

A STUDY OF MOLECULAR DIFFUSION IN ALCOHOL-WATER SOLUTION OF POLY-4-VINYLPYRIDINE BY FLUORESCENCE QUENCHING

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Abstract—Intra- and intermolecular diffusion processes in alcohol-water solutions of poly-4-vinylpyridine (PVPy) were studied by the fluorescence quenching method. It was prepared fluorescent-labelled PVPy (PVPy-F) by copolymerizing 4-vinylpyridine with anthrylmethylmethacrylate (F) (one chromophore group per 500 chain links in the copolymer), PVPy carrying both the chromophore and quenching chain links (quaternization of the pyridine residue by dimethylsulphate) (PVPy-FQ) and PVPy carrying only the quenchers (PVPy-Q).

Measurement of the mean lifetime of the excited state of the anthracene group for the polymers and for the monomer analogue (F) with increasing quencher concentration and use of the Stern-Volmer relation permit determination of the constants of bimolecular collisions between the monomer quencher (Q) (4-ethyl-N-methyl pyridinium sulphate) and F, between the monomer quencher and PVPy-F and between Q and F incorporated in the same polymer chain (PVPy-FQ). The values of the diffusion constant for the system of PVPy-F and Q and for the system of PVPy-FQ are approximately two and four times respectively less than that for the monomer pair (F + Q). For the latter system, the finding indicates some hindrance to the diffusion of the chain links with each other within the coil. Nevertheless, their mobility remains high and probably microdiffusion will not limit chemical reactions of functional groups within coils and with monomer molecules.

In studying the fluorescence quenching of the chromophore for PVPy-F in the presence of the polymer quencher, it was found that mutual interpenetration of the polymer coils is intensified markedly when the solution volume is filled entirely by the swelling coils.

INTRODUCTION

Some physico-chemical aspects of the behaviour of macromolecules and of their functional groups require quantitative knowledge of characteristics of intra- and intermolecular collisions for flexible polymer coils and of their collisions with small molecules in solution. Some papers have used kinetic [1-3] and equilibrium [4] approaches for solution of the problem. A theoretical basis for the experimental study of chain relaxation has been suggested [5].

It seems that fluorescence approaches [6, 7], particularly fluorescence quenching, might be useful for direct measurement of these characteristics since the process of quenching is largely diffusion-controlled. Comparisons of the rate constants of this process involving a fluorescent probe (F) and a quenching group (Q), attached either to the same chain or to different chains, with those for corresponding low molecular weight analogues are likely to provide interesting information about the diffusion mobility of chain links and about effects of the polymer coil on intra- and intermolecular translational diffusion of their functional groups.

Poly-4-vinylpyridine (PVPy) is useful for studying relaxation processes in coils since quaternization of fluorescent-labelled PVPy permits the preparation of copolymers carrying various amount of positively charged groups which, unlike free pyridine rings, are effective quenchers of anthracene fluorescence.

EXPERIMENTAL

Materials

Fluorescent-labelled PVPy (PVPy-F) was prepared by radical copolymerization of 4-vinylpyridine (65° at 15 mm, $n_D^{20} = 1.5520$) with anthrylmethylmethacrylate (AM) in methanol under vacuum with azobisisobutyronitrile as initiator. A fraction of PVPy-F with molecular weight 8×10^4 was used. The content of anthracene fragments in the polymer, by u.v.-spectroscopy, was about one per 500 links.

Copolymers containing both the fluorescent probe and the quencher (PVPy-FQ) were obtained in methanol solution at room temperature in the presence of the precise amount of dimethylsulphate required for preparation of polymers with the required degree of quaternization (β). The content of quaternized pyridine residues was determined from the ratio of absorptivity at 1600 cm^{-1} (due to pyridine groups) and at 1640 cm^{-1} (due to quaternized pyridine ring) in i.r. spectra of the copolymers. Copolymers (fraction of PVPy with molecular weight 6×10^4) containing only the quenching groups with $\beta = 26$ and 50 per cent were prepared similarly.

As monomer analogues of both the link involving the fluorescent probe and the quaternized pyridine ring, anthrylmethylmethacrylate (AM) and 4-ethyl-N-methylpyridinium methylsulphate (EtPy), prepared by quaternizing 4-ethylpyridine with dimethylsulphate, were used, respectively.

Methods

Mean lifetimes of the excited state of the anthracene fragment in the above compounds without (7, 1) and with

(τ) the quencher were measured with a phase spectrofluorometer in the Institute of Molecular Biology (Moscow), described by Tumerman [8]. The wavelength of the exciting light was 365 nm. The lifetime of the excited state for fluorescein as a standard (3.9 nsec) agrees with the published value (3.8 nsec) [9]. In all cases, 45 wt % aqueous ethanol was used, the viscosity of the solution being 2.8 sp at 20°.

The viscosities of polymer solutions were measured in Ubbelohde viscometers at $25 \pm 0.1^\circ$.

RESULTS AND DISCUSSION

In studying fluorescence of the anthracene ring incorporated into the PVPy chain, it was found that its intensity decreased strongly with increasing quaternized pyridine residues. It is evident that the latter are effective quenchers for the former, because its own counterions (such as CH_3SO_4^- and Cl^-) rather than iodide ions, are not the quenchers for the chromophore up to 0.1 M concentration in the solution. Recently pyridine quaternized by ethylbromide in water [10] and pyridinium chloride in ethanol [11] were found to quench fluorescence of pyrene and of anthracene with a bimolecular rate constants for collisions close to the diffusion constants for particles in these solutions. This point is very important since it supports the determination of the constants in the polymer systems.

Thus to elucidate the effect of polymer nature on the diffusion of chain links, it seems necessary to determine the rate constants of bimolecular collisions between the monomer analogues (system of F and Q), between PVPy-F and Q and between F and Q incorporated into the same coil (PVPy-FQ system). The latter constant will characterize mutual collisions of the links within the coil. It is of great interest for quantitative estimation of "internal viscosity" and for the understanding of mechanism of diffusion of macromolecules in solution. The "internal viscosity" concept for polymer chains was introduced by Kuhn [12] and developed in a series of papers [13, 14]. It was

suggested that the "internal viscosity" results in particular from resistance to segmental motion due to rotational energy barriers [14, 15].

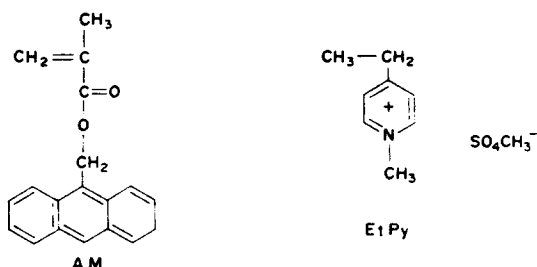
The well-known Stern-Volmer equation

$$\tau_0/\tau = 1 + K_{sv} [Q], \quad (1)$$

where τ_0 and τ are mean lifetimes of the excited state of the probe in the absence and presence of the quencher respectively, $[Q]$ is quencher concentration in solution and K_{sv} is the Stern-Volmer constant related to the fluorescence rate constant (k_q) by the relation

$$K_{sv} = k_q \tau_0, \quad (2)$$

allowed values of k_q for the collisions of the particles involved to be estimated. Figure 1 shows that dependence of τ_0/τ , for both F and PVPy-F, on the quencher concentration of Q follows the Stern-Volmer relation [1]. It becomes possible to calculate values of k_q . For the low molecular weight compounds



k_q is $3.2 \pm 0.3 \times 10^9 \text{ m}^{-1} \text{ sec}^{-1}$ at $\tau_0 = 2.7 \pm 0.2 \text{ nsec}$ for AM, indicating dynamic quenching in the solution with $\eta = 2.8 \text{ sp}$. As seen in Fig. 1, the Stern-Volmer constant (K_{sv}) in the case of PVPy-F and the monomer quencher (EtPy) is markedly higher than that for the system of F and Q. It is related to the longer lifetime of the excited state for the chromophore embedded in PVPy ($\tau_0 = 10.2 \pm 0.3 \text{ nsec}$). The phenomenon of the enhancement of τ_0 in polymers is well-known [16, 17]; it appears to be due to a change of local environment and/or segmental mobility. However, the k_q value for the system of PVPy-F and Q is $1.5 \pm 0.2 \times 10^9 \text{ m}^{-1} \text{ sec}^{-1}$.

According to simple Smoluchowski theory [18], the diffusion-controlled reaction rate constant, k_q , is proportional to $(D_f + D_q)$ where D_f and D_q are the diffusion coefficients of the fluorescent-labelled polymer and the monomer quencher, respectively. Attachment of the probe to a macromolecule will reduce the contribution of its translatory diffusion in the quenching process compared to that of the quencher. In the case, $D_f \ll D_q$, the ratio of the quenching rate constant for a fluorescent species attached to PVPy to that of the free molecule should be 0.5 for quenching by a common molecule. In our work the observed constant is half that when both reagents are small molecules (F + Q). The value of 0.47 ± 0.03 agrees adequately with that expected from consideration of the Smoluchowski equation [18]. Thus, the coincidence might be due to a lack of influence of steric factors associated with attachment of the probe to the polymer hampering the formation of the required "collisional complex". Moreover, it could indicate that most chemical reactions of the chain links with low molecular weight reagents in "good" solvents are not

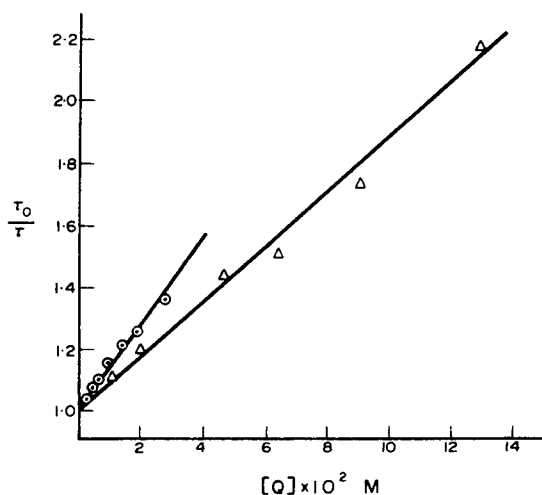
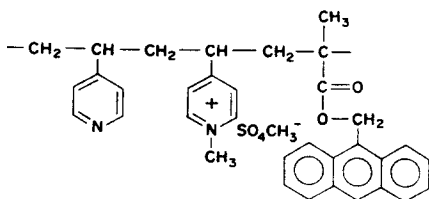


Fig. 1. Dependence of τ_0/τ for anthracene ring in fluorescent-labelled PVPy (PVPy-F) (○—○) and in the monomer anthrylmethylmethacrylate (Δ—Δ) on concentration of quencher. Experimental conditions: 45 wt % aqueous ethanol, $20^\circ \pm 0.1$, the probe $4 \times 10^{-6} \text{ M}$, the polymer $2 \times 10^{-3} \text{ M}$, the quencher is 4-ethyl-N-methylpyridinium sulphate.

limited by diffusion of small particles in coils. Recently, similar conclusions were suggested by studying the fluorescence quenching of anthracene attached to polystyrene (copolymer of styrene and 9-vinylanthracene) in toluene by carbon tetrachloride [19].

To estimate the intramolecular diffusion constant in the coils, the sample of PVPy-F was quaternized by dimethylsulphate. As a result, we can consider a series of copolymers (PVPy-FQ) carrying both a link involving the chromophore and quenching links in the amount of 1.0; 2.5; 7.0; 10.0; 12 and 17 per cent.



The mean life-time of the excited state (τ) was measured for each sample of PVPy-FQ at constant common concentration of the chain links in 45 wt % aqueous ethanol (2×10^{-3} M). It was demonstrated that a decrease of the polymer concentration (almost five-fold) does not change τ indicating predominant interaction between the quenchers and the chromophore within the coil and insignificant interpenetration of different coils at the particular concentration.

To plot the dependence of τ_0/τ on the quencher concentration, it is necessary to operate with a local concentration of the quenching links in the coil. To the best of our knowledge, experimental data concerning the local general concentration of chain links (C_{loc}) within a coil in solution are not available, and there are merely theoretical estimates of chain link density in Gaussian coils [20].

We attempted to determine the C_{loc} within PVPy coils with molecular weight 6.5×10^4 in the frozen alcohol-water solution by the spin-label procedure. Spin-labelling of PVPy was performed by quaternizing PVPy with 4-chloroacetamido-2,2,6,6-tetramethylpiperidine-*N*-oxyl ($\beta = 25$ per cent). A method for measuring a mean distance between monomer nitroxide radicals, based on broadening of the lines in EPR-spectra with increasing spin-label concentration in solutions, was suggested [21]. Using this approach, we calculated C_{loc} within the coil volume as 0.3 ± 0.1 M. It is fairly close to theoretical values of the chain density of flexible polymer coils [19]. It was used for estimation of the quencher concentration in the samples of PVPy-FQ. Attention should be paid to the fact that the dependence of τ_0/τ on the local quencher concentration follows the Stern-Volmer equation I (Fig. 2). The linear plot appears to be accounted for by the absence of conformational changes in the polymer molecule when varying the extent of quaternization. It was found that the intrinsic viscosity of PVPy-FQ with $\beta = 2.5$ per cent corresponds to that of PVPy-FQ with $\beta = 12$ per cent in the same solution involving added salt (0.01 M NaCl). It is noteworthy that the linear dependence of τ_0/τ on β remains only up to 20 per cent of the quaternization. The effect of high β on the quenching is being investigated.

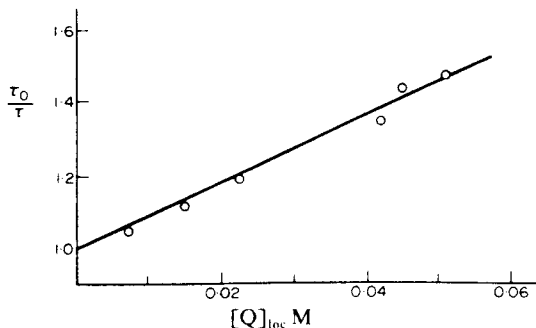


Fig. 2. Dependence of τ_0/τ for anthracene ring in a series of PVPy polymers containing the chromophore and various amounts of quencher (PVPy-FQ) on local concentration of quencher. C_{loc} of chain links in coils of PVPy takes 0.3 M. Polymer 2×10^{-3} M. 45 wt % aqueous ethanol.

The intramolecular diffusion constant in the dilute polymer solution is found to be $0.9 \pm 0.2 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. It is clear that the value is dependent on the chosen local concentration; for example, it changes from $1.3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ to $0.6 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ while C_{loc} varies from 0.2 to 0.4 M, and the choice of C_{loc} itself is somewhat indefinite. At the same time, the chosen value of C_{loc} appears to lead to the correct order of k_q .

It follows that the "internal viscosity" of the investigated polymers diminishes the rate constant of intramolecular collisions for chain links. The latter is about 4 times less than the diffusion constant for the monomer analogues. It demonstrates also that the diffusion of chain links within the coil remains rather high and, in the present case, cannot limit chemical reactions between separated links of the same chain.

Further, the approach of fluorescence quenching was used for studying the interaction of two macromolecular systems, viz. one having the fluorescent probe (PVPy-F or PVPy-FQ with $\beta = 26$ per cent) and the other having only the quenching groups (PVPy-Q). Figure 3 shows that, below some quencher concentration, quenching of fluorescence for the chromophore in PVPy-F does not occur. However, the quenching appears after the appropriate concentration of polymer quenchers is reached, the ratio of τ_0/τ beginning to increase. Consequently, the ability of macromolecular coils to interpenetrate with increasing polymer concentration changes differently (Fig. 3). At low concentrations, it remains rather insignificant. At some threshold concentration, which is likely to depend on the polymer nature and molecular weight, the ability for mutual interpenetration of the coils increases significantly. As a result, the k_q value calculated from the slope of the rising plot in Fig. 3 becomes close to the value of k_q characterizing intramolecular collisions in the coil. For the systems mentioned above, the thresholds of polymer concentration are 0.04 M for the system of PVPy-F and PVPy-Q with $\beta = 50$ per cent and about 0.075 M for the system of PVPy-FQ and PVPy-Q with $\beta = 26$ per cent. These values are in close agreement with reverse values of specific viscosity for PVPy-Q with $\beta = 26$ and 50 per cent evaluated in mole per litre under these conditions. The specific viscosities of these polymers in 45 per cent aqueous ethanol, at concentrations close to the threshold values, are

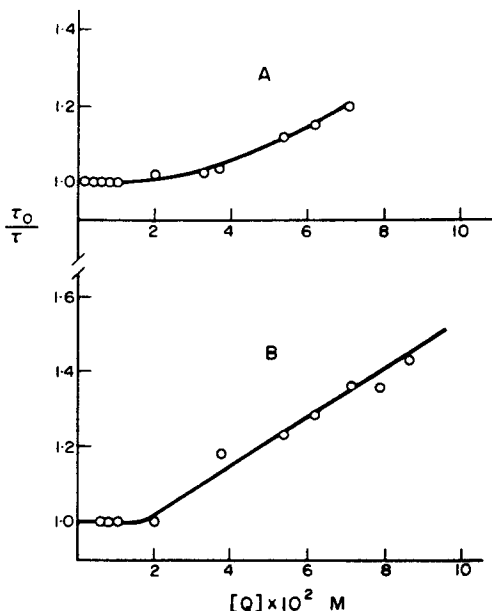


Fig. 3. Effect of quenching links in the PVPy-Q polymers with $\beta = 50$ per cent (A) and 26 per cent (B) on ratio of τ_0/τ for the chromophore in PVPy-F (A) and in PVPy-FQ with $\beta = 17$ per cent (B). Concentration of PVPy-FQ and of PVPy-F 3×10^{-3} M. Conditions are the same as those for Fig. 1.

1.1 dl/g for PVPy-Q with $\beta = 26$ per cent and 1.8 dl/g for PVPy-Q with $\beta = 50$ per cent. Probably, it indicates that the mutual interpenetration of coils is intensified markedly when a solution volume is completely filled by swelling coils.

The latter findings are very significant for the understanding of macromolecular motion in polymer solutions in that interpenetration of coils occurs only at concentrations beyond the non-contacting dilute situation. Thus the results demonstrate the value of the fluorescence quenching approach for measuring micro- and macrorelaxation processes in polymer solutions.

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REFERENCES

1. N. Goodman and H. Morawetz, *J. Polym. Sci.* **A2**, 9, 1657 (1971).
2. H. Morawetz, J.-R. Cho and P. J. Gans, *Macromolecules* **6**, 624 (1973).
3. J. R. Cho and H. Morawetz, *Macromolecules* **6**, 628 (1973).
4. D. J. Worsfold, *J. Polym. Sci.* **12**, 337 (1974).
5. G. Wilemski and M. Fixman, *J. chem. Phys.* **60**, 866, 878 (1974).
6. G. Oster and Y. Nishijima, *Newer Methods of Polymer Characterization*, (Edited by B. Ke), p. 169. Mir, Moscow (1966).
7. Y. Nishijima, *J. Macromol. Sci. Phys.* **B8**, 389 (1973).
8. A. Yu. Borisov and A. A. Tumerman, *Izv. Akad. Nauk SSSR* **23**, 97 (1959).
9. A. Müller, R. Lumry and H. Kokubun, *Rev. scient. Instrum.* **36**, 1214 (1965).
10. G. A. Davis, *Chem. Commun.* **19**, 728 (1973).
11. H. J. Pownall and L. C. Smith, *Biochemistry* **13**, 2, 594 (1974).
12. W. Kuhn and H. Kuhn, *Helv. chim. Acta* **29**, 71, 609, 830 (1946).
13. R. Cerf, *J. Polym. Sci.* **23**, 125 (1957).
14. E. R. Bazua and M. C. Williams, *J. chem. Phys.* **59**, 2858 (1973).
15. A. Peterlin, *J. Polym. Sci.* **B10**, 101 (1972).
16. E. V. Anufrieva, M. V. Volkenstein, Yu. Ya. Gotlib, M. G. Kravovjak, S. S. Skorohodov and T. V. Sheveleva, *Dokl. Akad. Nauk SSSR* **194**, 1108 (1970).
17. E. V. Anufrieva, Yu. Ya. Gotlib, M. G. Kravovjak and S. S. Skorohodov, *Vysokomolek. Soedin.* **A14**, 1127 (1972).
18. M. Smoluchowski, *Z. phys. Chem.* **92**, 129 (1917).
19. K. Brown and I. Soutar, *Europ. Polym. J.* **10**, 433 (1974).
20. C. Tenford, *Physical Chemistry of Macromolecules*, p. 207. Khimiya, Moscow (1965).
21. A. I. Kokorin, K. I. Zamaraev, G. A. Grigorjan, V. P. Ivanov and E. G. Rozantsev, *Biophizika* **17**, 34 (1972).