Self-diffusion in concentrated polystyrene solutions measured by fluorescence recovery after photobleaching*

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_ A polystyrene polymer of narrow molecular weight distribution was carboxylated, then reduced, and finally esterified with NBD–aminohexanoic acid [6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)–aminohexanoic acid]. The self-diffusion of the NBD-labelled polystyrene polymer in concentrated solutions of the unlabelled polystyrene polymer was measured by the method of fluorescence recovery after photobleaching over a concentration range from 0.017 g/ml to 0.41 g/ml at room temperature. In the semi-dilute region, the concentration dependence of diffusion coefficient was found to be in agreement with the predictions of scaling concepts.

INTRODUCTION

Experimental data on polymer self-diffusion are needed not only in many industrial applications of polymers but also in the efforts to develop molecular theory of polymer diffusion. It is therefore important to develop and refine experimental methods for polymer self-diffusion measurement.

Fluorescence recovery after photobleaching (FRAP) is a rapid and convenient method for measuring the diffusion coefficients of polymers in concentrated solutions. This method consists of inducing and observing fluorescence in a well-defined microscopic region of the polymer sample by irradiation with low-intensity light. After a high intensity pulse of light irreversibly destroys the fluorescent additive in this region, the rate of return of fluorescence due to diffusion of additive molecules from outside the region is measured. Diffusion coefficients are then calculated from the measured rates.

The FRAP method has been used by biophysicists for about 10 years to measure the translational diffusion coefficients of labelled molecules in cell membranes as well as in lipid bilayers[1-3]. However, to our knowledge, there have been no applications of the FRAP method to the measurement of polymer self-diffusion, aside from the work of Smith and coworkers[4] who used a related method called fluorescence redistribution after pattern photobleaching to measure the diffusion coefficients of fractionated poly(propylene oxide) polymers. We describe here a synthetic procedure for covalently attaching nitrobenzoxadiazole (NBD), a photobleachable dye, to commercially available polystyrene polymers of narrow molecular weight distributions. We then describe the procedure for measuring by the FRAP method the self-diffusion of an NBD-labelled polystyrene polymer in concentrated solution. And, finally, we compare the experimental values of the self-diffusion coefficient with the prediction of the scaling concepts[5,6].

EXPERIMENTAL

Polystyrene (PS) was purchased from Pressure Chemical (lot 4b)† and was used without further purification. It was specified to have the values of 110 000 and less than 1.06 for M_w and M_w/M_n respectively, where M_w and M_n are the weight-average molecular weight and the number-average molecular weight, respectively.

NBD-labelled polystyrene polymer (NBD–PS) was partially metalated in dry cyclohexane by n-butyllithium (n-BuLi) in the presence of N,N,N',N'-tetramethylethylenediamine (TMEDA). The initial concentration of n-BuLi was 0.1M at a monomer mole ratio for PS:TMEDA:n-BuLi of 2:2:1. The n-BuLi was forced into the mixture being stirred at 50°C under dry argon. After 30 min the reaction was then quenched in a dry ice/THF slurry[7]. The polymer carboxylate was purified by precipitation in acidified methanol from the toluene solution and then pumped dry.

A 1% solution of the polymer product in dry toluene was heated with stirring in the presence of sodium (2-methoxyethoxy) aluminum hydride for 1 h at 50°C and 15 min at 80°C. The mole ratio of the reducing agent to

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† In this report, to describe procedures adequately, we have occasionally identified commercial products and equipment. In no case does such identification imply NBS recommendation or endorsement, nor does it imply that the item identified is necessarily the best available for the purpose.

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the estimated carboxyl substituent was maintained in excess of 1.5 to 1 which is sufficient to verify reactivity. After diluting with toluene, the product mixture was shaken with a slight excess of 6N hydrochloric acid, and the cloudy toluene layer was clarified by treatment with anhydrous potassium carbonate. After filtering, the solution volume was reduced by pumping. The polymer was precipitated twice in methanol from the toluene solution and pumped dry.

The hydroxymethyl-substituted polystyrene polymer was esterified with a dye, 6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-aminohexanoic acid (NBDA). The catalyst, dicyclohexylcarbodiimide (DCC) in dry dimethyl-formamide (DMF) was added to a 5–10% polymer solution in DMF also containing NBDA and an accelerator, 4-dimethylaminopyridine (DMAP)[8]. Based on the initial polymer hydroxyl content (3.6 mol%), the mol ratio of reagents (DMAP/DCC/NBDA) was 4/2/1.7. After 3 h stirring at room temperature, the solvent was pumped off and the polymer was precipitated in acidified methanol from methylene chloride solution. The polymer was reprecipitated three times in methanol, pumped dry and excluded from light.

FRAP measurement

A polymer solution was prepared by mixing calculated amounts of PS, diethyl phthalate (DEP) and a small amount of NBD–PS (less than 0.01% of the unlabelled PS) dissolved in a sufficient amount of methylene chloride (CH_2Cl_2). A clear, uniform solution of PS and NBD–PS in DEP was obtained when CH_2Cl_2 was allowed to evaporate from the polymer solution. To prepare a sample for the FRAP measurement, a small amount of the PS/NBD–PS solution was deposited on a clean microscope cover slip and allowed to form a flat thin film. This cover slip was then placed on a microscope slide with the polymer film sandwiched in between. The thickness of the polymer film was about 5 μm.

The diffusion coefficient D of NBD–PS was measured by the FRAP method. In a FRAP experiment[2], a small area of the sample is illuminated with a weak beam of exciting light (monitoring beam). The fluorescence in this area is recorded as F. At a predetermined time, $t = 0$, the sample is momentarily illuminated with a strong laser beam (bleaching beam) to cause an irreversible bleaching of fluorophore. Following the bleaching, the fluorescence is again monitored by the monitoring beam. The fluorescence, $F(t)$, is initially very weak, but gradually increases as the fresh fluorescent molecules diffuse into the bleached area, and eventually recovers to its original intensity. From the rate of the recovery of fluorescence intensity, the diffusion coefficient can be determined.

In this experiment, the 488 nm emission of an argon ion laser was split by the first of two matched quartz flats into the bleaching and the monitoring beam. An electric shutter is placed in the path of the bleaching beam to control the amount of photobleaching. Both beams were subsequently recombined by the second quartz flat, and focused through the vertical illumination system (epi-illumination) onto the specimen plane. With a 40 × (0.65 NA) objective, the radius of the illuminated spot on the sample plane was about 1.45 μm. Through the partial reflectance coating on the quartz flats, the monitoring intensity was attenuated to 1/2000 of the bleaching beam.

Usually about 50% of the fluorophore molecules in the illuminated spot were bleached out. The time dependence of the recovery of fluorescence, due to the diffusive influx of the unbleached fluorophore molecules into the bleached spot, is measured with a photomultiplier whose output, after being electronically conditioned, was stored in a signal averager and simultaneously displayed on a CRT monitor. The time course of the recovery can then be analysed with the procedures of Axelrod and coworkers[2] to yield both the extent of recovery and the diffusion coefficient.

According to Axelrod and coworkers[2], D is related to the characteristic recovery time $\tau_{1/2}$ by

$$D = (\omega^2/4\tau_{1/2})\gamma \qquad (1)$$

where $\tau_{1/2}$ is the time for the fluorescence intensity to recover to half of its final intensity, and γ is a parameter dependent on the amount of initial photobleaching. (In this experiment, the value of γ varied between 1.15 and 1.30.) Laser beam diameter ω was determined by the methods of Jacobson and coworkers[3]. This results in an absolute uncertainty of 20% in D.

RESULTS AND DISCUSSION

Figure 1 gives the chemical structure of NBD–PS and the excitation and the emission spectra of its solution in methylene chloride. Microscope observations and FRAP measurements were made at room temperature (*ca.* 22°C). Under the fluorescence microscope, polymer samples exhibited uniform intensity, indicating that NBD–PS was uniformly distributed in the polymer solution. FRAP measurements yielded recovery curves characteristic of a single-component recovery and in most measurements the recovery was nearly 100%, indicating that all NBD–PS molecules were mobile. A typical fluorescence recovery curve is shown in *Figure 2*. The diffusion
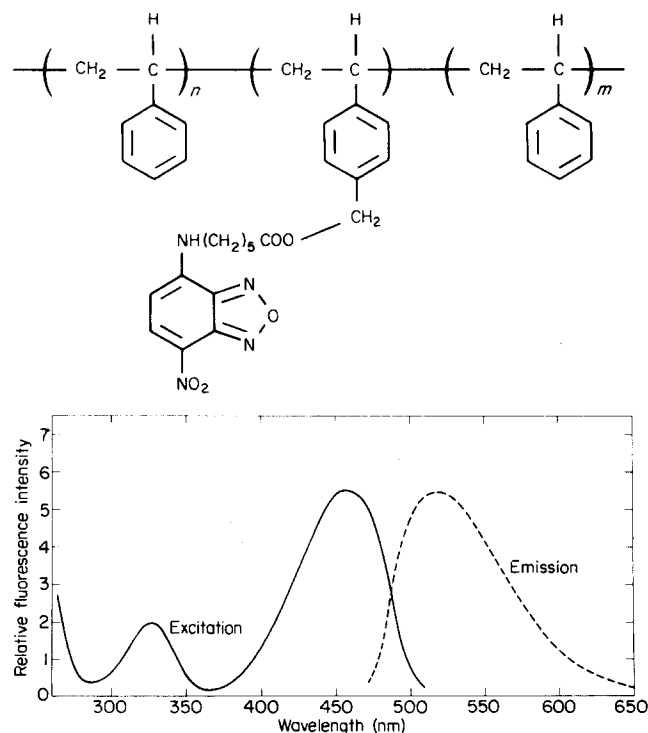

Figure 1 Chemical structure of NBD-labelled polystyrene, and the excitation and the emission spectra of NBD-labelled polystyrene in methylene chloride
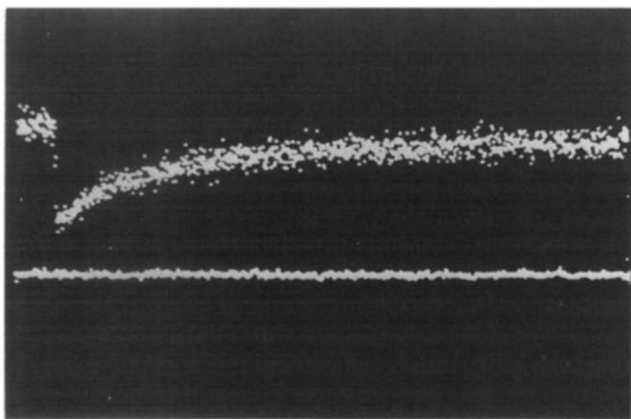
Figure 2 Typical fluorescence recovery curve for diffusive recovery showing a monotonic curve of gradually decreasing slope

Figure 3 Self-diffusion coefficient of NBD-PS as a function of polymer concentration. The least-square-fitted straight line has a slope of -1.82 ± 0.13

coefficient of NBD-PS was measured as a function of polystyrene concentration C over a range from 0.017 g/ml and 0.41 g/ml, and the result is shown in *Figure 3*. Each point represents the average of several measurements on three independently prepared samples. The uncertainties are the standard deviations and do not include the possible uncertainties inherited from the errors in ω.

To test the possible steric perturbation caused by the introduction of fluorescence probes, the diffusion coefficients in samples with mole PS/NBD-PS ratio varying from 1000/1 to 200/1 were measured and no statistically significant difference in the values of D was noticed. The effect of CH_2Cl_2 on polystyrene diffusion was examined on samples from which CH_2Cl_2 was allowed to evaporate for different time intervals. The value of D decreased progressively with evaporation time, but reached its terminal value after 48 to 72 h.

Figure 3 shows that the diffusion coefficient decreases with the polymer concentration. In this log D vs. log C plot, a definite change of the slope occurs near $C = (0.08 \pm 0.01)$ g cm^{-3}. This change allows us to give an estimate of $C^* = 0.08$ g cm^{-3} as the concentration at which the transition from the dilute to semi-dilute regime occurs. This value for C^* is in reasonable agreement with the predicted value[5] of 0.06 g cm^{-3} for our polystyrene with $M_w = 110\,000$.

Between $C = 0.094$ g cm^{-3} and $C = 0.287$ g cm^{-3}, the values of the diffusion coefficient can be fitted by the least-squares method to a power law, $D \sim C^{-\alpha}$, with an exponent $\alpha = 1.82 \pm 0.13$. This value is in good agreement with the prediction of de Gennes[6] that α is 7/4, and also with the result of Hervet and coworkers[9] who obtained by the forced Rayleigh scattering technique a value of 1.75 for α. At the highest concentration of this study ($C = 0.41$ g cm^{-3}), the value of D deviates from the linear behaviour. Such deviation indicates that we have perhaps reached the concentrated regime where many of the scaling arguments that lead to the power law are no longer valid because of the increase in local monomer–monomer interactions.
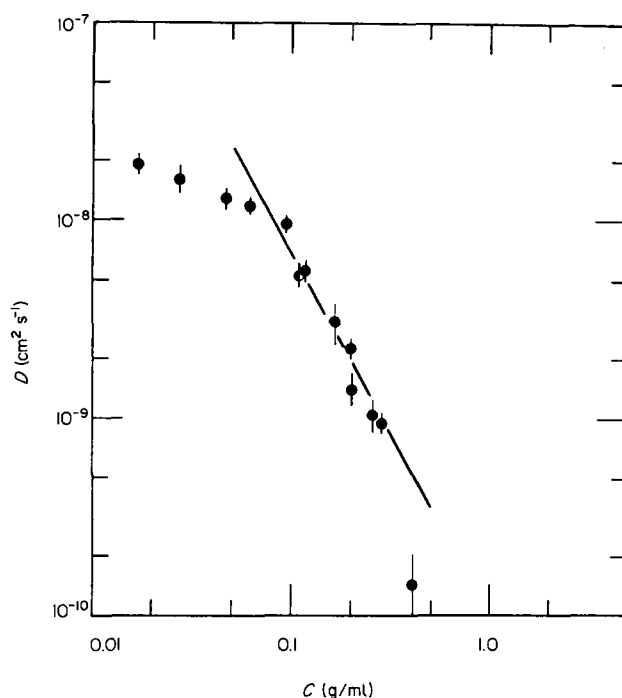
SUMMARY

To summarize, we have described the synthesis of a narrowly-distributed polystyrene polymer covalently labelled with nitrobenzoxadiazole, and have shown the procedure for measuring the self-diffusion in concentrated solutions by the method of fluorescence recovery after photobleaching. The results for the concentration dependence of the self-diffusion coefficient of a polystyrene polymer in diethyl phthalate are in good agreement with the predictions of the scaling concepts.

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