971).

(23) M. Doi, *Polym. J.*, **3**, 252 (1972).

(24) S. C. Liao and J. E. Mark, *J. Chem. Phys.*, **59**, 3825 (1973).

(25) B. Lemaire, G. Fourche, and E. Sanchez, *J. Polym. Sci., Polym. Phys. Ed.*, **12**, 417 (1974).

(26) Hopefully a water-jacketed Kerr cell will allow us to operate at elevated temperatures and permit us to determine ${}_mK_s$ for the higher molecular weight POE's in CCl_4 to make certain the chain length dependence observed by Aroney et al.[15] in benzene does not obtain in CCl_4 a better solvent[27] for the POE's, as preliminarily indicated in Figure 3.

(27) C. Sadron and P. Rempp, *J. Polym. Sci.*, **29**, 127 (1958).

(28) C. G. Le Fèvre and R. J. W. Le Fèvre, *J. Chem. Soc.*, 4041 (1953).

(29) G. D. Patterson and P. J. Flory, *Trans. Faraday Soc.*, **68**, 1098 (1972).

(30) A. D. Buckingham and J. A. Pople, *Proc. Phys. Soc., London, Sect. A*, **68**, 905 (1955).

(31) A. D. Buckingham, *Proc. Phys. Soc., London, Sect. A*, **68**, 910 (1955).

(32) C. G. Le Fèvre, R. J. W. Le Fèvre, and D. A. A. S. Narayana Rao, *J. Chem. Soc.*, 708 (1956).

(33) C. G. Le Fèvre and R. J. W. Le Fèvre, *J. Chem. Soc.*, 2670 (1959).

(34) A. E. Tonelli, *Macromolecules*, **10**, 153 (1977).

Energy Transfer in Labeled Polymer Chains in Semidilute Solutions

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ABSTRACT: We discuss the possibility of using Förster resonance energy transfer between chromophores attached to a polymer chain as a monitor of the distribution function of the end-to-end distance in the semidilute regime.

Recently, Daoud et al.[1] have shown that in the semidilute region of polymer statistics there exists a characteristic length $\xi(\rho)$ defining the average distance between entanglement points at monomer concentration ρ. These authors give the average square end–end distance of a polymer as

$$\langle R^2 \rangle = N_\xi \xi^2 \tag{1}$$

and assume a Gaussian distribution for the end-to-end distance

$$f(R) = \left(\frac{3}{2\pi\langle R^2\rangle}\right)^{3/2} \exp\left(-\frac{3R^2}{2\langle R^2\rangle}\right) \tag{2}$$

where N_ξ is the number of segments of characteristic length ξ. Since N_ξ and ξ depend on concentration ($N_\xi \propto \rho^{5/4}$ and $\xi \propto \rho^{-3/4}$), the end–end distance (eq 1) is a function of the concentration

$$\langle R^2 \rangle = B^2 \rho^{-1/4} \tag{3}$$

Here B depends on the molecular weight of a polymer (M_w); in the following argument, we assume $B = 115$ for a polystyrene with $M_w = 114\,000$ g/mol (ρ in Å^{-3} and R in Å). The conjecture of Daoud et al.[1] has been formulated to apply to all intra-chain segment distances in the semidilute region. These authors have compared their theoretical predictions to experimental measurements of the radius of gyration R_G determined from Zimm plots. However, the predicted concentration dependence is not so pronounced for this quantity.

In this note, we discuss a possible experimental method to make a direct measurement of the polymer end–end distance by measurement of non-radiative energy-transfer processes. As discussed below, the energy-transfer parameters are expected to be sensitive to the theoretically predicted concentration dependence. For simplicity we explicitly consider the case in which the optical labels are placed on the chain ends; our arguments will apply with appropriate modification to labeling in the interior of the chain.

The Förster theory of resonance excitation energy transfer[2] states that the rate of transfer (probability of transfer per unit time) from an excited donor molecule, D*, to an unexcited acceptor molecule, A, depends on the inverse sixth power of the distance, R, between the two molecules

$$\gamma = \frac{1}{\tau_D}\left(\frac{R_0}{R}\right)^6 \tag{4}$$

Here τ_D is the mean lifetime of D* in the absence of the acceptor and R_0 is the "critical transfer distance" determined by the overlap of the fluorescence spectrum of D* and the absorption spectrum of A; the value of R_0 is 20–50 Å for a typical pair of chromophores. From eq 4, the light intensity, $I(t)$, of the fluorescence of the excited donor molecule t is given by

$$I_D(t) = \frac{n_D(0)}{\tau_D} \int f(R) \exp\left\{-\frac{t}{\tau_D} - \frac{t}{\tau_D}\left(\frac{R_0}{R}\right)^6\right\} d^3\mathbf{R} \tag{5}$$

where $n_D(0)$ is the number of donor molecules excited at $t = 0$ and $f(R)$ is a normalized distribution function of the donor–acceptor distance R [e.g., eq 2].

In eq 4 and 5, the assumption has been made that during the donor lifetime, the donor and acceptor dipoles take on all their possible orientations so that the angular part of γ can be replaced by its average. In addition, we make the assumption that the end-to-end distance of each polymer in the ensemble changes very little during the donor lifetime so that the average over initial static polymer conformations is important. If the characteristic time for appreciable relative motion of the chain ends becomes comparable to τ_D, then there will be dynamic contributions to the emission lifetime.

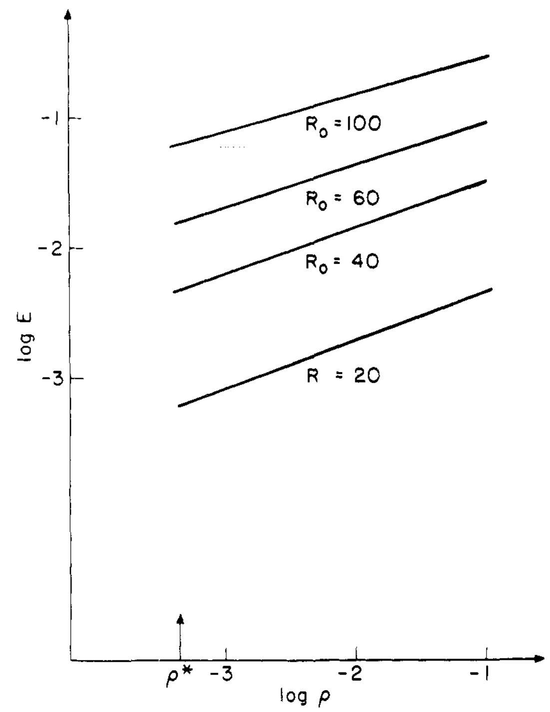

Figure 1. Efficiency vs. density for various values of R_0. Gaussian distribution [eq. 2] is assumed and B = 115 is used. ρ^* indicates the cross-over concentration from dilute to semidilute region.

Then, the efficiency of the energy transfer from donor to acceptor, E, is

$$E \equiv \frac{\int_0^\infty I_0(t)\mathrm{d}t - \int_0^\infty I_\mathrm{D}(t)\,\mathrm{d}t}{\int_0^\infty I_0(t)\,\mathrm{d}t}$$

$$= \int f(R)\frac{R_0^6}{R^6 + R_0^6}\,\mathrm{d}^3\mathbf{R} \quad (6)$$

where $I_0(t)$ is the light intensity of the fluorescence of D* without quenching by the acceptor,

$$I_0(t) = \frac{n_\mathrm{D}(0)}{\tau_\mathrm{D}}\exp(-t/\tau_\mathrm{D}) \quad (7)$$

The strong dependence of the probability of energy transfer on the separation R between donor and acceptor should be noted. This characteristic of long-range energy transfer has been used by many workers[3] to determine the distance between two labeled positions in a polypeptide or protein molecule. The experiments have been carried out by observing the fluorescence intensity of the donor molecule; a typical distance between donor and acceptor in these experiments is 20–80 Å, which gives an efficiency E of between 10 and 40%.

We have calculated the efficiency E [eq 6] for the end–end distance distribution of the polystyrene with M_w = 114 000 in the semidilute region [eq 1–3 with B = 115] and plotted the results in Figure 1. The result is that E is very small (order of 0.1–3% for R_0 = 20–40 Å). The reason for this is that the mean end–end distance $\langle R^2\rangle^{1/2}$ (100–300 Å) is much larger than R_0. Such a small E is very difficult to observe from the fluorescence light intensity measurement of the donor molecule, where a small deviation in the large magnitude of the fluorescence ($\int_0^\infty I_\mathrm{D}(t)\,\mathrm{d}t - \int_0^\infty I_0(t)\,\mathrm{d}t$) must be detected. Nevertheless, the efficiency E shows a concentration dependence of $\rho^{3/8}$ in the semidilute region. Indeed, eq 6 can be written as

$$E = \frac{4R_0^3}{\pi^{1/2}}\left(\frac{3}{2\langle R^2\rangle}\right)^{3/2}\int_0^\infty \mathrm{d}x e^{-3R_0^2x^2/2\langle R^2\rangle}\frac{x^2}{x^6+1}$$

For $\langle R^2\rangle \gg R_0^2$, we find

$$E \approx \rho^{3/8}\left(\frac{3\pi}{2}\right)^{1/2}\left(\frac{R_0}{B}\right)^3$$

Therefore, if we have a means of measuring such a small E, the Förster energy transfer mechanism will be very useful for the examination of the conjecture contained in eq 1–3.

The fluorescence of the acceptor may also be used to determine E. Unlike monitoring the donor fluorescence, only the absolute value of the intensity must be detected, and hence E can easily be obtained if we have a proper set of donor and acceptor chromophores.

The kinetic equations for the donor–acceptor system are given by

$$\frac{\mathrm{d}n_\mathrm{D}(t)}{\mathrm{d}t} = -\frac{n_\mathrm{D}(t)}{\tau_\mathrm{D}} - \gamma n_\mathrm{D}(t) \quad (8)$$

and

$$\frac{\mathrm{d}n_\mathrm{A}(t)}{\mathrm{d}t} = -\frac{n_\mathrm{A}(t)}{\tau_\mathrm{A}} + \gamma n_\mathrm{D}(t) \quad (9)$$

where the $n_\mathrm{D}(t)$ and $N_\mathrm{A}(t)$ are the numbers of excited donors and acceptors at time t, τ_D and τ_A are the intrinsic lifetimes of donor and acceptor, and the γ is the transfer probability given in eq 4. Solving for $n_\mathrm{A}(t)$, we obtain

$$n_\mathrm{A}(t) = \frac{\gamma n_\mathrm{D}(0)}{\left(\gamma + \frac{1}{\tau_\mathrm{D}} - \frac{1}{\tau_\mathrm{A}}\right)}[e^{-t/\tau_\mathrm{A}} - e^{-(\gamma+(1/\tau_\mathrm{D}))t}] \quad (10)$$

Then, the light intensity of the acceptor fluorescence is given by

$$I_\mathrm{A}(t) \equiv \int \frac{n_\mathrm{A}(t)}{\tau_A} f(R)\,\mathrm{d}^3\mathbf{R}$$

$$= n_\mathrm{D}(0)R_0^6\int\frac{\mathrm{d}^3\mathbf{R}f(R)\,\{e^{-t/\tau_\mathrm{A}} - e^{-(1+(R_0^6/R^6))t/\tau_\mathrm{D}}\}}{R_0^6\tau_\mathrm{A} + (\tau_\mathrm{A} - \tau_\mathrm{D})R^6} \quad (11)$$

The efficiency of the energy transfer can be obtained by integrating $I_\mathrm{A}(t)$, namely,

$$E = \int_0^\infty I_\mathrm{A}(t)\,\mathrm{d}t \Big/ \int_0^\infty I_0(t)\,\mathrm{d}t = \int\frac{\mathrm{R}_0^6}{R_0^6 + R^6}f(R)\,\mathrm{d}^3\mathbf{R} \quad (12)$$

which, of course, agrees with eq 6. Therefore, we can obtain E from the measurement of the fluorescence intensity of the acceptor molecule ($\int_0^\infty I_\mathrm{A}(t)\,\mathrm{d}t$).

The fluorescence intensity decay, $I_\mathrm{A}(t)$ [eq. 11], depends on the distribution function $f(R)$ and thus might give a good measure of it. As an example, we have calculated $I_\mathrm{A}(t)$ for a Gaussian distribution $f(R)$ of the same polystyrene [eq 1–3 with B = 115] and plotted the results in Figure 2 for the case $\tau_\mathrm{A} = \tau_\mathrm{D}$. As the polymer concentration ρ increases, the intensity $I_\mathrm{A}(t)$ increases, in agreement with E in Figure 1, and the functional form of $I_\mathrm{A}(t)$ becomes sharper. In general, we can calculate $I_\mathrm{A}(t)$ for any *known* distribution function $f(R)$.

The question of interest concerns the extraction of information about the *unknown* distribution function $f(R)$ from the experimental data of $I_\mathrm{A}(t)$. It has been shown by Grinvald et al.[4] that $f(R)$ can be extracted from the fluorescence decay measurement of the donor molecule $I_\mathrm{D}(t)$ by Laplace transformation, since

$$\frac{I_\mathrm{D}(t)}{I_0(t)} = \int f(R)\exp\left(-\frac{R_0^6}{R^6}\frac{t}{\tau_\mathrm{D}}\right)\mathrm{d}^3\mathbf{R} \quad (13)$$

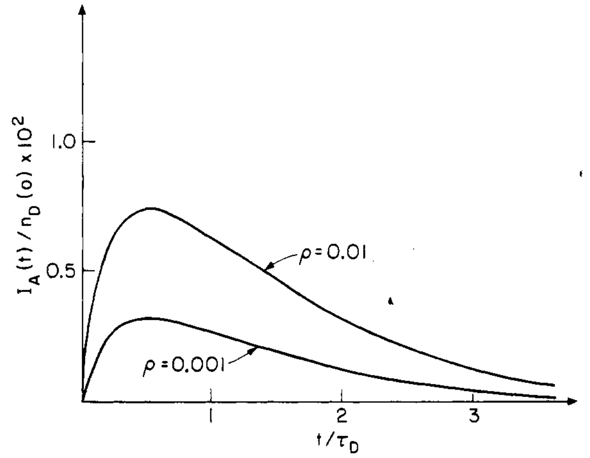

Figure 2. Time dependence of acceptor fluorescence intensity for $R_0 = 40$ Å. Gaussian distribution is assumed and $B = 115$ is used.

can be written as

$$\frac{I_D(t)}{I_0(t)} = \int g(s) \exp(-st)\, ds \tag{14}$$

where

$$s = \frac{R_0^6}{\tau_D R^6}, \qquad g(s) = -\frac{2\pi\tau_D R^9}{3R_0^6} f(R) \tag{15}$$

Since $I_D(t)/I_0(t)$ is the Laplace transformation of $g(s)$ [eq 14], we can obtain $f(R)$, in principle, by the inverse Laplace transformation of $I_D(t)/I_0(t)$. However, since this inversion is unreliable unless very precise data on $I_D(t)$ are available, the method of cumulant expansion[5] may be preferable. The cumulant expansion method can also be applied to eq 11 where inversion of $I_A(t)$ to find $f(R)$ is not possible (except in the case $\tau_A = \tau_D$) due to the complicated form of the integrand.

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References and Notes

(1) M. Daoud et al., *Macromolecules*, **6**, 804 (1975).
(2) Th. Förster, *Discuss. Faraday Soc.*, **27**, 7 (1959); *Z. Naturforsch., A*, **4**, 321 (1949).
(3) L. Stryer and R. P. Haugland, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 719 (1967); J. Yguerabide, H. F. Epstein, and L. Stryer, *ibid.*, **51**, 573 (1970); K. Beardsley and C. R. Cantor, *ibid.*, **65**, 39 (1970); C. R. Cantor and P. Pechukas, *ibid.*, **68**, 2099 (1971); R. Gennis and C. R. Cantor, *Biochemistry*, **11**, 2509, 2517 (1972); W. E. Blumberg, R. E. Dale, and J. Eisinger, *Biopolymers*, **13**, 1607 (1974); R. E. Dale and J. Eisinger, *ibid.*, **13**, 1573 (1974); *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 271 (1976); J. Eisinger, *Q. Rev. Biophys.*, **9**, 21 (1976); R. Guilland, M. Leclerc, A. Loffet, J. Loneis, B. Wilmet, and A. Englert, *Macromolecules*, **8**, 134 (1975); R. Guilland and A. Englert, *Biopolymers*, **15**, 1301 (1976).
(4) A. Grinvald, E. Haas, and I. Z. Steinberg, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2099 (1971).
(5) D. E. Koppel, *J. Chem. Phys.*, **57**, 4814 (1972).

Notes

On the Determination of Specific Retention Volumes of Polymer Stationary Phases

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Lichtenthaler et al.[2] have recently drawn attention to discrepancies in published retention volumes on poly(dimethylsiloxane) stationary phases.[2,3] An interlaboratory comparison[4] of retention volumes measured on seven poly(dimethylsiloxane) columns did not entirely resolve these problems, indicating the importance of packing procedure. In the present note, we wish to comment further on the accuracy of specific retention volumes determined on polymer stationary phases. Earlier studies from these laboratories[5] on polyethylene stationary phases showed a small but apparently real difference between the interactions of vapor phase probes with linear (LPE) and with branched (BPE) samples. (An average difference in the interaction parameter, χ, of 0.053 was found.)

Experimental Section

The experimental procedure and instrumentation were identical with those reported in earlier publications.[5,6] Calcinations were performed by first drying the sieved packing under vacuum for 48 h at 100 °C and then weighing and calcining at 1100 °C. Two-gram aliquots of the dried packing were calcined in porcelain crucibles for 3 h and cooled in a desiccator. All calcinations were done at least in duplicate.

Column characteristics are given in Table I. Polymers were linear polyethylene Marlex 6050 ($M_w = 92.2 \times 10^3$, $M_n = 7.4 \times 10^3$) and as earlier described.[5]

Table I
Column Characteristics of Polyethylene Stationary Phases

Column[a]	Polymer	Support	Polymer in total packing, %	Polymer mass[b]
1	Branched polyethylene	Chromosorb W AW-DMCS	12.07	0.7209
2	Branched polyethylene	Chromosorb W AW-DMCS	10.67	0.7929
3	Linear polyethylene Marlex 50	Chromosorb W AW-DMCS	6.66	0.5529
4	Linear polyethylene Marlex 50	Chromosorb W AW-DMCS	5.57	0.5943
5	Linear polyethylene Marlex 6050	Chromosorb W AW-DMCS	7.42	0.3057
6	Linear polyethylene Marlex 6050	Chromosorb G AW-DMCS	6.88	0.4058

[a] Columns 1 to 4 from ref 5. [b] All packings contained 0.1% 4,4′-thiobis(3-methyl-6-*tert*-butylphenol), based on polymer mass.