Effect of solvent on nanoscale polymer heterogeneity and mobility probed by single molecule lifetime fluctuations

R.A.L. Vallée [a,b,*], N. Tomczak [a,b], L. Kuipers [a], G.J. Vancso [b], N.F. van Hulst [a]

[a] *Applied Optics Group, MESA+ Research Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands*
[b] *Materials Science and Technology of Polymers, MESA+ Research Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands*

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Abstract

In this Letter, we address nanoscale heterogeneity in polymer mobility. Single molecule fluorescence lifetime fluctuations are exploited as a probe for local segmental dynamics in polymer films. Wide distributions in polymer mobility are observed, which depend on film fabrication, treatment and amount of solvent contained. Upon solvent evaporation mobile regions disappear, while more immobile regions settle, effectively increasing the glass transition temperature. Besides insight in polymer nanoscale dynamics and heterogeneity, our results bear specific relevance to photodynamic studies on single chromophores, where routinely polymer matrices are used for immobilization.
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1. Introduction

In the last decade the study of single fluorophores immobilized in polymeric thin films has received increasing research attention. Initially the polymeric matrix helped increase photostability and quantum efficiency of the fluorophores for single molecule studies [1–4]. More and more the situation has reversed and the polymer has become the object of study by single molecule methods [5–7]. Polymer mobility both in the supercooled liquid regime [5] and in the glassy state are addressed [7–9]. Recently we have shown that lifetime fluctuations of single molecules embedded in a polymer matrix reflect the local polymer mobility [7]. Free volume theories [10–13], generally applied to describe molecular properties and physical behavior of polymers, can now be confronted with nanoscopic experiments. Using the Simha–Somcynsky free-volume theory [12,13], we can relate the lifetime fluctuations directly to the number of segments involved in a rearrangement of the polymer chains constituting the immediate surrounding of the molecule. In our previous study [7], the films were annealed after spin coating of the dye-doped polymer solution on a glass substrate, in order to remove the residual solvent and erase the history of the films. In this Letter we analyze the nanoscale heterogeneity of polymer mobility as a function of time for freshly prepared films, particularly the effect of solvent evaporation. Our observations are particularly relevant for the single molecule community where photodynamic on single chromophores is studied and routinely polymer matrices are used for immobilization.

2. Methodology

The Simha–Somcynsky (S–S) theory [12] considers the polymer as a lattice of sites that can accommodate the chain segments of macromolecules. To account for molecular disorder, a temperature and volume dependent fraction h of holes is introduced. Knowing the configurational properties of the system, an equation of state has been established [13], which permits the determination of the fractional mean free-volume h_{av}

* Corresponding author. Present address: Department of Chemistry, Catholic University of Leuven, Celestijnenlaan 200 F, B-3001 Leuven, Belgium. Fax: +3216327990.
E-mail address: renaud.vallee@chem.kuleuven.ac.be (R.A.L. Vallée).

present in the system. However, due to thermal fluctuations, the free-volume varies both in time and at every position. As a consequence, the probe molecule sees its immediate surrounding fluctuating, mediated by a change of its local dielectric constant ϵ [15]: $\epsilon = h\epsilon_{vac} + (1 - h)\epsilon_{pol}$, where $\epsilon_{vac} = 1$ and $\epsilon_{pol} = 2.5$ designate the vacuum and polymer (polystyrene) dielectric constant, respectively. The fluctuations of the dielectric constant modulate the spontaneous emission rate $\Gamma(\epsilon)$ of the probe molecule, in accordance with [16]: $\Gamma(\epsilon) = \frac{9\epsilon^{5/2}}{(2\epsilon+1)^2}\Gamma_0$, where Γ_0 is the spontaneous emission rate of the molecule in vacuum. The radiative lifetime τ is the inverse of $\Gamma(\epsilon)$. A distribution of fluorescence lifetimes of the probe molecule is thus obtained as a result of the motion of the chain segments around the probe. Given the number (N_s) of these polymer segments, a mean-squared deviation from the mean free volume can be calculated and a gamma distribution of free volume is built [17,18]. This distribution of free volume is connected to the fluorescence lifetime distribution of the probe molecule, which is experimentally shown to be also best fitted by a gamma distribution [7]. Specifically, the shape parameters α of both distributions are shown to match within 5% accuracy. As a result, the shape parameter (α) of the lifetime-fitted gamma distributions gives directly the number N_s of chain segments involved in a rearrangement volume around the probe.

3. Experimental

Thin films (70 nm) of PS ($T_g = 373$ K, 89300 g/mol, polydispersity index (PI) of 1.06, Polymer Standard Service) with embedded 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine (DiD, Molecular Probes)

fluorescent probe molecules were prepared by either spin coating or casting the dye-doped polymer (5×10^{-10} M) in a toluene solution on a glass substrate at 295 K. For the duration of the experiment, the fresh samples were held isothermally in air and investigated using a confocal scanning fluorescence microscope. For the sake of comparison, an annealed film was also prepared, in vacuum, following a two stage procedure: 12 h at 323 K followed by 3 h at 378 K, in vacuum. The dye molecules were excited by 230 fs pulses at a wavelength of 647 nm, generated by a frequency doubled optical parametric oscillator pumped by a Ti:Sa laser (Spectra Physics). The fluorescence signal was collected in two orthogonal polarization channels in order to probe possible reorientation of the dye in the matrix. Fluorescence lifetime transients of single embedded molecules were acquired using time correlated single photon counting [14].

4. Results and discussion

Trajectories of both intensity and fluorescence lifetime trajectories of an individual DiD molecule in PS are shown in Fig. 1a. The intensity is constant within 15% during the investigated time window (60 s). While the fluorescence lifetime mostly stays around a value of 3 ns, it makes frequent excursions to longer lifetimes up to 7 ns. Fig. 1b shows the distribution of lifetimes and the correlation plot between intensity and lifetime. The correlation plot indicates that the lifetime is not correlated to the intensity, excluding the possibility that the lifetime fluctuations result from fluctuations in the available number of decay channels. The lifetime distribution is clearly asymmetric, with a tail towards longer lifetimes, which in fact rules out any non-radia-
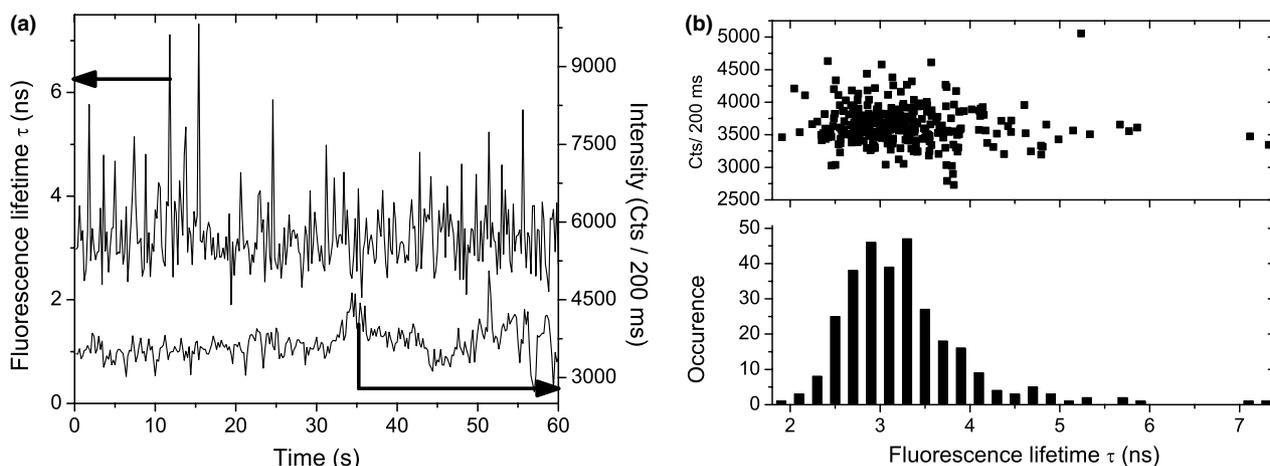

Fig. 1. (a) Transients of fluorescence intensity and lifetime for an individual DiD molecule embedded in a 70 nm thick film of PS obtained by spin coating of a dye-doped polymer solution on the glass substrate at room temperature. The molecule exhibits excursions towards longer lifetime values. (b) Corresponding fluorescence lifetime distribution and correlation plot between intensity and lifetime. While the intensity is constant within 15% the lifetime varies 100% from 3 up to 6–7 ns.

tive process as the cause of the fluctuations. Fluctuations of the fluorescence lifetime due to modifications of either the transition frequency or the transition dipole moment of a fluorophore [19], are prevented since the molecular conformation of the chosen carbocyanine dye is similar in both ground and excited state [20]. These observations are the prerequisites to attribute the lifetime fluctuations to polymeric density fluctuations [7].

On a daily basis, we collected 20–50 molecule lifetime transients in a time interval of 1 h. We fitted the lifetime distribution of each individual molecule with a gamma distribution to obtain the shape parameter, and consequently the number N_s of polymer segments rearranging around the probe molecule. From the obtained 20–50 values of N_s, we then constructed the distributions of N_s for each day. Fig. 2 shows the peak positions of the N_s distributions in time, for both spin coated (squares) and casted films (stars) of the dye-doped polymer solution on a glass substrate. Interestingly, both curves display an S-shape. Starting with 8–9 polymer segments involved in a rearrangement around the probe molecule for a freshly prepared sample, the curves stay flat as time evolves until four days are elapsed. At this point, the N_s values increase rapidly, to reach a plateau at $N_s = 12$ after a week.

Note that the number of segments $N_s = 12$ obtained two weeks after the spin coating or casting has been performed coincides with the N_s of a similar film when annealed. The fact that films obtained both by casting and spin coating give rise to the same behavior excludes the possibility that the S-shape is due to some relaxation process of the stresses induced by a spin coating pro-

cedure. The S-shape is thus attributed to the withdrawal of the solvent (toluene) trapped in the PS films during the coating. The effect of a given amount of solvent present in a polymer film is known to reduce the glass transition temperature T_g of the film [21–23]. We determined the temperature dependence of N_s as $dN_s/dT = -1$ segment per 9 K [7], so that a difference of three segments between the low- and high-N_s plateau corresponds to a difference of 27 K in T_g. The annealed film, free of solvent, has a glass transition temperature $T_{ga} = 373$ K. Consequently, the low-N_s plateau corresponds to a system PS with trapped solvent with a glass transition temperature $T_{gs} = 346$ K. The plasticizing effect of solvent is often described by: $k\omega_s = T_{ga} - T_{gs}$, where ω_s is the solvent weight fraction and k a constant, which for the PS/toluene system is equal to 500 K [24]. For a 27 K temperature difference between T_{gs} and T_{ga}, a solvent weight fraction $\omega_s = 5.5\%$ is trapped in the polymer film. This is the case for the film prepared by spin coating. Concerning the casted film, the values of N_s are slightly lower (Fig. 2) and the corresponding solvent weight fraction $\omega_s = 7\%$ is slightly higher than for the spin coated film, as expected.

The existence of a low-N_s plateau is probably due to the formation of a dense viscoelastic region at the exposed surface, at the early stages of the solvent desorption (during the spin coating or casting process) [25]. The formation of such a skin is undesirable in coating processes due to non uniformities in the polymer coating and a decrease in the drying rates [26]. The intrinsic dynamics of the system polymer chains-solvent and the presence of a drying front across the film thickness slowly react against the formation of the skin and drive the evaporation of the solvent.

So far we have discussed the development of the average properties of the polymer films in time. The exclusive advantage of our single molecule approach lies in the distribution. Each molecule reports on its nanoenvironment and by monitoring many molecules we find the nanoscale heterogeneity. Fig. 3 shows the N_s distributions for 4, 5 and 6 days after spin coating. Clearly a very broad distribution is observed, indicating the heterogeneity of the film ranging from mobile (low N_s) to immobile (high N_s) regions. The definition of T_g for the film only works on a macroscopic level as, on the nanoscale, domains with different N_s, i.e., different mobility and local 'effective T_g', occur. A detailed look at the distributions reveals how the average N_s increases day by day. The dominant changes occur at the low N_s side of the distribution where mobile regions vanish. This observation supports nicely the suggestion by Robertson [27] that regions of higher mobility should relax more rapidly than region with low mobility. On the contrary a certain fraction of low mobility regions is present from the beginning and becomes dominant as time elapses. In this context it is interesting to note that,
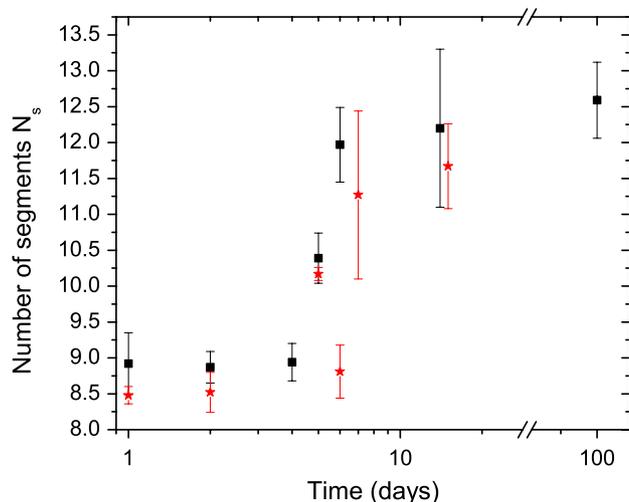

Fig. 2. Temporal evolution of the number of polymer segments N_s rearranging around DiD molecules embedded in a 70 nm thick polystyrene matrix. The values given correspond to the peaks of the distributions (Fig. 3), obtained on a daily basis after the spin coating (squares) or casting (stars) procedure has been initiated at room temperature 22 °C. The value pointed after break on the x scale corresponds to the peak value of the distribution of the number of segments obtained after annealing of the spin coated film.
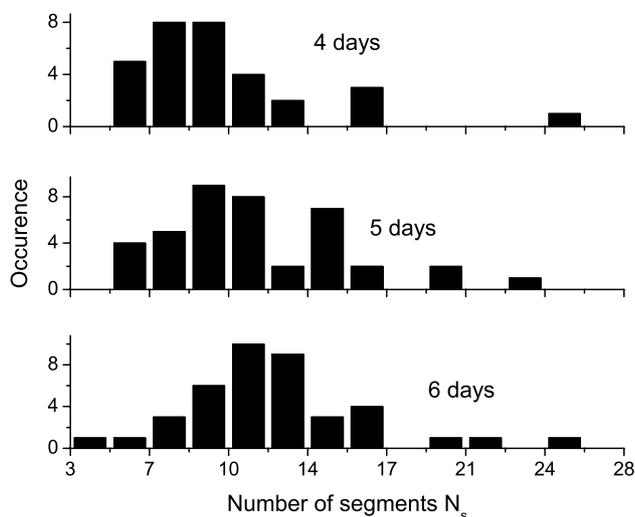
Fig. 3. Distributions of the number of segments N_s involved in a re-arrangement of the polymer chains in the immediate surrounding of DiD molecules embedded in a 70 nm thick film of PS for 4, 5 and 6 days after the spin coating procedure.

at any stage throughout the solvent evaporation process, the N_s distribution is wider than the actual shift occurring: the heterogeneity is larger than the effect we observe. Even for an annealed film with $T_{ga} = 373$ K nanoregions exist with a local effective T_g much lower and also higher than the bulk value.

The analysis described in this Letter is crucial for the single molecule community. Indeed, in this community, one usually uses a polymer matrix to immobilize fluorescence molecules. The matrix is most often prepared by spin coating a dye-doped polymer solution on a glass substrate. In this case, it is important to realize that the T_g of the material is not that expected, but is mostly reduced, due to the presence of solvents, thus increasing the mobility of the matrix. Furthermore, the molecules (and especially their fluorescence lifetime) under study are sensitive to the subsequent evaporation process, as shown in the current investigation.

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