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Solvation and Solvent Relaxation in Swellable Copolymers as Studied by Time-Resolved Fluorescence Spectroscopy

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The fluorescent probe dimethylaminonaphthylsulfonamide is covalently bound to the ends of the pol(ethylene glycol) chains of the swellable block copolymers poly(ethylene glycol)–polystyrene (PEG-PS) and poly(ethylene glycol)–poly(ethylene imine) (PEG-PEI) to investigate the molecular mobility inside the polymers, swollen by different liquids. Steady-state and time-resolved studies of the Stokes shift between absorption and fluorescence spectra reveal that the probe is solvated by both the polymer matrix and the liquid phase. The extent of solvation by the liquid and the mobility of the microenvironment of the probe depend on both the swelling volume of the polymer and the solubility of the probe in this liquid. Steady-state and time-resolved fluorescence depolarisation measurements show that the polymer matrix forms a very rigid solvent cage, which almost completely immobilizes the probe. Upon solvation of the probe by the liquid, the mobility of the probe increases. In PEG-PEI swollen by polar solvents, the mobilities of the probe itself and of its microenvironment, although not reaching the values observed in homogeneous solution, are significantly higher than in PEG-PS, due to the hydrophilic nature of the polymeric backbone in PEG-PEI.

KEY WORDS: DANSyl-labeled copolymers; swelling volume; time-resolved fluorescence; solvent relaxation; rotational depolarization.

INTRODUCTION

Poly(ethylene glycol)–polystyrene (PEG-PS)- and poly(ethylene glycol)–poly(ethylene imine) (PEG-PEI)-block copolymers form swellable macromolecular networks, which differ with respect to the hydrophobicity and degree of cross-linking of their polymeric backbones (Fig. 1). Thus, PEG-PEI is of interest as a water-"super" absorbing material, whereas PEG-PS is only little swollen by polar liquids. By bonding reactive centers to the terminal amino groups of the PEG chains, the polymers are turned into microreactors for, e.g., peptide synthesis [1]. Reaction rates in these microreactors are limited mainly by the accessibility of the reaction centers. Solvation and mobility of the active centers are frequently used as measures of their accessibility [2–10]. Substitution of the reactive centers by fluorescent probes (Fig. 1) allows the quantification of mobility and solvation dynamics inside the polymer network. The rotational mobility of the probes is obtained by measurements of fluorescence anisotropy [3,4,6–8]. Using, e.g., diphenylhexatriene (DPH) as a fluorescent probe of pronounced hydropho-

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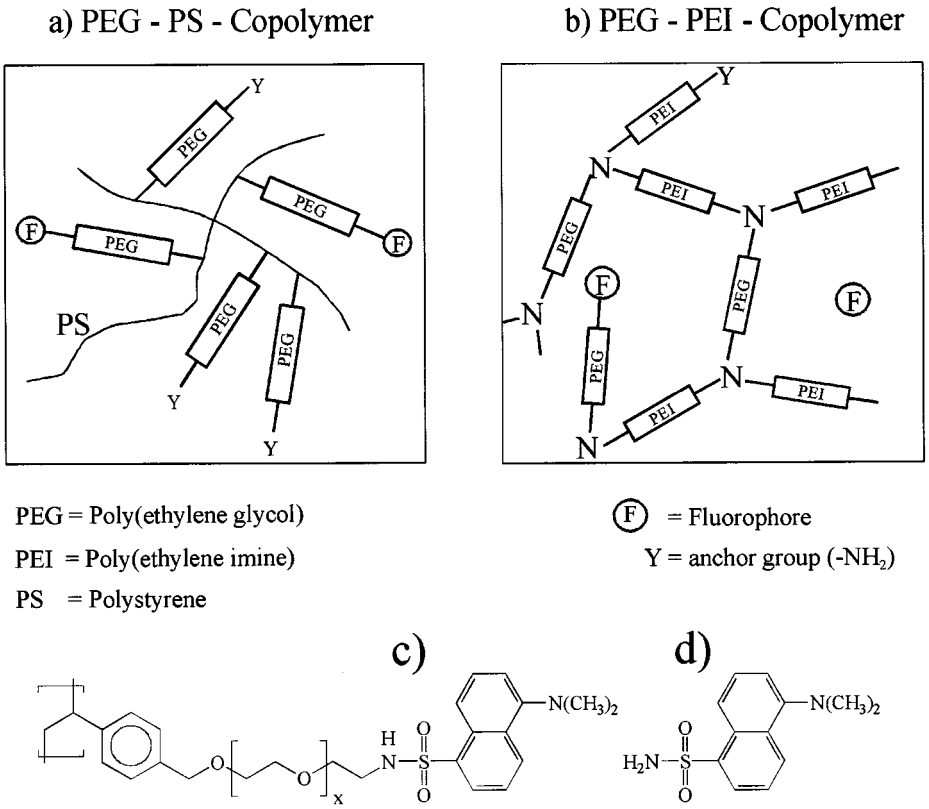

Fig. 1. Schematic structure of the block copolymers PEG-PS (a) and PEG-PEI (b). PEG, poly(ethylene glycol); PEI, poly(ethylene imine); PS, polystyrene; Y, $-NH_2$-anchor group at the end of the PEG chains; F, fluorophore. DANS-PEG-PS ($x \approx 75$) (c); DANSamide (d).

bicity, it could be shown that the rotational mobility of the PEG headgroup depends not only on the extent of swelling of the PEG-PS network in a certain solvent, but also on the solvation of the headgroup by this solvent [3,4]. The translational mobility in polymer networks has been determined from the extent of pyrene excimer formation [9,10]. The Stokes shifts of the fluorescence maxima of the probe molecules yield the reorientational mobility of the molecules solvating the probe [5].

In this work, the fluorescent probe dimethylamino-naphthylsulfonamide (DANSamide) was chemically bound to PEG-PS and PEG-PEI copolymers to investigate the polarity and the mobility of its microenvironment in the presence of different liquid phases. Polarity of the microenvironment and mobility of the solvent molecules are determined by steady-state and time-resolved measurements of the Stokes shift between fluorescence and absorption maxima. The mobility of the probe itself is measured by steady-state and time-resolved measurements of fluorescence anisotropy.

EXPERIMENTAL

Sample Preparation

DANSylchloride (Fluka) and PEG-PS (Tentagel microspheres of $d = 90$ μm; Rapp Polymere, Tübingen, Germany) were used as received. In Tentagel, the fraction of PEG chains, each consisting of ca. 75 oxyethylene units, was 70–80% (w/w). The degree of cross-linking is given by the mole fraction of 1–2% divinylbenzene. The relative swelling volume of Tentagel microspheres is defined as the ratio of the volume of the sedimented, swollen bead and the volume of the dry bead. Details of the determination of the swelling volume are given elsewhere [11]. PEG-PEI was synthesized according to a procedure described in the literature [12]. The mole fraction of PEG chains amounts to $x = \frac{1}{3}$.

In both copolymers, the PEG chains are terminated by $-NH_2$ groups. The functionalization of the polymers with DANS was performed by shaking 2 g of the polymer

in a dry dichloromethane solution of 1 mg DANSylchloride for 2 h at room temperature. The quantity of bound fluorophores was chosen to cover 0.1–1% of all available amino groups.

Fluorescence Measurements

Functionalized Tentagel microspheres (≈1 mg) were suspended in spectroscopy-grade solvents. The suspensions were stirred during the measurement to avoid sedimentation. PEG-PEI polymer was swollen with solvent until it completely occupied the cuvette space.

Fluorescence spectra and steady-state anisotropy measurements were performed on a SPEX Fluorolog 222 fluorometer equipped with two calcite Glan–Thompson polarizers. Fluorescence decay times were obtained by the single-photon counting method using a thyratron-controlled nanosecond flashlamp as excitation source. The instrument response provides a temporal resolution of $\Delta_t = 0.5$ ns at $\lambda = 337$ nm. Average fluorescence decay times (τ_F) were obtained from a double-exponential decay analysis, $I_F(t) = A_1\exp(t/\tau_1) + A_2\exp(t/\tau_2)$ and $\langle\tau_F\rangle = (A_1\tau_1^2 + A_2\tau_2^2)/(A_1\tau_1 + A_2\tau_2)$. Rotational correlation times were calculated from mean fluorescence lifetimes $\langle\tau_F\rangle$ and steady-state anisotropies r by inserting $\langle\tau_F\rangle$, r, and the maximum anisotropy, r_0, into the Perrin equation,

$$\tau_R = \frac{\langle\tau_F\rangle}{(r_0/r) - 1} \tag{1}$$

Time-resolved anisotropy decay measurements were performed at the Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, with single-photon counting equipment providing a time resolution of $\Delta t = 50$ ps. Details of the setup have been described previously [4]. Anisotropy decay curves were obtained by fitting $r(t) = D(t)/S(t)$, where $D(t) = I_{vv}(t) - I_{vh}(t)g$ and $S(t) = I_{vv}(t) + I_{vh}(t)g$. The scaling factor $g = I_{hv}/I_{hh}$ was determined for each decay measurement.

Time-resolved solvent relaxation measurements were performed with an Edinburgh Instruments 199S lifetime fluorometer. The pulse width of the thyratron-controlled nitrogen flashlamp at $\lambda = 337$ nm of $\Delta t = 2.0$ ns limits the temporal resolution of the setup to $\Delta t = 0.5$ ns. Fluorescence decay curves $D(t, \lambda)$ were fitted by a sum of exponentials. The time-resolved emission spectra (TRES) at times t after excitation, $S(t, \lambda)$, were reconstructed from the steady-state fluorescence spectra $S_{st}(\lambda)$ and the fluorescence decay curves $D(t, \lambda)$ [5,13],

$$S(\lambda, t) = S_{st}(\lambda) \cdot D(t, \lambda) \Big/ \int_{t=0}^{\infty} D(t, \lambda)dt \tag{2}$$

RESULTS AND DISCUSSION

Solvation Dynamics of DANSamide in Solution

The lowest electronic transition of DANSamide has considerable charge transfer (CT) character, due to the combination of the electron donating amino group and the electron withdrawing sulfonyl substituent [14,15]. Consequently, the absorption maximum is blue-shifted upon increasing the polarity and hydrogen-bonding character of the solvent. Upon excitation of the DANS fluorophore, its dipole moment increases, which leads to reorientation of the surrounding solvent molecules. The solvent relaxation in turn causes a red shift of the fluorescence spectrum, which increases with the polarity of the solvent. In homogeneous solutions solvent relaxation takes place on a picosecond time scale [13]. In restricted environments, as in lipid membranes and on metal oxide surfaces, strong interactions between the matrix and the solvent slow down the relaxation process by several orders of magnitude [5,16].

The Stokes shift between fluorescence and absorption maxima of DANSamide increases from $\Delta\tilde{\nu} \approx 8000$ cm^{-1} in nonpolar solvents to $\Delta\tilde{\nu} \approx 13000$ cm^{-1} in water. In the absence of specific interactions the Stokes shift $\Delta\tilde{\nu}$ depends linearly on Onsager's function, $f(D, n)$,

$$\Delta\tilde{\nu} = \frac{2(\mu_a - \mu_g)^2}{hc_0r^3} f(D, n) \tag{3}$$

where μ_g and μ_e are the dipole moments of the fluorophore in the ground and excited states, respectively, h is Planck's constant, c_0 is the velocity of light in vacuum, and r is the radius of the solvent cage [17, 18]. Onsager's function, $f(D,n)$, is given by

$$f(D,n) = (D - 1)/(2D + 1) - (n^2 - 1)/(2n^2 + 1) \tag{4}$$

where D and n are the dielectric constant and refractive index, respectively, of the solvent.

The Stokes shifts observed for DANSamide in different solvents obey relationship (3), except for solvents forming hydrogen bonds. However, a linear relationship between spectral positions and polarity of the environment is obtained for all solvents, including ethanol and

water, when absorption and fluorescence maxima are plotted against the empirical $E_T(30)$ polarity scale (Fig. 2) [19].

Solvation Dynamics of DANSamide Bound to PEG-PS and PEG-PEI Polymers

Steady-State Measurements of Solvent Relaxation

Similarly to DANSamide in solution, the Stokes shift of DANS bound to PEG-PS polymer matrices increases with the polarity of the solvents in which the polymer is suspended (Fig. 3), suggesting that the probe is at least partially solvated by the liquid phase. However, the Stokes shifts observed for polymer-bound DANS are always smaller than those observed in homogeneous solutions (Fig. 3). Obviously, the microenvironment of DANS in the swollen polymer is less polar and/or less mobile than in solution. A quantitative measure for the extent of solvation by mobile solvent molecules is provided by the solvation fraction SF,

$$SF = \frac{\Delta\tilde{\nu} - \Delta\tilde{\nu}_{dry}}{\Delta\tilde{\nu}_{sol} - \Delta\tilde{\nu}_{dry}} \qquad (5)$$

where $\Delta\tilde{\nu}_{sol}$, $\Delta\tilde{\nu}$, and $\Delta\tilde{\nu}_{dry}$ are the Stokes shifts of DANSamide in solution and of DANS bound to suspended and dry PEG-PS polymer, respectively. The SF values given in Table I clearly show that the solvation of PEG-PS-bound DANS by a certain solvent depends on both the swelling of the polymer beads and the solubility of the probe in this solvent. Swelling of the beads is necessary for the solvent molecules to penetrate into the beads and reach the probe. The number of solvent molecules available for solvation of the probe grows with the volume fraction of solvent sorbed by the polymer, i.e., with the extent of swelling. In the case of cyclohexane and diethylether, which do not swell PEG-PS, the probe is solvated by the polymer matrix rather than by the liquid phase. The degree of solvation of the probe by the solvent sorbed

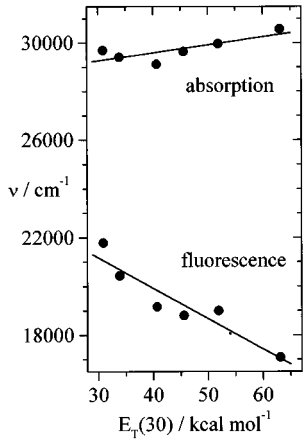

Fig. 2. Spectral positions of absorption and fluorescence maxima of DANSamide in different solvents plotted vs $E_T(30)$ solvent polarity values ($c_{DANSamide} = 10^{-5}$ *M*). Points correspond to experimental values in cyclohexane, toluene, dichloromethane, acetonitrile, ethanol, and water [in the order of increasing $E_T(30)$ values]. Lines are linear fits to the experimental data.

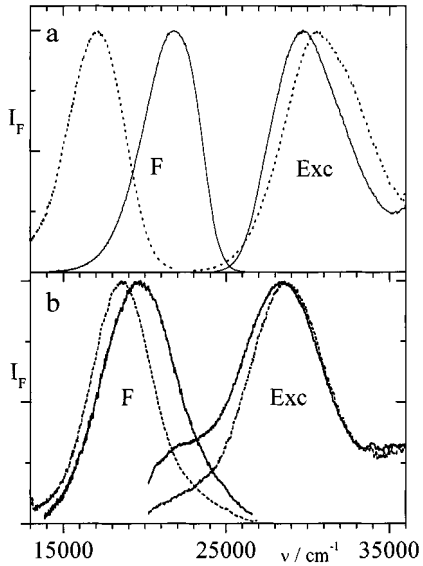

Fig. 3. Fluorescence (F) and fluorescence excitation spectra (Exc) of (a) DANSamide in water (dashed line) and in cyclohexane (solid line) and (b) DANS-PEG-PS suspended in acetonitrile (dashed line) and in cyclohexane (solid line). Local concentration of DANS in the PEG-PS microbeads $c_{DANS} = 6{\cdot}10^{-4}$ *M*. Excitation at $\tilde{\nu}_{ex} = 28{,}500\ cm^{-1}$; fluorescence observed at $\tilde{\nu}_{em} = 19{,}600\ cm^{-1}$.

Table I. Relative Solvation SF, Relative Swelling Volume and Relative Stokes Shift, $\Delta\tilde{\nu}$, of DANS-PEG-PS Microbeads in Solvents of Different Polarity and Stokes Shifts of DANSamide in Solution, $\Delta\tilde{\nu}_{sol}$[a]

Solvent	Polarity [E_T (30)]	Rel. swelling volume	$\Delta\tilde{\nu}_{sol,rel}$ (cm^{-1}) (DANS-NH_2)	$\Delta\tilde{\nu}_{rel}$ (cm^{-1}) (DANS-PEG-PS)	SF
Dry		1.0		0	
Cyclohexane	30.9	1.0	−600	300	
Toluene	33.9	3.4	500	600	1.20
Acetonitrile	45.6	3.0	2200	1600	0.73
Ethanol	51.9	1.2	2500	1500	0.60
Water	63.1	2.5	5000	1200	0.24

[a] For the sake of clarity, all Stokes shifts are given relative to the Stokes shift in dry DANS-PEG-PS, $\Delta\tilde{\nu}_{dry}$: $\Delta\tilde{\nu}_{rel} = \Delta\tilde{\nu} - \Delta\tilde{\nu}_{dry}$ and $\Delta\tilde{\nu}_{sol,rel} = \Delta\tilde{\nu}_{sol} - \Delta\tilde{\nu}_{dry}$.

into the polymer matrix is determined by the solubility of the probe in this solvent. This is evident from the comparison of the SF values of DANS-PEG-PS in ethanol and water, which both solvate the PEG chains but not the PS backbone. Although the relative swelling volume of PEG-PS in water is much larger than in ethanol, the SF value in ethanol clearly exceeds that obtained in water. This is due mainly to the low solubility of DANS in water. As the available water (some seven water molecules per oxyethylene unit) solvates the PEG chains rather than the hydrophobic DANS moieties, DANS is partly "solvated" by the PS matrix, owing to strong $\pi-\pi$ interactions.

Cyclohexane, although not swelling PEG-PS, causes a Stokes shift which is slightly larger than in dry Tentagel. As only a small fraction of the terminal amino groups of the PEG chains in Tentagel is reacted with DANSylchloride, and this reaction occurs faster than the diffusion of DANSylchloride into the polymer beads [20,21], DANS is probably bound preferrably to the outer regions of the beads, where it can be solvated by cyclohexane. This interpretation is supported by measurements of Tentagel labeled with diphenylhexatriene, which is distributed more homogeneously over the bead and which shows no measurable solvation by cyclohexane [4].

In contrast to DANS-PEG-PS, the Stokes shifts of DANS-PEG-PEI and of DANSamide dissolved in suspensions of PEG-PEI are close to those of DANSamide in the corresponding homogeneous solutions (Fig. 4). The Stokes shifts listed in Table II show that the microenvironments of the probe in PEG-PEI copolymers suspended in acetonitrile and water are highly polar and highly mobile. This is due mainly to the large volume fraction of sorbed solvent of ca. 96% in the highly swellable polymer and the absence of hydrophobic polymer segments to which the probe could be adsorbed in the presence of highly polar solvents.

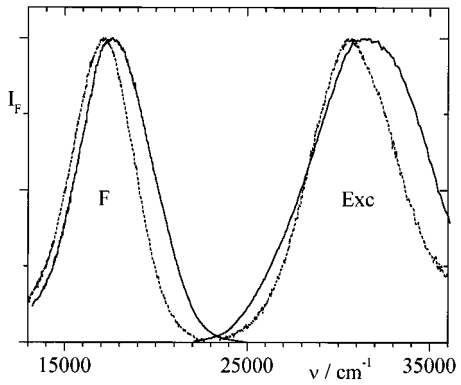

Fig. 4. Fluorescence (F) and fluorescence excitation spectra (Exc) of DANS-PEG-PEI suspended in water (solid line) and of DANSamide in suspensions of PEG-PEI in water (dashed line). Excitation at $\tilde{\nu}_{ex}$ = 30,000 cm^{-1}; fluorescence observed at $\tilde{\nu}_{em}$ = 18,200 cm^{-1}. Concentration of DANSamide in water $c_{DANSamide} = 1 \cdot 10^{-5}$ *M*; local concentration of DANS in PEG-PEI microbeads $c_{DANS} = 2 \cdot 10^{-4}$ *M*.

Table II. Relative Stokes Shifs $\Delta\tilde{\nu}_{rel}$ (Relative to the Stokes Shift in Dry DANS-PEG-PS) of DANS-PEG-PEI, $\Delta\tilde{\nu}_{rel}$, in Solvents of Different Polarity, and of DANSamide in Suspensions of PEG-PEI, $\Delta\tilde{\nu}_{sol,rel}$, in Solvents of Different Polarity

Solvent	Polarity [$E_T(30)$]	$\Delta\tilde{\nu}_{sol,rel}$ (cm^{-1}) (DANS-NH_2)	$\Delta\tilde{\nu}_{rel}$ (cm^{-1}) (DANS-PEG-PEI)
Dry		700	1000
Acetonitrile	45.6	2100	2600
Water	63.1	5400	4800

Time-Resolved Measurements of Solvation Dynamics

Measurements of time-resolved emission spectra (TRES) yield both the polarity and the mobility of the environment solvating the probe, if the linear relationship between spectral shift of the fluorescence maximum and micropolarity given by Eq. (1) is valid. A quantitative measure for solvent relaxation is the autocorrelation function $C(t)$

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \tag{6}$$

where $\nu(0)$, $\nu(\infty)$, and $\nu(t)$ represent the frequencies of the fluorescence maxima before excitation by the light pulse, at infinitely long times and at arbitrary times t after excitation, respectively. The mean solvent relaxation time, τ_s, is obtained as the time constant of the autocorrelation function. It is in the range of 10^{-10} and 10^{-13} s in homogeneous solutions [13] but may rise to several nanoseconds in highly viscous environments, e.g., in lipid membranes [5].

The solvent relaxation of the microenvironment of DANS bound to Tentagel has been determined in four solvents: water, acetonitrile, cyclohexane, and diethylether. For a complete interpretation of the TRES results, an estimation of the $t = 0$ spectra in all four cases is desirable. Since the determination of absorption spectra of these strongly scattering systems with their inhomogeneous distribution of absorbers is not reliable, the "Maroncelli method" [13] cannot be applied for this purpose. Thus for a rough estimation of the $t = 0$ spectra the fluorescence emission spectra at 77 K have been recorded (Fig. 5). The emission maxima (water, 21,200 cm^{-1}; acetonitrile, 21,000 cm^{-1}; cyclohexane, 21,400 cm^{-1}; diethylether, 21,650 cm^{-1}) differ only slightly from solvent to solvent. In all four cases the freezing of the solvent/

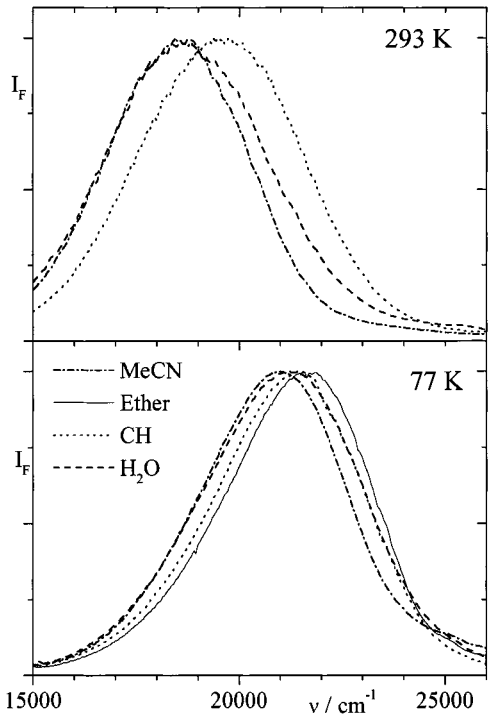

Fig. 5. Fluorescence spectra (excited at $\tilde{\nu}_{ex}$ = 29,000 cm^{-1}) of DANS-PEG-PS in four solvents at T = 273 K (top) and at T = 77 K (bottom). The high-temperature spectrum in diethylether is omitted, because it is practically identical to that in cyclohexane. MeCN, acetonitrile; ether, diethylether; CH, cyclohexane.

tentagel system slows down effectively the reorganization of the DANS microenvironment and leads to fluorescence from singlet states which are close to the Franck–Condon states. Comparison with the time-resolved data, however, strongly indicates that a substantial portion of solvent relaxation is still present at 77 K.

Time Evolution of the Half-Widths of the Reconstructed TRES

The time evolution of the half-widths of the TRES (Fig. 6) reconstructed from the steady-state fluorescence spectra and the fluorescence decay curves according to Eq. (2) yields information to which extent the solvent relaxation can be monitored by the time resolution of the experimental setup. In the case of water and cyclohexane the half-widths increase with time after excitation and reach a maximum. At longer times after excitation the half-widths decrease. The observed time dependence indicates that during the lifetime of the excited state solvent relaxation completes and thus the major part of the solvent relaxation process is detectable with the given subnanosecond time resolution of the experimental setup

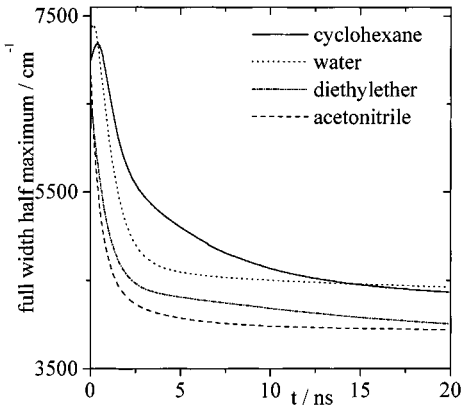

Fig. 6. Full widths at half-maximum of the time-resolved fluorescence spectra of DANS-PEG-PS in four solvents plotted vs time after the exciting light pulse.

[5]. In the case of acetonitrile and diethylether the half-widths reach their maximum at very short times after excitation. This shows that a major part of the solvent relaxation occurs much faster than the time resolution of our experiment and, thus, cannot be detected. It can be concluded that in the case of acetonitrile and diethylether, a major part of the solvent relaxation is occurring on the picosecond time scale. The solvent relaxation in the water and cyclohexane systems appears to be considerably slower: at least a major part of the overall solvent relaxation occurs on the nanosecond time scale.

Time Evolution of Maxima of the Reconstructed TRES

The temporal evolutions of the maxima of the TRES are shown in Fig. 7a. The solvent relaxed state of the acetonitrile system appears to be the one with the lowest energy of all four investigated systems. This observation indicates that the microenvironment of DANS is most polar when acetonitrile is present. The highest polarity should lead to the largest overall time-dependent Stokes shift $\Delta\tilde{\nu}$. The determined value of $\Delta\tilde{\nu}$ = 550 cm^{-1} is the smallest of all solvents and much smaller than the shift between the fluorescence maxima at T = 77 K and at room temperature. This supports the conclusion that the major part of the solvent relaxation occurrs on the picosecond time scale and that the solvent relaxation in the acetonitrile system is the fastest of all the solvent systems under investigation.

The water system appears to be the second most polar one. From the temperature dependence of the fluorescence maxima and the time dependence of the TRES half-widths, it can be concluded that a major part of the solvent relaxation occurrs on the nanosecond time scale.

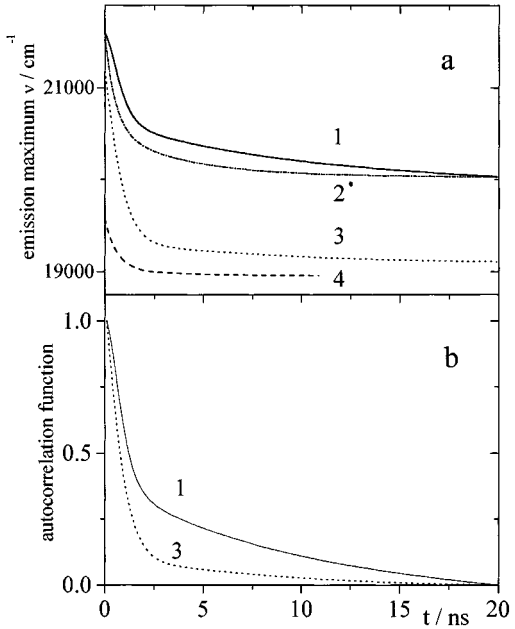

Fig. 7. Spectral positions of the maxima of the time-resolved fluorescence spectra of DANS-PEG-PS in four solvents plotted vs time after the exciting light pulse. (1) Cyclohexane; (2) diethylether; (3) water; (4) acetonitrile. (b) Autocorrelation functions of DANS-PEG-PS in water and in cyclohexane, obtained from Eq. (6) using the data in a.

The large total Stokes shift of about 1900 cm^{-1} is evidence for a highly polar DANS microenvironment in this system. The fact that the major part of the solvent relaxation can be characterized by the time resolution of our experimental setup allows for the calculation of the autocorrelation function by Eq. (6) (Fig. 7b). The time required for the correlation function $C(t)$ to decay to $1/e$ is about 0.9 ns. A biexponetial fit to the $C(t)$ function yields the correlation times $\tau_s = 0.8$ ns and $\tau_s = 17$ ns, thus giving evidence for the existence of a second, slow solvent relaxation process.

The cyclohexane system is the third most polar one. Since, again, the major part of the solvent relaxation is detectable, its kinetics are displayed by the correlation function (Fig. 7b). The time required for the correlation function $C(t)$ to decay to $1/e$ is about 2.5 ns. Two processes contribute to the overall solvent relaxation: a fast process characterized by a solvent relaxation time of $\tau_s = 0.9$ ns and a second, slow process with a relaxation time of $\tau_s = 17$ ns. In contrast to the other three solvent systems, the slow component of the reorganization process dominates the overall solvent relaxation and can still be detected up to 40 ns after excitation.

In the diethylether system the DANS microenvironment is the one with the lowest polarity and a major part of the solvent relaxation cannot be detected with the available nanosecond time resolution.

Mobility of the Fluorescent Probe in Solution and Bound to Polymers

The rotational mobility of the DANS moiety (Fig. 1) in PEG-PS and PEG-PEI interphases is determined by fluorescence anisotropy measurements. Steady-state measurements of fluorescence anisotropy serve as a qualitative measure for the mobility of the probe. In heterogeneous systems, where nonexponential fluorescence and fluorescence anisotropy decay curves as well as fluorescence depolarization by light scattering are encountered, a detailed description of the rotational motion of the probe can be obtained only by time-resolved anisotropy measurements.

The rotational correlation times of DANSamide in different homogeneous solutions, determined by time-resolved experiments, are given in Table III. Steady-state measurements of DANSamide in rigid media [4] yield maximum anisotropies of $r_0 = 0.30–0.32$, which are in accordance with values obtained from time-resolved measurements [22,23].

Steady-State Measurements of Fluorescence Anisotropy

Figure 8 shows the fluorescence excitation and fluorescence anisotropy spectra of DANS-PEG-PS in the dry state and suspended in different solvents. Two different fluorescing species with maxima at $\tilde{\nu} \approx 28{,}000$ cm^{-1} and $\tilde{\nu} \approx 22{,}000$ cm^{-1} are identified.

The maximum at $\tilde{\nu} \approx 28{,}000$ cm^{-1} corresponds to monomeric, i.e., nonaggregated DANS moieties. The rotational correlation times of monomeric DANS obtained by inserting mean fluorescence decay times and steady-state anisotropies given in Table IV into the Perrin equation [Eq. (1)] show the same dependence on the nature of the solvents as observed for the Stokes shifts. In solvents like cyclohexane, which do not swell PEG-PS, there is no measurable mobility of DANS. The highest mobility of DANS is observed in toluene, which swells

Table III. Rotational Correlation Times, τ_R, of DANSamide in Homogeneous Solutions

Solvent	τ_R (ns)
Water	0.10 ± 0.01
Acetonitrile	0.09 ± 0.01
Dichloromethane	0.08 ± 0.01

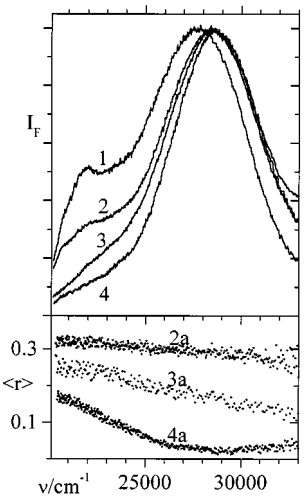

Fig. 8. Fluorescence excitation spectra (1–4) and fluorescence anisotropy spectra (2a–4a) of DANS-PEG-PS (local concentration of DANS in polymer, beads $c_{DANS} \approx 6 \cdot 10^{-4}\,M$). (1) Dry; (2, 2a) in cyclohexane; (3, 3a) in water; (4, 4a) in acetonitrile.

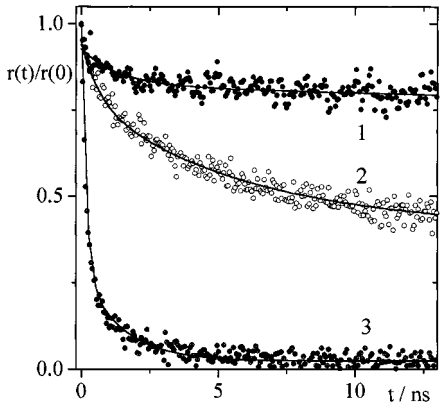

Fig. 9. Fluorescence anisotropy decay curves of DANS-PEG-PS microbeads suspended in cyclohexane (1), water (2), and acetonitrile (3). The decay curves are normalized at $t = 0$ to eliminate effects due to fluorescence depolarization by light scattering. Points, experiment; lines, fits of experimental data to Eq. (7).

both the PEG and the PS parts of Tentagel and also is a good solvent for DANS. Despite the large swelling volume of PEG-PS in water, the mobility of DANS is lower than that in acetonitrile by an order of magnitude, due to the inferior solvation of the DANS moiety by water. The same dependence of the mobility of hydrophobic groups bound to the ends of the PEG chains on the extent of their solvation by the liquid phase has also been observed in ^{13}C NMR experiments [11].

The maximum at $\tilde{\nu} \approx 22{,}000\ cm^{-1}$ is assigned to $(DANS)_x$ aggregates [4]. The extent of aggregate formation increases with the loading of the particles with DANS [4] and decreases with the solvation of the DANS moiety and with the swelling volume of the PEG-PS beads. The exception is cyclohexane, where aggregation is significantly reduced compared to the dry beads, although cyclohexane does not swell Tentagel. As pointed out above, DANS and especially $(DANS)_x$ might be concentrated in the outer regions of the beads, where they are accessible also to nonswelling solvents. The aggregates show no measurable mobility. Their steady-state anisotropy, $\langle r \rangle$, measured in the dry state, closely approaches the intrinsic anisotropy, r_0, of DANS. The lower values of $\langle r \rangle$ observed in water and acetonitrile are rather due to the overlap of aggregate and monomer spectra than to higher mobilities of the aggregates.

Table IV. Mean Fluorescence Decay Times $\langle \tau_F \rangle$, Steady-State Fluorescence Anisotropies $\langle r \rangle$ at $\tilde{\nu}_{exc} = 28{,}000\ cm^{-1}$ and Rotational Correlation Times, τ_R, Calculated by Eq. (1), of DANS-PS-PEG in Different Solvents

Solvent	$\langle \tau_F \rangle$ (ns)	$\langle r \rangle$	τ_R (ns)
Dry	11		
Cyclohexane	13	0.31	$\rightarrow\infty$
Toluene	9	0.03	0.9
Acetonitrile	16	0.03	1.7
Ethanol	13	0.05	2.4
Water	15	0.17	17

Time-Resolved Measurements of Fluorescence Anisotropy

DANS-PEG-PS. In Fig. 9 the fluorescence anisotropy decay curves of DANS-PEG-PS in cyclohexane, water, and acetonitrile are compared. All three decay curves are clearly nonexponential. At the moment it cannot be decided whether the nonexponential behavior is due to locally different mobilities in the heterogeneous system or to the complicated type of motion performed by fluorophores covalently bound to the ends of PEG chains and physically attached to some part of the polymer matrix [24]. In the latter case, short and long components are assigned to the rotational motion of the fluorophore itself and to the motion of the polymer matrix (e.g., swaying motions of the PEG chains), respectively. Thus, the normalized decay curves $r(t)/r_0$ are fitted to a biexponential decay,

$$\frac{r(t)}{r_0} = \sum_{i=1}^{2} A_i \cdot \exp\left(-t/\tau_{R,i}\right) + \frac{r_\infty}{r_0} \qquad (7)$$

where $\tau_{R,i}$ are the rotational correlation times and r_∞ is the residual anisotropy at infinetely long times after excitation. The results are summarized in Table V. In acetonitrile, the highest mobility of DANS is observed. However,

Table V. Rotational Correlation Times, $\tau_{R,i}$, Relative Amplitudes, A_i, of the Anisotropy Decay Components of DANS-PEG-PS in Different Solvents (ν_{exc} = 27,800 cm^{-1})[a]

Solvent	$\tau_{R,1}$ (ns)	$\tau_{R,2}$ (ns)	A_1	A_2	r_∞/r_0	θ (deg)
Cyclohexane	0.4 ± 0.1	4.7 ± 1.9	0.11 ± 0.02	0.07 ± 0.01	0.80 ± 0.01	22
Water	0.5 ± 0.1	5.0 ± 0.4	0.16 ± 0.02	0.40 ± 0.01	0.42 ± 0.01	42
Dichloromethane	0.52 ± 0.04	4.3 ± 0.39	0.68 ± 0.04	0.32 ± 0.03	0	90
Dimethylformamide	0.25 ± 0.02	1.20 ± 0.09	0.82 ± 0.05	0.18 ± 0.02	0	90
Acetonitrile	0.22 ± 0.01	3.4 ± 0.2	0.94 ± 0.04	0.06 ± 0.01	0	90

[a] r_∞/r_0 represents the ratios of residual and initial anisotropies, θ gives the cone angle of the wobbling motion calculated from r_∞/r_0 by Eq. (8). Local concentration of DANS in polymer beads $c_{DANS} = 6 \cdot 10^{-5}$ *M*.

even in acetonitrile the rotational correlation times, $\tau_{R,i}$, are significantly longer than for DANSamide in homogeneous solutions. In water, despite the observation of two short components, the mobility of DANS is greatly reduced compared to acetonitrile. The presence of a residual anisotropy, r_∞, implies that the rotational motion of the DANS fluorophore is restricted to a finite solid angle. As the mobilities of the PEG chains in acetonitrile and water are comparable, the constrained mobility of DANS in water must be caused by incomplete solvation of DANS by water. Obviously, the hydrophobicity of the fluorophore results in partial solvation by the polymer matrix, namely, by the PS parts. This interpretation is supported by time-resolved anisotropy measurements of DPH-labeled PS-PEG in water, where no short-lived component is observed [4]. In cyclohexane, practically no fluorescence depolarization is observed within the lifetime of the fluorophore, except for a weak short component, which is probably due to DANS bound to the outer regions of the beads. DANS moieties in the inner regions of the beads, which are not reached by cyclohexane, are immobilized by a rigid solvent cage formed by the polymer matrix. In the absence of solvent, the PEG chains are at least partially present in crystalline form below T = 329 K, as deduced from the results of differential scanning calorimetry experiments [11].

From the residual anisotropies, r_∞, the available space for rotational motion of the fluorophores can be obtained. The "wobble in cone" model is a simple approximation, which assumes that the fluorophore undergoes free rotational diffusion within a cone of semiangle θ. This angle is related to the residual anisotropy by [24]

$$2\left(\frac{r_\infty}{r_0}\right)^{\frac{1}{2}} = \cos^2\theta + \cos\theta \qquad (8)$$

For DANS-PEG-PS in water and cyclohexane, values of $\theta \leq 42°$ and $\theta \leq 22°$ are obtained, respectively.

PEG-PEI. The fluorescence anisotropies of DANSamide in PEG-PEI-suspensions and of DANS-PEG-PEI in water decay much faster than in DANS-PEG-PS (Fig. 10). The results of biexponential fits of the PEG-PEI systems in three solvents are listed in Table VI. In contrast to DANS-PEG-PS, the decay curves are always dominated by the short component. Also, the cone angles, θ, obtained from the residual anisotropies by Eq. (8), are much wider (Table VI), indicating almost unrestricted motion of DANS in solvent swollen PEG-PEI. The free probe DANSamide in PEG-PEI reaches a rotational correlation time, which is very close to that of DANSamide in homogeneous solution. However, the mobility of DANS bound to PEG-PEI in water is still significantly lower than in acetonitrile and in dichloromethane despite the large volume fraction of water in swollen PEG-PEI of V_w/V_{tot} = 0.96.

CONCLUSION

Solvation and mobility of DANSamide bound to the ends of the PEG chains in PEG-PS and PEG-PEI copoly-

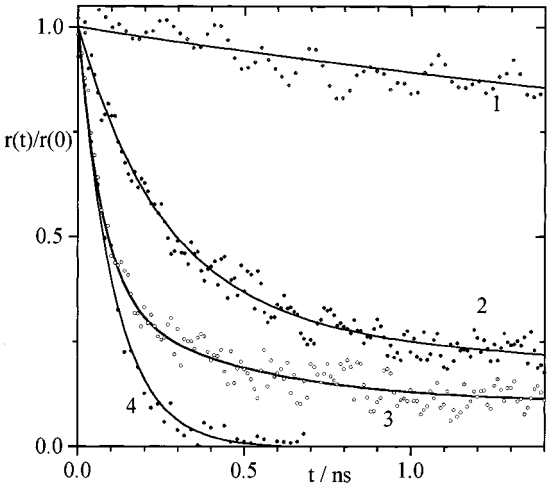

Fig. 10. Fluorescence anisotropy decay curves of DANS-PEG-PS (1), DANS-PEG-PEI (2), and DANSamide/PEG-PEI suspension (3) and DANSamide (4) in water (excited at $\tilde{\nu}_{ex}$ = 27,800 cm^{-1}). Points, experiment; lines, fits of experimental data to Eq. (7).

Table VI. Rotational Correlation Times, $\tau_{R,i}$, and Relative Amplitudes, A_i, of the Anisotropy Decay Components of DANS Bound to PEG-PEI in Different Solvents and of DANSamide in Different Suspensions of PEG-PEI (ν_{exc} = 27,800 cm^{-1})[a]

System/solvent	$\tau_{R,1}$ (ns)	$\tau_{R,2}$ (ns)	A_1	A_2	r_∞/r_0	θ (deg)
			DANS-PEG-PEI			
Water	0.21 ± 0.02	1.07 ± 0.06	0.63 ± 0.04	0.22 ± 0.02	0.15 ± 0.01	59
Acetonitrile	0.21 ± 0.04	1.54 ± 0.3	0.61 ± 0.13	0.25 ± 0.10	0.14 ± 0.02	60
Dichloromethane	0.17 ± 0.01	1.79 ± 0.18	0.73 ± 0.03	0.25 ± 0.02	0.03 ± .002	74
			DANSamide in PEG-PEI suspension			
Water	0.07 ± 0.01	0.44 ± 0.04	0.64 ± 0.01	0.26 ± 0.01	0.10 ± 0.01	64
Acetonitrile	0.13 ± 0.01	—	0.80 ± 0.03	—	0.19 ± 0.01	56
Dichloromethane	0.10 ± 0.02	0.84 ± 0.33	0.75 ± 0.08	0.18 ± 0.06	0.12 ± 0.01	62

[a] r_∞/r_0 represents the ratios of residual and initial anisotropies. θ gives the cone angle of the wobbling motion calculated from r_∞/r_0 by Eq. (8).

mers are determined by the relative contributions of liquid phase and polymer matrix to the solvation of the probe. The solvent cage formed by the polymer matrix is rigid and thus almost completely immobilizes the probe, as shown by steady-state and time-resolved fluorescence anisotropy measurements. Solvation of the probe by the liquid phase requires both, swelling of the polymer and high solubility of the probe in this solvent, as could be shown by steady-state and time-resolved measurements of solvent relaxation. By time-resolved measurements of solvent relaxation, it could be shown that the environment of the probe becomes highly polar upon swelling the polymers in solvents of high dielectric constants. In acetonitrile, which actually solvates the probe, solvent relaxation is significantly faster than in water. In water, DANSamide is partly solvated by the polymer matrix, namely, the PS parts, due to its relatively strong hydrophobicity. In all solvents under investigation, solvent relaxation in the PEG-PS copolymer is slower than in homogeneous solution by two orders of magnitude, indicating strong interactions between solvents and the polymer matrix.

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