Direct observation of single molecule mobility in semidilute polymer solutions

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We determine the mobility of dye-labeled polystyrene molecules in solution by fluorescence correlation spectroscopy (FCS) over a wide range of concentrations and molecular weights (ranging from 3.9×10^3 to 1550×10^3 g/mol). In order to obtain absolute values of the diffusion coefficient, which can be compared to diffusion coefficients determined by other methods, the size of the focal volume has been determined by independent experiments and theoretical calculations. All data demonstrate that FCS is uniquely suited to explore polymer dynamics in solution. The mobility of the chains as expressed through the self-diffusion coefficient is significantly slowed down above the overlap concentration c^*. The dependence of c^* on molecular weight is well described by the power law $c^* \propto M_w^{1-3\nu}$ (ν: Flory exponent). A comparison with the data taken from the literature demonstrates that the overlap concentration presents a robust concept that holds for a wide range of molecular weights.

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I. INTRODUCTION

Diffusion and transport in polymer solutions is among the oldest subjects of polymer science [1–12]. It is generally accepted that at least three different concentration regimes must be distinguished when discussing the dynamics in polymer solution: (i) a dilute regime in which the diffusion coefficient $D_0 = kT/6\pi\eta R_H$ is fully governed by the hydrodynamic radius R_H, (ii) a semidilute regime in which the coils start to overlap, and finally (iii) a concentrated regime in which the chains form a heavily entangled mesh. Evidently, the onset of mutual interaction will slow down considerably the diffusional motion of coils. Stronger overlap between the coils will lead to entanglements of different chains and motion of single chains proceeds by reptation [5]. Hence, the self-diffusion coefficient D is expected to decrease considerably with increasing polymer concentration. Moreover, its dependence on molecular weight must change when moving from the dilute to the concentrated regime.

This transition from dilute to concentrated polymer solutions has been the subject of a number of theoretical and experimental studies. Graessley presented a study of the dynamics of polymers and considered the entanglement of the chains [6,7]. Phillies proposed a universal formula for the whole concentration range and neglected reptation [9,10]. In contrast, Hess explicitly distinguished three regimes and proposed an influence of the entanglement on the polymer diffusion [11,12]. The most successful concept in this field has been the overlap concentration c^* which delineates the crossover between the dilute and semidilute regimes in which the chains start to overlap. For a polymer with degree of polymerization N and Flory radius R_F de Gennes proposed the following expression for the overlap concentration c^* [3–5]:

$$c^* \cong \frac{N}{R_F^3} = \frac{1}{a^3 M^{1-3\nu}} M_w^{1-3\nu}. \tag{1}$$

Here a is the length of a single polymer segment, M the molecular weight of a monomer unit, and M_w the weight-average molecular weight of the chain. The Flory exponent ν assumes 1/2 in a θ solvent and a value of 3/5 in good solvents [5].

An experimental test of these predictions requires the precise determination of the self-diffusion coefficient of polymers in solutions of varying concentration. Methods used so far include dynamic light scattering (DLS) [13,14], pulsed field gradient nuclear magnetic resonance (PFG NMR) [15,16], and forced Rayleigh scattering (FRS) [17,18]. More recently, fluorescence correlation spectroscopy (FCS) became available which monitors the motion of single polymer chains. The enormous potential of this method was shown by several investigations of biological macromolecules, mostly single- and double-stranded DNA in aqueous solution [19–22]. With the combination of confocal microscope setups and efficient lasers and detectors the quality of FCS measurements was significantly improved [23–26]. However, for synthetic polymers in organic solvents this technique was only rarely used. Up to now this method has been predominantly used to study the aggregation of block copolymers in organic solvents [27–29]. Recently, Liu *et al.* used FCS to measure in a very broad range the concentration dependence of the diffusion coefficient for a single molecular weight

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[30]. The rare use of FCS in organic solvents is certainly due to the difficulty of generating a well-defined observation volume. In particular, Enderlein *et al.* [35] recently demonstrate that small changes in the refractive index of the solvent lead to marked changes of the focal volume. This in turn may lead to problems when determining the diffusion coefficient of polymers in organic solvents.

Here we present a comprehensive study on molecular motion in dilute and semidilute solutions using FCS measurements of dye-labeled polymer chains. The goal of the present investigation is twofold: First, we compare the size of the focal volume calculated by the method of Enderlein *et al.* with experimental values obtained as described recently [31]. This comparison provides a solid basis for the application of the FCS in organic solvents. Second, we explore the transition regime between the dilute and semidilute regimes for a wide range of molecular weights M_w. In this way we check the validity of the concept of the overlap concentration c^* as introduced by Eq. (1).

II. THEORY AND EVALUATION OF DATA

In FCS a laser beam is focused by an objective with high numerical aperture (typical NA $\geqslant 0.9$) and excites fluorescent molecules entering the illuminated observation volume. The emitted fluorescent light is collected by the same optics and separated from scattered excitation light by a dichroic mirror. The emitted light is detected by an avalanche photodiode. The time-dependent intensity fluctuations are analyzed by an autocorrelation function

$$G(\tau) = \frac{\langle I(t)\rangle\langle I(t+\tau)\rangle}{\langle I(t)\rangle^2}. \tag{2}$$

The autocorrelation function depends on the average time (τ_{diff}) a molecule needs to diffuse through the observation volume and the average number (N) of molecules in the observation volume. These two values can be extracted by fitting the following equation to the experimental data:

$$G(\tau) = \frac{1}{N}\frac{1}{1+\tau/\tau_{diff}\sqrt{1+(w_{x,y}/w_z)^2(\tau/\tau_{diff})}} + 1, \tag{3}$$

where $w_{x,y}$ is the dimension of the observation volume perpendicular to the optical axis and w_z is the dimension along the optical axis. The average diffusion time τ_{diff} is related to the diffusion coefficient D by

$$D = \frac{w_{x,y}^2}{4\tau_{diff}}. \tag{4}$$

More details of FCS have been published in several review articles [26,32–34].

To describe the dynamic behavior of polymer chains in the dilute regime often the friction and the respective friction coefficient of the chains are analyzed. For infinite dilution the friction coefficient is calculated from the reciprocal to the diffusion coefficient to $f_0 = kT/D_0 = 6\pi\eta R_h$. Hence, the measured diffusion time τ_{diff} is a direct measure for the friction of a single polymer chain [Eq. (4)]. For dilute solutions only a hydrodynamic interaction between the coils is expected which may be treated within the frame of the Kirkwood-Riseman theory [1]. Because of these interactions, f_0 and D_0 have to be corrected. For dilute solutions the diffusion coefficient is approximated by a linear dependence on concentration:

$$D = D_0(1 - k_f c). \tag{5}$$

Here D_0 is the diffusion coefficient at infinite dilution and k_f is a correction factor.

TABLE I. Molecular weight and polydispersity of the polystyrenes (PS) used in the present study.

PS	MW [kg/mol]	PDI
PS4	3.9	1.10
PS11	11.5	1.03
Ps17	17.3	1.03
Ps67	67	1.05
PS264	264	1.02
PS1.5M	1550	1.06

III. EXPERIMENT

A. Dye-labeled polystyrene

Polystyrenes with narrow molecular weight distribution were synthesized by living anionic polymerization. The polymers were end-capped by ethyleneoxide. A small portion of the polymers were subsequently labeled by rhodamine-B via a polymer analogous coupling reaction. Details of the synthesis and the characterization of the polymers are reported elsewhere [31]. The solutions for the FCS experiments were prepared in toluene by blending a constant concentration of 10^{-8}-M dye-labeled polystyrene with varying amounts of unlabeled polystyrene of the same molecular weight—i.e., from the same synthesis batch. The molecular weight and polydispersity of the polymers are summarized in Table I.

B. FCS experiments

For the FCS measurements we modified the commercial ConfoCor II setup (Carl Zeiss, Jena Germany) [34]. A 40× Plan Neofluar objective with a numerical aperture of 0.9 was used to focus the laser beam into the sample and to collect the emitted fluorescence light. The rhodamine-B-labeled PS chains were excited by an Ar-ion laser at 514 nm. The size of the illuminated volume was determined as described by Zettl *et al.* [31]. Quantitative information on the characteristic diffusion time τ_{diff} is revealed by fitting the measured autocorrelation data using the Levenberg-Marquardt algorithm to the autocorrelation function shown in Eq. (3). This function assumes that all molecules have the same diffusion coefficient which is related to τ_{diff} as described in Eq. (4).

To avoid evaporation of the organic solvent during the measurement we designed a well-sealed sample chamber with a 0.14-mm-thick cover glass at the bottom. The polymer solutions were freshly prepared with toluene p.a. grade and

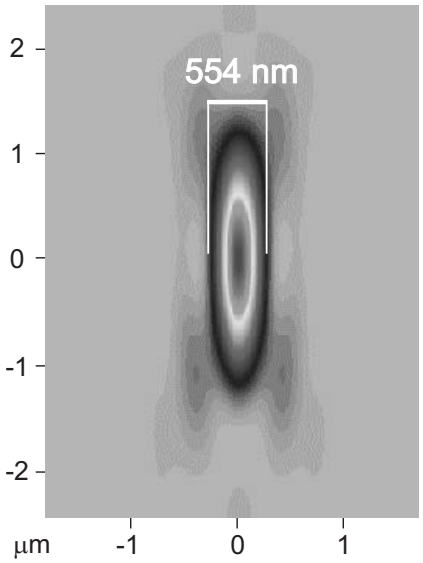

FIG. 1. Calculated intensity distribution of the used confocal setup. The white bar indicates 2 times the measured waist radius ($w_{x,y}$).

investigated immediately. For each molecular weight three independent measurements were performed. The duration of each measurement was varied depending on the concentration and molecular weight of the sample.

IV. RESULTS AND DISCUSSION

Most commercially available objectives and FCS setups are designed for investigations in aqueous environments. Enderlein *et al.* demonstrated that with these objectives already small changes in the refractive index leads to strong effect in the diffusion coefficient [35]. These findings make it necessary to find proper experimental setups and suitable procedures to determine the size of the observation volume for experiments in organic solvents.

Here we analyze the size of our observation volume by calculations and calibration measurements. The calculations are done on the basis of fundamental wave optical considerations. Seminal calculations of the intensity distribution of focused laser beams were done by Richards and Wolf [36,37]. Their calculations were expanded to confocal setups by Sheppard and Török [38], Török *et al.* [39,40], and Enderlein [41]. In Fig. 1 the calculated intensity distribution for our setup is shown.

For the experimental determination of the size of the focus the diffusion time τ_{diff} of dye-labeled polystyrenes with different molecular weights was determined by FCS measurements in dilute solution [31]. The diffusion time τ_{diff} as the function of the known molecular weight then could be used to calculate the waist radius $w_{x,y}$ by comparing our result with published data for the molecular weight dependence of the diffusion coefficient. This procedure yielded a value of 277 nm for $w_{x,y}$. The white bar in Fig. 1 indicates 2 times the experimental determined waist radius ($2w_{x,y}$ =554 nm). Figure 1 demonstrates that the findings of theory and experiment are in excellent agreement. Hence, the present FCS setup leads to absolute values for the diffusion coefficient of individual polymer chains over a broad range of concentrations.

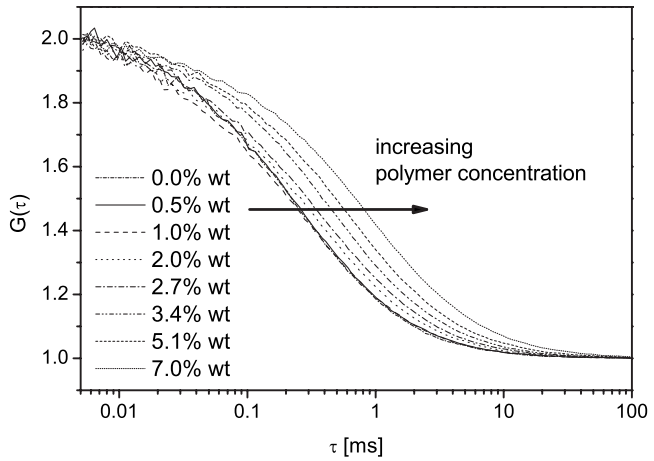

FIG. 2. Normalized FCS autocorrelation curves of a 67 kg/mol PS. The polymer concentration was increased from 0 wt. % (solid line) up to 7 wt. % (dashed line). For polymer concentrations below 1 wt. % we received identical autocorrelation curves. Above the concentration the curves shift to higher correlation times with increasing polymer concentration.

In the following, we will focus on the crossover between the dilute and semidilute concentrations and study these region for various molecular weights in detail. The normalized autocorrelation curves of a polystyrene with a molecular weight of 67 kg/mol show a clear concentration dependence. In Fig. 2 the concentration of the polymer solutions is varied in a range between 0 and 7 wt. % of unlabeled polymer. The autocorrelation curves for polymer concentrations below 2 wt. % are nearly identical. Polymer concentrations of 2 wt. % and higher show a clear shift of the measured curves to higher correlation times τ. To enable quantitative conclusions we fitted Eq. (3) to the data and revealed the diffusion time τ_{diff} at the respective concentration. In the low-concentration region (below 2 wt. %) we observe a diffusion time of around τ_{diff}=240 μs which only slightly increases with increasing polymer concentration. At concentrations above 2 wt. % the diffusion time increases linearly with the polymer concentration up to τ_{diff}=820 μs for 7 wt. % polymer solution.

In order to determine the overlap concentration we applied a linear fit to each of the two regimes and defined the point of intersection as the *overlap concentration* c^*. This procedure was applied for the determination of the overlap concentration of all polymers listed in Table I. The results are summarized in Fig. 3. It should be noted that the linear fit above c^* holds only true for a small range of concentrations; for still higher concentrations, a nonlinear increase is to be expected. For the present purpose, however, this approximation is justified since we only aim at the determination of c^*.

The fit in the regime of lowest concentrations can be used to determine the self-diffusion coefficient D_0 at infinite dilution and the coefficient k_f of Eq. (5). With increasing molecular weight the diffusion time of the polymer in the dilute solutions increases and τ_{diff} increases markedly. In the

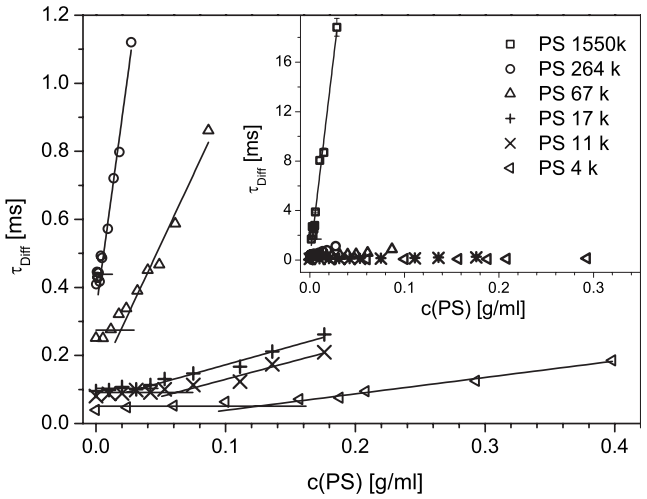

FIG. 3. The change of the diffusion time τ_{diff} with increasing polymer concentration for six different molecular weights. For low polymer concentrations τ_{diff} stays nearly constant. Above a certain concentration the diffusion time increases linearly with increasing concentration. To each of these two regions a linear fit was applied, and from the point of intersection the overlap concentration c^* was determined. In the inset the 1550 kg/mol polymer is included.

following the two regimes clearly visible in Fig. 3 will be discussed.

A. Polymer diffusion in the dilute regime

The intercept of the y axis of Fig. 3 yields directly D_0 which refers to polymer chains devoid of any mutual interaction. Hence, diffusion is solely determined by the size of the coils in the respective solvent (see Fig. 4) and $D \propto M^{-0.607\pm0.029}$. From the present data we obtain an exponent of 0.6. This is in the range expected for a good solvent [5].

Figure 5 displays the slope k_f of the linear regime. The quantity k_f reflects the binary hydrodynamic interaction of polymer coils in solution and is expected to increase with molecular weight as $k_f \propto M^{0.8}$ (see the discussion of this point in Ref. [16]). Indeed we obtained an exponent of 0.72±0.15

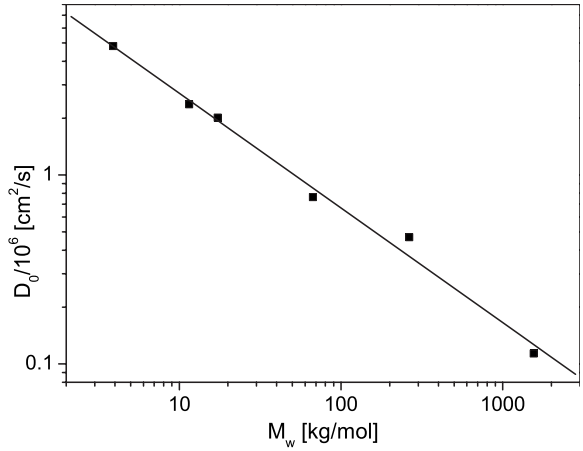

FIG. 4. Diffusion coefficient D_0 at infinite dilution as the function of the molecular weight of the polymer.

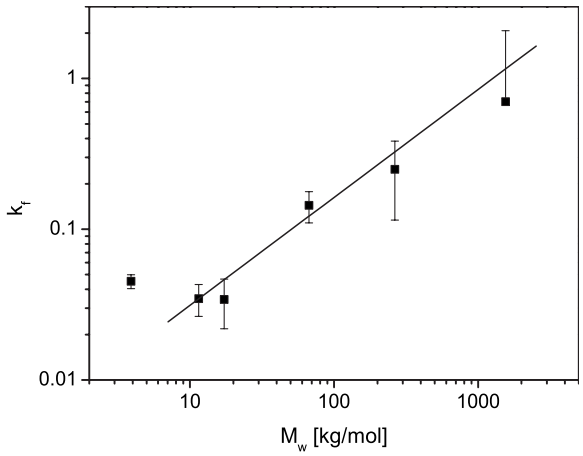

FIG. 5. Friction coefficient versus molecular weight. For the analysis of the dependence of the friction coefficient on the molecular weight the data of the smallest molecular weight (4 kg/mol) have been excluded.

which is in agreement with this prediction. Only the smallest molecular weight is not fitted by this relation. The polymer with a molecular weight of 3.9 kg/mol is too small, and its diffusion cannot be treated in terms of scaling laws. These results are in agreement with the findings of Callaghan and Pinder [16].

From this section we conclude that the diffusion coefficient of single polymer coils can be obtained precisely by FCS measurements in the dilute regime. In particular, secure data of k_f can be obtained by this method for a broad range of molecular weights. This is due to the fact that measurements can be done for the smallest concentrations without sacrificing the accuracy of the data.

B. Polymer overlap concentration

In Fig. 6 the overlap concentration versus the molecular weight is plotted. We see a power law dependence of the overlap concentration on the molecular weight. From a fit to our data this yields

$$c^* = 10^{(3.94\pm0.27)} M^{0.79\pm0.06}. \quad (6)$$

A comparison of Eq. (6) with Eq. (1) leads to ν=0.59. This finding is in excellent agreement with the exponent predicted by theory for a polymer in a good solvent (ν=0.6). Up to now, only a few groups have studied the overlap concentration of polystyrene in good solvents. In Fig. 6 all values published so far are summarized and compared to our results. Liu *et al.* determined the overlap concentration of polystyrene with a molecular weight of 309 kg/mol in toluene by dynamic light scattering and compared their results with FCS measurements. They found a good agreement between the two methods. Other groups studied polystyrene in dichlormethane or in benzene. Hervet *et al.* [17] and Brown and Mortensen [13] measured the change of the diffusion coefficient by forced Rayleigh scattering or dynamic light scattering and determined the change the overlap concentration of polystyrene in benzene and in dichlormethane. Brown and Mortensen additionally calculated c^* from values for the

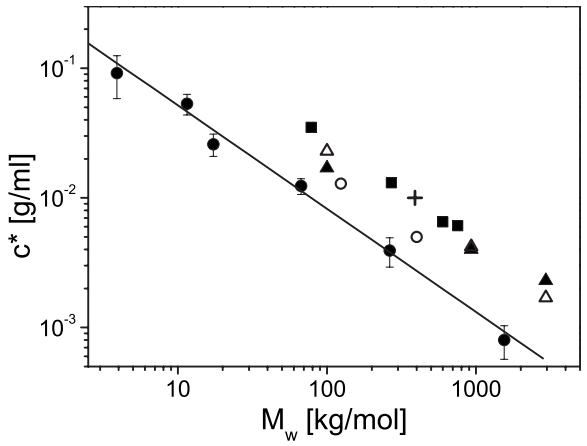

FIG. 6. Molar mass dependence of the overlap concentration c^* of polystyrene. Experimental results found here (●) are compared with the overlap concentration of polystyrene taken from literature: +: Liu *et al.* [30], DLS in toluene. ■: Hervet *et al.* [17], FRS in benzene. ○: Raspaud *et al.* [14], static light scattering in benzene. ▲: Brown and Mortensen [13], DLS in dichloromethane. △: Brown and Mortensen [13], viscosity measurements in dichloromethane.

chain dimension determined by rheological measurements. Raspaud *et al.* [14] measured the radius of gyration of polystyrene by static light scattering in dilute polymer solutions and calculated with this dimensions the overlap concentration.

Figure 6 demonstrates that all investigations published so far are restricted to a small range of molecular weights. In particular, data referring to low molecular weights have been missing so far. The FCS technique employed here expands the accessible range of molecular weights much beyond the range of molecular weights used previously. The comparison of all data of c^* obtained so far shows that the absolute magnitude of the overlap concentration may depend on the particular method used for its determination. However, our present data fully verify the power law, Eq. (1), and demonstrate the general validity of the concept of the overlap concentration over a wide range of molecular weights. Moreover, the exponent obtained from the present data is in good agreement with all published data shown in Fig. 6. It hence becomes evident that the overlap concentration c^* presents a robust concept that may be used to discuss the mobility of polymers at intermediate concentrations.

V. CONCLUSION

We demonstrated that the mobility of single-labeled polystyrene molecules in solution can be determined by FCS over a wide range of concentrations and molecular weights (ranging from 3.9×10^3 to 1550×10^3 g/mol). All data demonstrate that FCS is uniquely suited to explore polymer dynamics in solution. It is found that the mobility is significantly slowed down above the overlap concentration c^* as expressed through Eq. (1). The dependence of c^* on molecular weight is described by the power law, Eq. (1), in an excellent fashion. A comparison with data of c^* taken from literature demonstrates that the overlap concentration may depend to a certain extend on the method used for its determination. A survey of all data, however, reveals that the concept of an overlap concentration is highly useful to assess the slowing down of mobility at the crossover from the dilute to the semidilute regime.

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