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Single-Chain Diffusion Coefficient of F-Dextran in Poly(vinylpyrrolidone)/Water: Fluorescence Recovery after Photobleaching Experiments

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ABSTRACT: Fluorescence recovery after photobleaching (FRAP) experiments have been performed on a fluorescent dextran/poly(vinylpyrrolidone) (PVP) mixture in water. The experiments were made at several total polymer concentrations C_T , from dilute to semidilute solutions (0.1–10C*) in the homogeneous and disordered state. The composition of the “visible” polymer fluorescent dextran in the mixture was around 0.5% in the semidilute regime. The dynamics of such a system as revealed by FRAP exhibit one relaxation mode identified as a self-diffusion coefficient. This mode has been compared to the extracted D_S value from the two relaxation modes (cooperative and interdiffusive) obtained using dynamic light scattering (DLS; *Macromolecules* 1993, 26, 2592). As expected in both techniques, the measured (FRAP) and the extracted (DLS) D_S decrease with the concentration. Comparison between the results shows that in the semidilute range of concentration the agreement is reasonable between both D_S . As the concentration decreases, the agreement becomes less and less satisfactory. This result confirms that the extrapolation to infinite dilution of D_S (deduced from DLS) is not valid and shows the importance of the hydrodynamic interactions in the dilute ternary systems.

I. Introduction

In previous papers, it has been shown theoretically,^{1–7} and experimentally,^{8–19} that the dynamic behavior of ternary polymer mixtures in a good solvent (two polymers and solvent) is characterized by two relaxation modes. Under certain conditions, these two dynamical processes are interpreted as follows: The first represents the total polymer concentration fluctuations and is referred to as the cooperative mode. It depends on the excluded-volume parameter and increases with the total polymer concentration C_T . The second describes the relaxation of the composition fluctuations and is known as the interdiffusive mode. It is the only relaxation mode which exists in the melt state.^{20–22} It depends on the interaction parameter χ_F and decreases with C_T . Several experimental studies showed that the variations of the amplitudes (A_C , A_I) and the association diffusion coefficients (D_C , D_I) with C_T and the composition $x = C_1/C_T$ are in good agreement with theoretical predictions.^{1–7} Recently,^{23,24} it has been shown that, besides the fact that two relaxation modes describe the dynamic behavior of ternary mixtures, it is possible to have also direct access to values of important parameters such as χ_F and the self-diffusion coefficient D_S . The determination of χ_F with a good accuracy was suggested by Benmouna and Borsali²⁵ when using dynamic light scattering (DLS) under “zero average contrast” (ZAC) conditions expressed by the equation:

$$x \left[\frac{\partial n}{\partial c} \right]_1 + (1-x) \left[\frac{\partial n}{\partial c} \right]_2 = 0 \quad (1)$$

where $x = C_1/C_T$ is the composition (weight fraction) of polymer 1 and $(\partial n/\partial c)_i$ ($i = 1$ or 2) is the increment of the refractive index of species i . Relation (1) means that the average refractive index of the two polymers matches exactly the refractive index of the solvent. Under such conditions the interpretation of the dynamics is easier than it is in the case of an arbitrary good solvent. Moreover, at $x = 1/2$, the situation becomes even more simple since

only one relaxation mode should be observed. The second mode (cooperative) disappears because its amplitude is zero ($A_C = 0$). This behavior has been already observed^{12,15} using NSE and DLS experiments, respectively, and confirms the usefulness of the ZAC condition.

Recently,¹⁹ complementary experiments using DLS on ternary mixtures in different solvents, where the ZAC is not fulfilled, suggest that one can also have direct access, with a good accuracy, to the χ_F interaction parameter when simply analyzing the variation of the cooperative and interdiffusive diffusion coefficients as a function of composition. The determination of χ_F under these conditions was found in good agreement with that obtained using static light scattering under “optical Θ condition”. This result confirms the independence of the frequencies from the optical properties of the system and allows the determination, with good accuracy, of important parameters such as χ_F in arbitrary solvents.

Very recently²³ DLS experiments on a poly(vinylpyrrolidone) (PVP)/dextran/water mixture were performed, and the values of the interaction parameter χ_F and those of the self-diffusion D_S as well as their concentration dependencies were deduced from the analysis of the two relaxation modes as a function of concentration and composition.

In this work we compare the value of the self-diffusion coefficient D_S measured using fluorescence recovery after photobleaching (FRAP) to that deduced from the DLS experiments in the same system, i.e., dextran/PVP/water.

The investigated system is, of course, not ideal and the choice of F-dextran/PVP/water may appear ill-chosen, but the aim of this work is not only to measure using FRAP the single-diffusion coefficient of the labeled chain but also to compare these values to existing ones extracted by the DLS technique on the same system.²³

For this purpose, all the measurements were carried out at a relatively small weight fraction of the “visible” polymer: fluorescent dextran, $x < 0.01$, in the semidilute solution. In fact, depending on the method of preparation and the procedure of filtration, x varies from 0.0017 to

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0.0068 in the semidilute regime. Only for the lowest concentration, i.e., $c = 8.66 \times 10^{-4}$ g/cm³, x is about 0.023.

Zero average contrast is, of course, useful for extracting the "interdiffusion mode" which is identified as the single-chain diffusion coefficient at small x . These conditions correspond to the case where the solvent (a good solvent) has a refractive index intermediate between those of the two polymers.

In our system, this condition cannot simply be fulfilled because water does not meet this condition and, to our knowledge, no good solvent is known with a refractive index between those of PVP and dextran.

As has been shown theoretically^{1,8} and experimentally,^{14,19} only one relaxation mode characterizes the dynamics of the ternary system in the small x -range. The second mode characterizing the total polymer concentration fluctuations disappears because its amplitude vanishes. The only observable relaxation frequency is the interdiffusion mode, which is identified as the self-diffusion coefficient at sufficiently small weight fraction.

The model we have used for the interpretation of the relaxation modes in ternary mixtures has been developed by Akcasu, Benmouna, and Benoit.¹⁻⁸ Using the random-phase approximation, this model has explained experimental results for two homopolymers in solution⁸⁻¹⁹ and diblock copolymer-solvent^{9,16,17} systems. For convenience, it is useful to recall some relations that are of interest for this work. In any ternary mixtures of two polymers in solvent, the total dynamic scattering function $S_T(q, t)$ can be expressed as a sum of two exponentials:

$$S_T(q, t) = A_1 e^{-q^2 D_1 t} + A_2 e^{-q^2 D_2 t} \quad (2)$$

where t is time and q the wavevector defined as $q = (4\pi/\lambda)n \sin(\theta/2)$, with λ the wavelength of the incident beam, n the refractive index, and θ the scattering angle. In the small x -range, one expects a single decay relaxation of the dynamic scattering function, namely,

$$S_T(q, t) = A_1 e^{-q^2 D_1 t} \quad (3)$$

When the two polymers have different degrees of polymerization ($N_1 \neq N_2$), have different excluded-volume parameters v_{ij} , and are slightly incompatible ($\chi_F \neq 0$), the expression of D_1 is^{1,3,7,23}

$$D_1(q) = D_{av}(q) + [D_{av}^2(q) - \Delta(D)]^{1/2} \quad (4a)$$

where

$$D_{av}(q) = \frac{D_{11}(q) + D_{22}(q)}{2} \quad (4b)$$

$$\Delta(D) = D_{11}(q) D_{22}(q) - D_{12}(q) D_{21}(q) \quad (4c)$$

and

$$D_{11} = D_{S,1}(c) \frac{1}{P_1(q)} [1 + 2x C_T A_{2,1} M_{w,1} P_1(q)] \quad (5a)$$

$$D_{22} = D_{S,2}(c) \frac{1}{P_2(q)} [1 + 2(1-x) C_T A_{2,2} M_{w,2} P_2(q)] \quad (5b)$$

$$D_{12} = 2D_{S,1}(c) x C_T A_{2,12} M_{w,1} (m_2/m_1) \quad (5c)$$

$$D_{21} = 2D_{S,2}(c) (1-x) C_T A_{2,12} M_{w,2} (m_1/m_2) \quad (5d)$$

$D_{S,i}(c) = kT/N_i \zeta_i(c)$ ($i = 1, 2$), ζ_i is the friction coefficient

Table 1. Properties of Polymer Samples

| sample | $10^{-3} M_w$ | $I = M_w/M_n$ | R_g (Å) | A_2 (mol·cm ³ g ⁻²) |
|---------|---------------|---------------|-----------|--|
| PVP K90 | 310 | 1.5 | 216 | 4.2×10^{-4} |
| DT500 | 485 | 1.95 | 260 | 4.6×10^{-4} |
| FDT500 | 433 | 1.9 | | |

per monomer i which depends on the concentration, $P_i(q)$ is the form factor of polymer species i , $A_{2,ij}$ ($i, j = 1, 2$) are the second virial coefficients, and m_i ($i = 1, 2$) are the monomer molecular weights of the two species.

It is straightforward to show from the above relations (4) and (5) that in the small x - and q -ranges ($x \rightarrow 0$, $q \rightarrow 0$; $P(q) = 1$) the measured diffusion coefficient is

$$D_1(q, c)|_{(q,x) \rightarrow 0} = D_{S,1}(c) \quad (6)$$

which is identified in our experimental investigations as the self-diffusion of the fluoresceinated dextran molecule. We have investigated the fluorescein dextran/PVP/water system at several total polymer concentrations in the small x -range, and after analyzing the results, we have obtained a value of D_S for the dextran polymer in the PVP matrix and its variation with the total polymer concentration. The results are discussed and compared to those deduced from DLS experiments, in the framework of the model described here, after the Experimental Section.

II. Experimental Section

Materials and Sample Preparation. PVP was supplied by Fluka AG, and dextran FDT500 labeled with FITC (fluorescein isothiocyanate) was obtained from Sigma. The amount of dye in FDT500 is 0.007 mol/mol of glucose. The characteristics of these polymers are listed in Table 1. The weight-average molecular weights M_w , the radii of gyration R_g , and the second virial coefficients $A_{2,i}$ of these samples were determined by static light scattering measurements in water. Several fluorescent dextran/PVP/water solutions were prepared at different total polymer concentrations (namely, $8.66 \times 10^{-4} < C_T$ (g/cm³) $< 12.08 \times 10^{-2}$) by dilution. All the measurements were done at a relatively small composition of the visible polymer: fluorescent dextran, $x < 0.01$, in the semidilute range of concentration; x varies from 0.0017 to 0.0068 in the semidilute regime. Only for the lowest concentration, i.e., $c = 8.66 \times 10^{-4}$ g/cm³, x is about 0.023. At higher concentrations and in this range of composition the system is still homogeneous and in the disordered state. One notes that the phase separation or the concentration at "the cloud" point in the ternary mixture is approximately $C_{cp} \approx 8.1 \times 10^{-2}$ g/cm³ at $x = 0.5$ as has been shown in our earlier paper.²³ The overlap concentrations are $C^*_{PVP} \approx 1.22 \times 10^{-2}$ g/cm³ and $C^*_{F-dextran} \approx 0.976 \times 10^{-2}$ g/cm³, assuming the polymer chains are flexible and in a good solvent: $C^* = M_w/(N_{av} 4\pi R_g^3/3)$. After adding fluorescent polymer, the solutions were allowed to mix for a few days by rigorous shaking. They were filtered with 0.22- μ m Millipore filters and allowed to equilibrate for a short time prior to measurement.

Equipment and Data Analysis. The self-diffusion coefficient of fluorescent-labeled dextran molecules was measured by a fringe pattern bleaching technique similar to the one described by Davoust et al.²⁶ The light beam of an etalon-stabilized monomode Ar laser (1 W at 488 nm) was split, and the two beams crossed in the sample cell, providing illumination in a deep interference fringe pattern. The fringe spacing $d = 2\pi/q$ is set by crossing the angle θ and ranged from 8 to 58 μ m, defining the diffusion distance.

Fluorescence bleaching of the labeled polymers in the illuminated fringes was obtained by producing a $1/2$ s full intensity pulse by depolarizing the beam with a Pockel's cell at a location between nearly crossed polarizers. The decay of the amplitude of the fringe pattern after bleaching was detected by the modulation of the illuminating fringe position using a piezoelectrically modulated mirror and lock-in detection of the emerging fluorescence. The preaveraged data were analyzed using

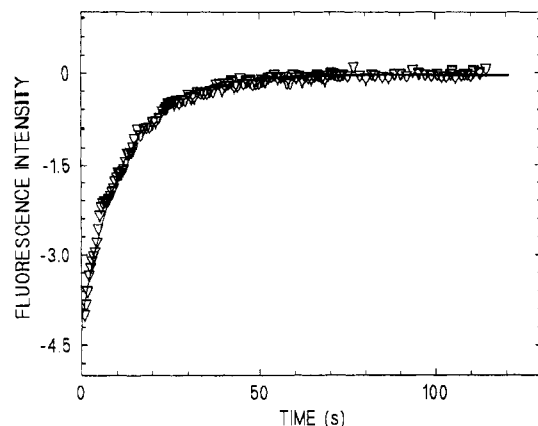


Figure 1. Typical fluorescence intensity function measured using FRAP for the ternary mixture F-dextran/PVP/water at the total polymer concentration $C_T = 6.79 \times 10^{-2} \text{ g/cm}^3$, $x = 0.3\%$, and $q = 0.2216 \times 10^5 \text{ cm}^{-1}$. The triangles represent the experimental data, and the solid line represents the monoexponential fit according to relation (3).

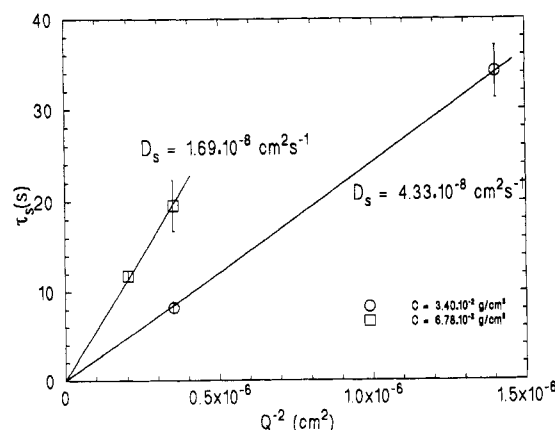


Figure 2. Variation of the relaxation time τ_s as function of $1/q^2$ for the ternary mixture F-dextran/PVP/water for the total polymer concentrations and compositions shown.

a single-exponential function, and the fit was satisfactory as it will be discussed in the next section.

III. Results and Discussion

The experiments were performed at the temperature $t = 20 \pm 0.1^\circ \text{C}$ at different total polymer concentrations, from the dilute to the semidilute regime and in the small x -range, i.e., $8.66 \times 10^{-4} < C_T (\text{g/cm}^3) < 12.08 \times 10^{-2}$, and far away from the phase separation. The dynamic functions were measured at several scattering vectors q . A typical fluorescence intensity signal obtained from fluorescein dextran/PVP/water for $x = 0.003$, $C_T = 6.79 \times 10^{-2} \text{ g/cm}^3$, and $q = 0.2216 \times 10^5 \text{ cm}^{-1}$ is displayed in Figure 1. The triangles represent the experimental data, and the solid line represents the monoexponential fit according to relation (3). It was clear from the fit-control parameters that the monoexponential fit is satisfactory and there were no reasons to fit the data with two-exponential decays or a stretched exponential. Similar observations were made at the other concentrations. The angular variation of the unique frequency called hereafter $\Gamma_s = 1/\tau_s$ shows a q^2 dependency indicating a diffusive motion. This behavior is illustrated in Figure 2 for the concentrations shown. In Figure 3 are plotted the associated self-diffusion coefficients $D_s = (\Gamma_s/q^2)_{q \rightarrow 0}$ as a function of the total polymer concentration, and these are listed in Table 2. On the same figure are also reported, for comparison, the values of the self-diffusion coefficient deduced from the DLS experiment on the same system

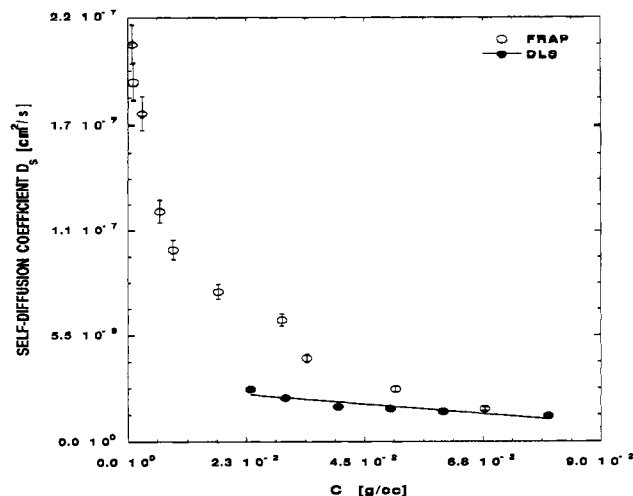


Figure 3. Variation of the self-diffusion coefficients D_s measured in the ternary polymer mixture PVP/dextran/water as function of the total polymer concentration C_T : (●) DLS;²³ (○) FRAP (this work).

Table 2. Values of the Single-Chain Diffusion Coefficients D_s as a Function of Concentration: FRAP and DLS Experiments

| conc ($\times 10^{-2} \text{ g/cm}^3$) | $10^{-8} \text{ cm}^2/\text{s } D_s$ | |
|--|--------------------------------------|-------------------|
| | FRAP | DLS |
| 0 | 18.54 ^a | 2.89 ^a |
| 0.087 | 20.6 | |
| 0.1046 | 18.70 | |
| 0.274 | 17.10 | |
| 0.612 | 12.00 | |
| 0.866 | 10.00 | |
| 1.712 | 7.79 | |
| 2.33 | | 2.7 |
| 2.93 | 6.31 | |
| 3.00 | | 2.25 |
| 3.40 | 4.33 | |
| 4.00 | | 1.80 |
| 5.00 | | 1.70 |
| 5.096 | 2.72 | |
| 6.00 | | 1.54 |
| 6.788 | 1.69 | |
| 8.00 | | 1.32 |
| 12.084 | 0.297 | |

^a Extrapolated values.

(see ref 23). Within experimental error, a satisfactory agreement between both D_s FRAP (this work) and DLS (earlier work)²³ seems to be reached in the semidilute regime: i.e., $C_T > 5 \times 10^{-2} \text{ g/cm}^3$. As the concentration is decreased, this agreement fails. This result was already anticipated in our earlier paper²³ and is confirmed in these experiments.

The reasons for this discrepancy are as follows: If one assumes that the FRAP gives the "true" values of D_s , this means that the model we have used to extract the self-diffusion coefficients from DLS experiments is not valid in the dilute system and a fortiori at $C_T = 0$ by means of extrapolation. Indeed, in earlier DLS experiments²³ the extrapolation had given $D_s(C_T \rightarrow 0) = 2.89 \times 10^{-8} \text{ cm}^2/\text{s}$ while the diffusion coefficient measured in the binary system dextran/water is $D_s = 1.2 \times 10^{-7} \text{ cm}^2/\text{s}$; the agreement is rather poor. In the present experiments and in the dilute range of concentration, the FRAP measurements show that the self-diffusion coefficient D_s is about $1.85 \times 10^{-7} \text{ cm}^2/\text{s}$, rather close to the DLS binary measurements. These results confirm that the extrapolated value at $C_T = 0$ of the self-diffusion in DLS experiments is wrong. Apart from the polydispersity effect, one of the major contributions in this discrepancy

is the hydrodynamic interaction.²⁷ Indeed, in the model we have used to analyze the DLS data²³ we neglected such an interaction and these complementary results show that these effects are very important. These results do support and do show that such interactions are important in the dilute range of concentration since there is a disagreement between both D_S values measured using FRAP and those extracted from DLS experiments in the dilute regime.

Indeed, we recall that the D_S values obtained from DLS were extracted from a model where hydrodynamic interactions are neglected. Therefore, if one assumes that the true values of D_S correspond to those measured using FRAP, which is probable, this means that the hydrodynamic interactions are of great importance in the dilute systems since, as the concentration decreases, the agreement becomes less satisfactory. The hydrodynamic version of this model has been already developed^{2,7} including the screening of hydrodynamic interactions.^{28,29} A more rigorous analysis which takes into account such interactions in the dilute system involving mixtures of polysaccharides in aqueous solutions is under investigation.

As far as the concentration dependency of the single-chain diffusion coefficient is concerned, we do not think that a scaling law could be extracted from the available data since less than 1 decade in the concentration range was investigated.

IV. Conclusion

We have investigated the dynamic behavior of the fluoresceinated dextran/PVP mixture in water using fluorescence recovery after photobleaching (FRAP) experiments. In the small range of composition and in dilute to semidilute solutions, one relaxation mode describes the dynamics of the mixture. This frequency has been identified as the self-diffusion coefficient. The results show that D_S decreases with the concentration, which is qualitatively in agreement with earlier DLS experiments, and confirms the slowing down of the motion of a single labeled chain i in a matrix of unlabeled j polymers.

The values of the self-diffusion coefficients measured directly using this technique (FRAP) have been compared to those deduced from DLS experiments. The agreement is found reasonable in semidilute solutions, confirming the validity of the model used to extract the self-diffusion coefficient from DLS experiments which is based on the RPA. However, as the concentration is decreased, this agreement becomes increasingly unsatisfactory, showing that the deduced values of the self-diffusion coefficients in the dilute system by extrapolation to infinite dilution ($C_T \rightarrow 0$) are wrong. This is to some extent comforting since the model is valid above C^* . Finally, to extract the true value of the self-diffusion coefficient in the dilute ternary system when using DLS experiments, one should

analyze the results within a model which takes into account bulk flow effects (hydrodynamic interactions). Such models exist^{7,26-29} and the analysis is under study on other systems involving mixtures of polysaccharides in aqueous solutions where little information is available in terms of mobility and compatibility. This will be discussed in a forthcoming paper.³⁰

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