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A Fluorescence Correlation Spectroscopy Study on the Self-Diffusion of Polystyrene Chains in Dilute and Semidilute Solution

Ruigang Liu, Xia Gao, Jörg Adams, and Wilhelm Oppermann*

Institute of Physical Chemistry, Clausthal University of Technology, Arnold-Sommerfeld-Strasse 4, 38678 Clausthal-Zellerfeld, Germany

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ABSTRACT: The self-diffusion coefficient D_s of dye-labeled polystyrene chains having $M_w = 3.90 \times 10^5$ g/mol and $M_w/M_n < 1.10$ was measured over almost 4 decades of polymer concentration in toluene solution, from 10^{-4} to 0.4 g/mL, by fluorescence correlation spectroscopy. In the dilute regime up to 5×10^{-3} g/mL, D_s remains practically constant. The transition to the semidilute regime is observed around 0.01 g/mL, and from 0.02 to 0.4 g/mL D_s decreases strongly with rising concentration. This decrease is well represented by a power law, $D_s \sim c^{-1.75}$, as predicted by scaling and reptation theory. As an alternative, a good fit over the whole concentration range can be obtained with a stretched exponential function, $D_s = D_0 \exp(-15c^{0.65})$, with $D_0 = 2 \times 10^{-7}$ cm²/s and c given in g/mL.

1. Introduction

Much effort has been devoted to obtain a comprehensive understanding of the dynamics of polymer chains in dilute, semidilute, and concentrated solution or in the melt from both experimental and theoretical aspects. For entangled chains occurring in semidilute and concentrated polymer solutions, the reptation model and the scaling ideas contributed greatly to the development of theories and inspired research in this field.[1,2]

The physical processes determining polymer self-diffusion are fundamentally different in different concentration regimes. In dilute solution, the polymer molecules move nearly independently of each other, and the self-diffusion coefficient is primarily determined by the size of the polymer coils quantified, e.g., by the radius of gyration, R_G. In the semidilute or concentrated regime, the chains are topologically constrained in tubes formed by the surrounding chains. Diffusion then occurs as a curvilinear (one-dimensional) movement along their own contour.[1−4] Here, the scaling and reptation theory leads to the well-known predictions $D_s \sim M^{-2}c^{-1.75}$ and $D_s \sim M^{-2}c^{-3}$ for good-solvent and θ-solvent conditions, respectively.[1] The crossover from the dilute to the semidilute regime takes place at the overlap concentration c^*.

Hess[5,6] extended and revised the basic scaling and reptation theory by introducing the entanglement transition concentration, c_e, in the semidilute regime. When $c^* < c < c_e$, there should be coil overlap, but no effective entanglements, and the diffusion coefficient should scale as $D_s \sim M^{-1}c^{-0.5}$. For $c > c_e$, the result $D_s \sim M^{-2}c^{-1.75}$ of the basic scaling and reptation theory is recovered.

On the basis of the consideration that the interchain hydrodynamic interactions significantly outweigh the effects of topological constraints, Phillies[7,8] proposed the equation $D_s = D_0 \exp(-\alpha c^u)$ to account for the concentration dependence of the self-diffusion coefficient in a wide range from dilute to semidilute solutions, where $0.5 \le u \le 1$, and α and u depend on the molar mass of the polymer chains.

Among the growing number of experimental data, the scaling and reptation prediction is supported in a limited concentration range.[9−20] On the other hand, it was shown that Phillies' equation describes many data extremely well.[7,8,18−26] On grounds of the published results, there is no clear argument either for or against the relevance of reptation in semidilute or moderately concentrated polymer solutions. Measurements covering a much broader concentration range might be helpful in this respect.

One experimental method to determine diffusion coefficients is fluorescence correlation spectroscopy (FCS).[27−30] FCS has gone through significant evolution and found widespread application in the past decade because of the development of confocal microscopes or other confocal detection setups with highly sensitive detectors as well as suitable dye labels and labeling procedures. However, most FCS measurements are being performed on biomacromolecules in an aqueous environment.[31−35] There are only very few reports on the application of FCS to polymers in organic media.[36]

We used the advantages of FCS to measure the diffusion coefficient of labeled polystyrene in a polystyrene solution in toluene over a broad range of concentrations. To do so, it was necessary to develop a suitable method to label monodisperse polystyrene with Rhodamine B. The observation volume of the FCS experiments was calibrated by comparing the results of dynamic light scattering (DLS) measurements with FCS measurements for highly dilute solutions. We shall show that reliable data for the diffusion coefficient could be obtained by FCS over more than 3 decades of concentration.

2. Experimental Section

Labeling of Polystyrene. Polystyrene ($M_w = 3.90 \times 10^5$ g/mol and $M_w/M_n < 1.10$, Pressure Chemical Co.) was labeled with Rhodamine B isothiocyanate according to Scheme 1. In the first step, 0.4 g of polystyrene dissolved in 10 mL of freshly distilled dichloromethane was reacted under argon with 2 mg of *N*-chloromethylphthalimide (**I**) to obtain the random copolymer (**II**). The reaction was catalyzed by 0.2 mL of $SnCl_4$ and took place at 30 °C for 4 h. Copolymer **II** was precipitated in methanol and dried. It was then dissolved in 10 mL of 1,4-dioxane, 0.5 mL of a 40% aqueous solution of CH_3NH_2 was

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* To whom correspondence should be addressed. E-mail: wilhelm.oppermann@tu-clausthal.de.

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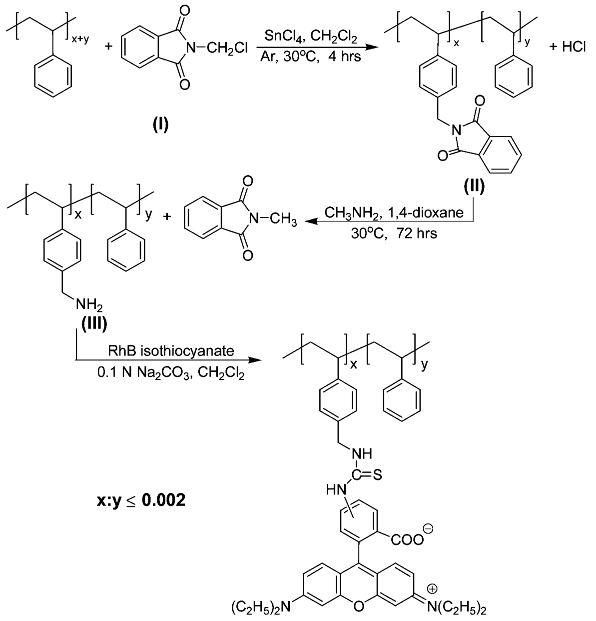
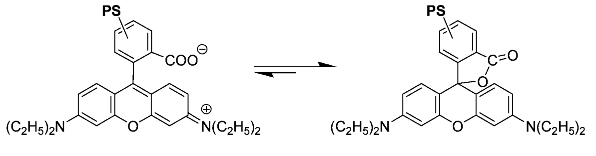
Scheme 1. Labeling Process of Polystyrene



Scheme 2. Zwitterion−Lactone Equilibrium of Rhodamine B



added dropwise, and the mixture was stirred for 72 h at 30 °C to achieve an exchange of the phthalimide groups by amine groups. After precipitation in methanol and drying, 0.39 g of aminomethylized polystyrene (**III**) was obtained. The aminomethyl groups were subsequently reacted with Rhodamine B isothiocyanate (Aldrich): 0.2 g of **III** was dissolved in 20 mL of dichloromethane and mixed with 5 mL of a solution of 20 mg of Rhodamine B isothiocyanate in 0.1 M aqueous Na_2CO_3. While the two-phase mixture was vigorously stirred at room temperature for 2 h, the organic phase became pink in color. Finally, the organic phase was separated from the aqueous phase, and the polymer was precipitated in methanol and thoroughly washed with ethanol to remove free dye.

The maximum degree of labeling is determined by the amount of **I** utilized in the first stage and should be about 0.2 mol % with regard to repeat units. ^{1}H NMR measurements performed on similar samples having a higher degree of labeling showed that the reaction proceeds almost quantitatively. For the actual sample, the NMR signals of the substituents were too weak to be quantified. Hence, we can only state that the degree of labeling is below 0.2 mol %.

Rhodamine B can exist in two isomeric forms depicted in Scheme 2. The zwitterionic form prevails in polar media, particularly in an aqueous environment, and is responsible for the absorption and fluorescence of visible light. Nonpolar media favor the nonfluorescent lactonic form showing absorption only in the UV (316 nm).[37] In toluene, used as a solvent in the present work, the equilibrium between the two forms is shifted toward the lactone.

Figure 1 shows the normalized fluorescence excitation and emission spectrum of the labeled polystyrene in toluene (1 × 10^{-4} g/mL) measured with a photon counting spectrometer (SPEX Fluorolog 2) in 90° geometry. The maxima of the excitation and emission peaks are at 555 and 576 nm, respectively. This compares closely with the data reported for Rhodamin B in ethanol (554 and 589 nm). However, the fluorescence intensity is about a factor of 40 smaller than that of a solution of Rhodamine B in water having the same dye concentration. These results indicate that Rhodamine B is linked to the polystyrene chains, but only a small fraction of the labeled chains carry active labels contributing to fluorescence because the zwitterion−lactone equilibrium (Scheme 2) lies to the right.

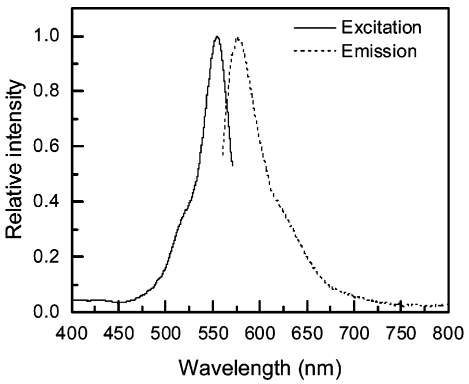


Figure 1. Excitation and emission spectra of Rhodamine B labeled polystyrene in toluene (concentration: 1 × 10^{-4} g/mL).

Preparation of Solutions. The labeled polystyrene was dissolved in toluene (spectroscopy grade, Aldrich) at a concentration of 1 × 10^{-4} g/mL. To portions of this solution, various amounts of unlabeled polystyrene ($M_w = 3.35 \times 10^5$ g/mol, $M_w/M_n = 2.1$, BASF) were added and dissolved. To make sure that the solution was homogeneous, at least 2 days was allowed for dissolution, with substantially more time for the higher concentrations. According to this procedure, a series of polystyrene solutions ranging from the dilute to the semidilute regime were prepared, where a small part of the polystyrene consists of labeled species. These solutions were filled into solvent-tight sample chambers sealed with a cover glass of 0.14 mm in thickness for the FCS measurements.

Dynamic Light Scattering. Dynamic light scattering measurements were performed on dilute toluene solutions (10^{-4} g/mL) of the labeled and the corresponding unlabeled polystyrene in the scattering vector range of 0.77×10^5−2.87×10^5 cm^{-1} (in the scattering angle range of 30°−150°) on an ALV/CGS-3 compact goniometer system with ALV/LSE5003 multiple tau digital correlator at 22 °C. The solutions were filtered through 0.2 μm PTFE membrane directly to into the dust-free light scattering cuvette having 18 mm i.d. The acquisition time for each run was 10 min, and the experiments were repeated five times at each angle.

FCS Measurements. FCS measurements were performed on a confocal laser scanning microscope (Leica TCS SP2) with FCS extension (Leica FCS2). A 63× objective immersed in water with a numerical aperture of 1.2 was used. The diameter of the detection pinhole was set to 100 μm. Excitation of the chromophore was achieved by a He−Ne laser at 543 nm.

3. Results and Discussion

In FCS experiments, the fluorescence intensity, $I(t)$, emitted by the fluorescent molecules in the observation volume is measured and autocorrelated to yield[27,28]

$$G(\tau) - 1 = \frac{\langle \delta I(t)\, \delta I(t+\tau)\rangle}{\langle I(t)\rangle^2} \qquad (1)$$

$\delta I(t) = I(t) - \langle I(t)\rangle$ is the instantaneous deviation of the measured intensity from its time average, $\langle I(t)\rangle$. For a freely diffusing monodisperse and uniformly fluorescent particle, the correlation function has the following analytical form:[28−30]

$$G(\tau) - 1 = \frac{1}{N(1 + \tau/\tau_D)(1 + (\omega_1/\omega_2)^2(\tau/\tau_D))^{1/2}} \qquad (2)$$

where N is the average number of particles in the observation volume. The effective observation volume,

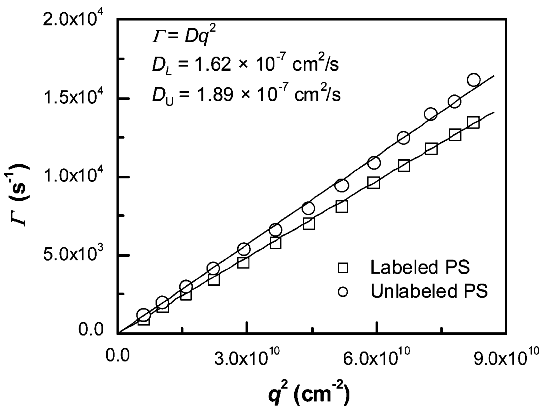


Figure 2. Scattering vector dependence of the first cumulant Γ of solutions of labeled and unlabeled polystyrene in toluene; $M_w = 3.90 \times 10^5$ g/mol, $c = 1 \times 10^{-4}$ g/mL.

$V = \pi^{3/2}\omega_1{}^2\omega_2$, is approximated by a three-dimensional Gaussian profile with ω_1 and ω_2 being measures of the radius of the focused beam spot and the focal depth along the optical axis, respectively. τ_D is the characteristic decay time for the diffusion process. When the observation volume is calibrated so that ω_1 and ω_2 are known, the diffusion coefficient D_s of the fluorescent species can be determined by[29]

$$\tau_D = \omega_1{}^2/4D \qquad (3)$$

The calibration for a particular experimental setup is usually done by measuring the characteristic diffusion time of molecules or particles whose diffusion coefficients are exactly known from literature data. For organic solvents, reliable data are rarely available. In this work, we therefore used the dye-labeled polystyrene itself for calibration. Its diffusion coefficient in dilute toluene solution was determined by dynamic light scattering. Then the same solution was studied by FCS measurements to obtain the fluorescence correlation function, and a fit to eq 2 gave τ_D.

Figure 2 shows the dependence of the first cumulant Γ determined by dynamic light scattering as a function of the square of the scattering vector q ($c = 1 \times 10^{-4}$ g/mL, T = 22 °C). The measurements were performed on the dye-labeled polystyrene and on the unlabeled primary material as well. It is seen that Γ is proportional to q^2; this observation indicates a pure diffusion process of the polymer molecules. From fitting the data according to $\Gamma = Dq^2$, the self-diffusion coefficient of the labeled polystyrene in dilute toluene solution was obtained to be $D_L = 1.62 \times 10^{-7}$ cm^2/s, while for the unlabeled polystyrene $D_U = 1.89 \times 10^{-7}$ was obtained. The latter value is consistent with relationships between diffusion coefficient and molecular weight given in the literature for polystyrene in toluene solution.[38] A variation of the order of ±10% between different sources is common. It is not quite clear why there is such a large difference between the labeled and the unlabeled polystyrene. One possibility is that the Rhodamine B labels influence the molecular dimensions in solution. This seems rather unlikely in view of the small fraction of labeled repeat units. Another possibility is a fractionation which could have taken place during the derivation. Since the loss of material is below 3%, this can account only to part of the 15% change of D.

FCS experiments performed on the same solution of the labeled polystyrene gave a characteristic decay time $\tau_D = (6.5 \pm 0.2) \times 10^{-3}$ s by fitting the correlation function to eq 2. From this fit and from eq 3, the parameters quantifying the observation volume are obtained: $\omega_1 = 0.63 \pm 0.02$ μm and $\omega_2/\omega_1 = 3.8$.

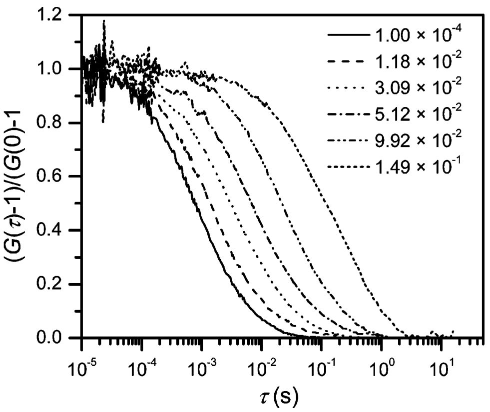


Figure 3. Typical normalized FCS correlation functions of labeled polystyrene in toluene solutions having different polymer concentrations as indicated (in g/mL).

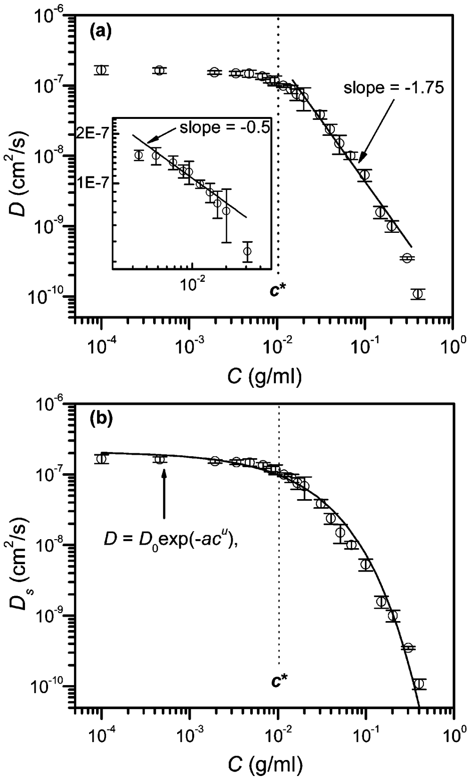


Figure 4. Diffusion coefficient of labeled polystyrene in toluene solutions as a function of polymer concentration: (a) showing predictions according to reptation and scaling theory; (b) showing a fit according to Phillies' equation.

Figure 3 shows some typical normalized FCS correlation functions of the dye-labeled polystyrene in toluene solutions having different concentrations of unlabeled polystyrene. The results clearly show that the shape of the correlation functions does not change perceptibly with concentration, but they are shifted toward longer times with increasing polymer concentration. Fitting of the correlation functions by eq 2 and combination with eq 3 yields the self-diffusion coefficient D_s of the dye-labeled polystyrene chains. The results are shown in Figure 4 in double-logarithmic format. (Figure 4a,b shows the same data; they are used to discuss different aspects.) The error bars in the figure indicate the variation of the results of 15−20 independent measurements; the scatter is usually within ±10−20%.

In the very dilute regime up to a concentration of 5×10^{-3} g/mL, the diffusion coefficient remains practically constant. Then there is a transition regime around 0.01 g/mL, and from 0.02 to 0.40 g/mL the diffusion coefficient decreases strongly with rising concentration.

In Figure 4a, the experimental results are compared with the predictions of scaling and reptation theory. The overlap concentration c^*, where the transition from the dilute to the semidilute regime occurs, can be estimated according to

$$c^* = \frac{3M_\mathrm{w}}{4\pi N_\mathrm{A}(R_\mathrm{G})^3} \qquad (4)$$

where M_w is the weight-average molar mass of the polymer, N_A is Avogadro's number, and R_G is the radius of gyration. Results of several light scattering experiments led to the relationship $\langle R_\mathrm{G}^2\rangle = 1.38 \times 10^{-4} M_\mathrm{w}^{1.19}$ nm^2 for polystyrene in toluene at 25 °C.[39] Hence, the molar mass of $M_\mathrm{w} = 3.35 \times 10^5$ g/mol of matrix polystyrene corresponds to $R_\mathrm{G} = 22.8$ nm, which translates into an overlap concentration $c^* = 0.01$ g/mL, as indicated in Figure 4a. (It was assumed that the slight temperature difference can be ignored.) This calculated c^* coincides fairly well with the experimentally observed crossover from the dilute regime where D_s is independent of concentration to the regime where a strong concentration dependence occurs.

For $c \geq 0.02$ g/mL, the data follow closely the power law relationship for the good solvent case, $D_\mathrm{s} \sim c^{-1.75}$ (solid line in Figure 4a), with the exception of the uppermost concentrations.[1,2] This deviation is expected because θ conditions are reached and a stronger concentration dependence with an exponent −3 is predicted.[1] Other authors give an exponent −7/3 for θ conditions.[40]

The crossover from the dilute to the semidilute regime is predicted to occur between c^* and a second characteristic concentration, c_e. Above c_e, which is typically 2−5 times higher than c^*,[41] entanglements dominate the rheological behavior, and transport by reptation is considered the major mechanism. At $c^* < c < c_\mathrm{e}$, the solution is semidilute but unentangled. According to Hess,[5,6] the transition range should be rather wide and D_s should scale as $c^{-0.5}$. In the inset in Figure 4a, this range is shown with greater resolution. We have tentatively drawn a line with slope −0.5, which represents the data between 0.005 and 0.02 g/mL reasonably well, although a smooth transition from $D_\mathrm{s} \sim c^{-0.5}$ for $c \leq 0.005$ g/mL to $D_\mathrm{s} \sim c^{-1.75}$ for $c \geq 0.02$ g/mL seems more appropriate.

In Figure 4b, the experimental data are fitted to the stretched-exponential equation proposed by Phillies.[7,8] The parameters used are $u = 0.65$ and $\alpha = 15$. The crossover is fitted rather well. On the other hand, in the higher concentration range the experimental concentration dependence of D_s seems to be less curved than the fit function.

There has been an extensive discussion in the literature on whether experimental data support the scaling predictions, which are applicable only in certain limited concentration regimes, or are better described by continuous functions over the entire concentration range. A universal representation in reduced variables of the concentration and molar mass dependence of the diffusion coefficient has also been put forward, in some instances requiring a correction via the monomeric friction coefficient.[22,24,41−43]

It is obvious that the data presented here are far too limited to permit for a critical inspection of the controversial points. In fact, our data are compatible with either viewpoint. Our main emphasis is to show that the FCS technique allows the accurate determination of the diffusion coefficient over almost 4 decades of polymer concentration with constant sensitivity. In that respect it is superior to most of the other techniques which seldom cover more than 2 orders of magnitude in concentration and are sometimes limited to certain polymer/solvent systems.

4. Conclusions

By fluorescence correlation spectroscopy, the self-diffusion coefficient of labeled polystyrene chains was measured over more than 3 decades of polymer concentration, from 10^{-4} to several 10^{-1} g/mL. It was thus possible to cover wide ranges of the dilute regime and the semidilute regime by the same experimental method. Our data do not indicate a wide crossover regime governed by a power law with an exponent of $-1/2$. In the semidilute regime, the self-diffusion coefficient decreases with increasing polymer concentration as $D_\mathrm{s} \sim c^{-1.75\pm0.1}$, in agreement with the theoretical prediction of scaling and reptation theory. On the other hand, an equally acceptable fit can be obtained with the continuous stretched exponential function.

Note Added after ASAP Publication. This article was released ASAP on September 20, 2005. Equation 2 has been revised. The correct version was posted on September 29, 2005.

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