Heterogeneity during Plasticization of Poly(vinylpyrrolidone): Insights from Reorientational Mobility of Single Fluorescent Probes

Sukanya Bhattacharya, Dharmendar Kumar Sharma, Suman De, † Jaladhar Mahato, and Arindam Chowdhury*‡

Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

ABSTRACT: While dynamics of single-molecule (SM) fluorescent probes have been used to investigate the structure and relaxation processes in polymers near the glass transition temperature (Tg), it is difficult to perform SM imaging at elevated temperatures which restricts such studies to a limited number of polymers for which Tg is close to room temperature (RT). Plasticization, solvent (or additive) induced lowering of Tg offers an alternate avenue to access various effective temperatures in the glassy and rubbery phases of polymers under ambient conditions. By investigation of the reorientational propensity of individual Rhodamine 6G (Rh6G) probes, which is governed by rigidity/dynamics of the polymer cavities, we have explored the extent of spatiotemporal heterogeneity during moisture induced plasticization of poly(vinylpyrrolidone) (PVP), far below and near (below and above) bulk Tg. Lack of any probe reorientation suggests that the matrix remains extremely rigid up to a certain level of hydration, as expected for probes buried deep within the glassy state. At intermediate levels of hydration, SMs undergo a wide variety of rotational dynamics ranging from being static/wobbling motion to slow, hindered large-angle reorientation, as well as facile, intermittently hindered fast rotation, which reflects that swelling/softening of network cavities is spatiotemporally extremely diverse as the effective Tg approaches RT. SM probes exhibit temporally nonuniform rotational mobility even at relatively high moisture contents of the matrix beyond which probes can undergo translational motion, which indicates that relatively slow time scale polymer segmental motion can be operational for plasticized PVP (in the rubbery state). Our inferences are supported by the non-Gaussian nature of angular jump distributions for dipolar reorientation, similar to those reported for translational diffusion of SM tracers in polymers and cellular media, suggesting the existence of slow time-varying local environmental changes around individual probe molecules during plasticization.

INTRODUCTION

Single-molecule fluorescence microscopy (SMFM) has emerged as an important tool for the study of inhomogeneity in complex systems as individual molecular probes act as reporters for fluctuations in the local environment in nanometer length scales. 1–3 For instance, changes in electronic properties and spatiotemporal dynamics of single-molecule (SM) probes embedded in polymer thin film matrices have been used to investigate the heterogeneity in morphology and relaxation processes that occur during transition from a glassy to a rubbery state. 2,4,5 To study polymeric systems near the glass transition temperature (Tg), SM measurements have been performed at different temperatures ranging from below through above Tg which is often challenging especially when high-Tg polymers are being probed. Even for polymers with Tg close to room temperature (RT), such as poly(methyl acrylate) (PMA, Tg ~ 283 K) or poly(vinyl acetate) (Tg ~ 310 K), the vast majority of SM diffusion dynamics has been performed in a relatively narrow temperature range ~Tg—10 K to ~Tg + 25 K. 6–8 Such limitation is primarily due to the workable temperature range of high numerical aperture objectives required to detect feeble SM fluorescence signals. This not only restricts the study of a variety of high-Tg polymers near glass transition, but also limits our understanding of the extent of spatiotemporal heterogeneity beyond a certain temperature range close to Tg. Therefore, it would be interesting to carry out SM diffusion dynamics measurements on high-Tg polymeric materials if the Tg could be lowered by alternate means such that both glassy and rubbery states can be accessed at RT.

Addition of low-molecular weight additives (plasticizers) to high-Tg polymers can have pronounced effects in lowering the effective Tg close to or below RT (plasticization), which transforms a high-Tg polymeric material into a rubbery state. 4,6 Plasticization is used to generate flexible and moldable polymeric materials from brittle high-Tg polymers, which renders them utilisable for various practical applications ranging from cables to coating of pharmaceuticals as well as personal care products such as contact lenses. 11–13 Very often, plasticizers are “good” solvents for a particular high-Tg polymer that weaken inter-/intra-chain interactions (in effect, they “soften” the polymer) and allow for primary relaxation

Received: August 25, 2016
Revised: November 2, 2016
Published: November 4, 2016

DOI: 10.1021/acs.jpcl.6b08584
J. Phys. Chem. B 2016, 120, 12404−12415
Article

The Journal of Physical Chemistry B

processes to be operational at temperatures far below the $T_g$ of the unmodified polymer. In fact, different proportions of appropriate solvents can be used to tune the $T_g$ for various high-$T_g$ amorphous polymers over an extremely wide temperature range, and thereby access different states of high-$T_g$ polymers under ambient conditions. Such a process can therefore facilitate investigation of the extent of heterogeneity far below, near and above the $T_g$ without the need to perform variable temperature SMFPM measurements. However, there are very few reports where SM dynamics has been utilized to study high-$T_g$ polymers with increasing solvent absorption.

Poly(vinylpyrrolidone) (PVP), a high-$T_g$ (428 K) amorphous polymer with applications in pharmaceutical coatings, is known to spontaneously absorb moisture from the environment and undergo plasticization. Due to its wide usage for storage, stability, and delivery of drugs in humid/aqueous environments, the physical properties of PVP at different moisture contents have been studied extensively at various ambient relative humidity (RH). It has been shown that $T_g$ of bulk PVP decreases continuously with moisture uptake, and at an RH of ∼70%, the polymer is effectively transformed into a rubbery state at RT. Interestingly, for both powder PVP and solution cast films, the extent of $T_g$-lowering has been found to be nonuniform over the entire range of moisture absorbed (0–50% by wt); at low ambient RH (∼10% by wt), the bulk $T_g$ of PVP is lowered by ∼90 K (to ∼337 K), whereas it requires considerably more moisture (∼30% by weight) to reduce the $T_g$ below RT. While ensemble measurements and theoretical models have been reasonably successful in describing moisture induced lowering of $T_g$ in hydrophilic polymers such as PVP, it is unclear how plasticization is initiated at low solvent contents. Moreover, it would be interesting to find out whether polymer segmental mobility (or primary relaxation) is operational at slow time scales even far below $T_g$ and probe how network cavities evolve as the effective $T_g$ of the partially hydrated polymer approaches RT.

We have earlier shown that individual Rhodamine 6G (Rh6G) tracer molecules exhibit diverse translational dynamics inside plasticized (rubbery) PVP thin films at RT (effective $T_g < RT$, ∼225–273 K). The large variation of SM diffusivities and anomalous probe-diffusion behaviors pointed out that the local microviscosity of the matrix is very nonuniform along spatial locations of the hydrated thin-film matrix. It was also observed that translational motion of SM tracers is initiated only above a threshold hydration level of the PVP film (beyond RH ∼55%, referred to here as RH$_{trans}$), indicative of adequate swelling of the polymer cavities where primary relaxation processes are operational near submillisecond time scales. Although SM diffusion has been utilized to extract distribution of certain physical properties (such as microviscosity) for PVP above $T_g$, the extent of spatiotemporal heterogeneity at low moisture contents of the matrix (far below and slightly above effective $T_g$) has not been explored as of yet.

SM rotational mobility experiments offer a direct way to interrogate the PVP network cavities below and close to the $T_g$, as it is known that reorientational dynamics of individual fluorescent probes are primarily governed by the size/shape of the polymer network cavities as well as relaxation processes operational therein. For instance, in the glassy state far below the $T_g$, SM probes (of ∼1 nm size) are expected to be entangled (or tightly wrapped) by the compact polymer network. In contrast, if the $T_g$ is lowered close to ambient temperature via solvent absorption, the network cavities (or mesh size) become slightly larger and relaxation processes occur at relatively faster time scales so as to allow for dipolar reorientation, the dynamics of which is dictated by the nature (size/shape) of the network cavities and the polymer segmental motion therein.

In this work, our aim is to understand how moisture induced plasticization is initiated in PVP films and investigate the extent of spatiotemporal heterogeneity as the matrix undergoes evolution in physical properties in terms of rigidity/swelling/rearrangement of network cavities as well as polymer segmental motion/relaxation processes. Control of ambient RH between 10 and 50% allowed us to interrogate the network at different hydration levels below the critical moisture content for the advent of probes’ translational motion (RH Trans). To understand the network behaviors during the initial stages of plasticization, we have studied the reorientation propensities of single Rh6G molecules sparsely embedded in a thin-film matrix at three regimes of relatively low ambient humidity. Analyses of SM angular trajectories at different moisture contents of the matrix provide insights into the diversity of rotational mobility, and thereby provide information on the spatiotemporal heterogeneity of the network behaviors from the glassy (far below $T_g$) through to the rubbery state (close to $T_g$) of PVP.

# MATERIALS AND METHODS

**Materials.** PVP (MW 40000, Sigma) and Rhodamine 6G (Radiant Dyes, Germany) were used as received. Glass coverslips (No. 1, Corning) were used as substrates to spin-cast the polymer from aqueous solution (using MilliQ water).

**Sample Preparation.** Thin film samples of PVP sparsely doped with Rh6G were prepared as described previously. To minimize interfacial effects and embed the probes within the polymer, a drop of PVP solution (2 mg/mL) was spin-coated over precleaned glass coverslips, followed by one drop of ∼300 pM solution of Rh6G premixed with PVP and eventually one more drop of PVP solution was spin-cast on top (each step at 2000 rpm for 1 min). For removal of residual solvent and relaxing the polymer film, the samples were annealed at ∼120 °C for 2 h under evacuated conditions. The thickness of the films was determined to be ∼200 ± 30 nm using both atomic force microscopy and ellipsometry. The humidity was regulated by controlling a flow of argon gas bubbled through water into the home-built enclosed chamber mounted on top on the sample stage (Figure 1a). The RH was monitored using a probe suspended ∼1 mm above the sample with an accuracy of ±2%. The PVP sample was exposed to a fixed ambient RH for 30 min before data collection, and the RH was kept constant (within ±2%) during data acquisition. A flow of argon also facilitated in maintenance of an inert atmosphere, which reduced photo-bleaching of Rh6G to some extent.

**SM Microscopy and Data Analysis.** SM imaging measurements were performed in a home-built epifluorescence setup (Figure 1b) based on an inverted microscope (Nikon TE2000), the details of which can be found elsewhere. In brief, the sample was illuminated (∼2 mW below objective) using a circularly polarized (using a λ/4 wave plate) laser line (532 nm DPSS) and the emission was collected through a dichroic mirror and an emission filter before imaging using in a cooled (∼−25 °C) interline CCD camera (DVC, 1412AM). Defocused imaging (exposure of 1 s) was performed by moving the objective by ∼1 μm toward the sample, and the three-dimensional (3D) orientations of individual dipoles could be extracted by comparison of the obtained non-Gaussian
I observed defocused image patterns. However, high acquisition could also be inferred from the shapes of the emission signal into two mutually orthogonal components (s- and p-). The relative position of the CCD camera was chosen for analyses. Polarization-resolved movies provide dynamic information on the in-plane orientation (φ) of SM dipoles as a function of acquisition time, as calculated from the reduced linear dichroism (LD): 

\[ LD(t) = \frac{I_s(t) - I_p(t)}{I_s(t) + I_p(t)} = \cos 2\phi(t). \]

Here, φ is the azimuthal angle subtend by the projection of the dipole in the sample plane. Emission spots with total intensity \( I_s(t) = I_p(t) + I_p(t) \) less than 10 cts/pix/100 ms were excluded for further analysis to avoid artifacts in estimation of LD(t). Frequency distributions of LD, \( P(LD(t)) \), and that of absolute angular velocities \( \omega_l(t) \), \( P(\omega_l(t)) = P(\omega(t)/dt) \) at each ambient RH were generated for individual SMs (at 0.2 and 2° bins, respectively) which did not photobleach for 5 s, whereas the ensemble \( P(LD(t)) \) and \( P(\omega_l(t)) \) at each ambient RH were calculated for ~50 different SMs at various spatial locations over their entire time-trajectories. The ensemble \( P(\omega_l(t)) \) (plotted in semilogarithmic scale, 4° bins) were fit to Gaussian functions, whereas the wings (tails) of the distributions were fit to single exponential decay. Because of the limited number of data points (typically 50–200) obtained for each SM before photobleaching, as well as the nonuniform rotational mobility for each SM in different acquisition time-windows, autocorrelation functions for LD(t) were unreliable to extract rotational correlation times and therefore were not calculated. Mean-square angular displacement against lag-time for most SMs exhibited saturation behaviors and was unreliable for extracting long-time angular diffusivities.

## RESULTS AND DISCUSSIONS

**Probe Reorientational Propensity at Different Hydration Levels of PVP.** Figure 2 shows representative defocused images for Rh6G in PVP thin films under three different ambient RH, ranging from very dry conditions (RH ~ 1%) to a hydration level (RH of ~50%), which is slightly below that for the limit for onset of SM translational diffusion (RH \( \geq 55% \)). Under dehydrated conditions (Figure 2a), an overwhelming majority of SMs are found to exhibit nonsymmetric (or bilobal) emission patterns, the long axis of which (various SMs) are isotropically oriented along the xy plane, characteristic of immobilized dipoles randomly distributed in 3D. With a slight increase of the ambient RH to ~30% (Figure 2b), a lesser proportion of SMs exhibit bilobal patterns, whereas the remaining molecules display more symmetric, quasicircular emission which arise due to reorientation of dipoles faster than the data acquisition time scale (1 s). At an ambient RH of ~50%, the occurrence of bilobal emission is remarkably reduced, suggesting that the rotational mobility of SMs is augmented significantly.

Whereas defocused imaging allows for determination of 3D orientations of single dipoles embedded in a rigid matrix, high excitation powers and exposure times are necessary to generate high-quality images required for comparison with simulations. Thus, SM imaging was performed using a polarized beam splitter, which enabled simultaneous detection of orthogonally polarized emission from individual molecules in the same FOV at relatively faster time scale. Figure 2d–f shows polarization-resolved images via the two detection channels (designated s- and p-) under dry conditions (Figure 2d) of the matrix and in the presence of moisture (Figure 2e–f). The time-average emission intensities \( I_s(t) \) and \( I_p(t) \) of ~25 arbitrarily chosen Rh6G probes (at each RH) obtained from the two
detection channels are plotted in Figure 2g−i. At an RH of ∼10% (Figure 2g), most of the emission spots are prominent in either the s- or p-channel due to markedly different $I_s$ and $I_p$, and 90° rotation of the analyzer results in switching of the SM emission intensities between the s- and p-channels (data not shown). Such behaviors are noticed up to an RH of ∼20%, indicating that a negligible fraction of probes are able to reorient over tens of seconds. With a slight increase in ambient RH to 30% (Figure 2e,h), a significant proportion (∼30%) of Rh6G molecules have comparable $I_s$ and $I_p$, which suggest that rotational mobility is initiated between an RH of 20 and 30%. On the contrary, at an RH of 50% (Figure 2f,i), more than ∼80% of SMs exhibit comparable $I_s$ and $I_p$, implying a dramatic increase in the population of rotationally labile Rh6G molecules.

Although SM imaging (Figure 2) provides a qualitative idea on the overall rigidity of the polymer network, and, to some extent, the spatial variability at different stages of plasticization, information on dynamic fluctuations of the polymer network could be obtained by investigating rotational dynamics of SMs at a higher temporal resolution. As a wide variety of reorientational propensity was observed at intermediate and moderately high moisture contents of the PVP matrix (vide infra), the behaviors observed for the majority subset of SMs at each RH level is presented here, exemplified using reorientational dynamics of nine characteristic SMs (designated M1−M9) which lasted 15−35 s. Figure 3 shows the polarization-resolved intensity traces of representative Rh6G molecules which encompass the dynamic behaviors of the majority of probes in the RH range of 10−50%. Up to an RH of ∼20%, orthogonally polarized SM emission intensities ($I_s(t)$ and $I_p(t)$) are typically dissimilar (Figure 3a−c) as expected of fixed dipolar orientations. However, with slight increase of ambient RH by ∼10%, a wide range of rotational dynamics can be noticed. Whereas about a quarter of the SMs are found to...
remains rotationally immobile, a larger population ($\sim$45%) exhibit anticorrelated switching of $I_1(t)$ and $I_p(t)$ (Figure 3d–f) for considerable time periods. Furthermore, the rotational mobility for the remaining population of SMs (at an RH of $\sim$30%) is remarkably augmented, similar to that observed at an RH of $\sim$50% (Figure 3g–i), where correlated fluctuation of $I_1(t)$ and $I_p(t)$ occur during the majority of the acquisition time, and only at certain intermittent time windows (spanning 0.1–0.3 s), are anticorrelated emission intensity fluctuations observed.

Dual-polarization SM traces (Figure 3) can often be challenging to interpret in terms of molecular reorientation, as $I_1(t)$ and $I_p(t)$ are not only a function of the in-plane projection of the transition dipole, but also depend on the out of plane motion, as well as laser power fluctuations. Therefore, the reduced LD was computed for each polarization-resolved SM trajectory, the temporal evolution of which is used to extract in-plane angular displacements ($\phi(t)$) as well as azimuthal angular velocities ($\omega(t)$). Figure 4 depicts the temporal evolution of LD($t$) and $\omega(t)$ for the nine SMs shown in Figure 3. The P(LD($t$)) and P($\omega(t)$) at various RHs, over entire trajectories for individual SMs (M1–M9) are presented in Figure 5a–f, along with the ensemble distributions of LD($t$) and $\omega(t)$ constructed from the trajectories of many spatially segregated individual Rh6G probes (Figure 5g–l).

SM Rotational Dynamics during Various Stages of Plasticization. PVP Films at Very Low Moisture Contents (up to an RH of 20%). Under relatively dry conditions of the film, LD($t$) for individual Rh6G probes is usually found to fluctuate slightly around a fixed non-zero value (Figure 4a–f). For the majority of SMs, the standard deviations ($\sigma$) obtained from $P(LD(t))$ (Figure 5a) are typically in the range of 0.10–0.14, which is slightly higher than the minimum measurable deviations ($\sigma_{min}$ $\sim$ 0.08) in LD($t$) obtained for a few SMs (with comparable $I_L$ and photobleaching time). Considering that $\sigma_{min}$ is the inherent noise in LD($t$) arising from completely (rotational) immobile dipoles, it is reasonable to infer that in the presence of low moisture contents (an RH up to $\sim$20%), the polymer network cavities provide just enough space for Rh6G molecules to undergo a low amplitude (within 20°) wobbling/wiggle type motion, while restricting whole molecule reorientation (or tumbling) to occur. This is likely to be a consequence of subnanometer local fluctuations in the polymer network which involve small conformational changes of the pyrrolidone side chain, such as a twisting motion around the C–N bond (Figure 6a). However, it is difficult to comment on whether the observed wobbling motion of SMs reflect slow components of secondary ($\beta$) relaxation processes, which are known to occur at fast time scales (>1 kHz) deep within the glassy state. Nonetheless, it is clear that the PVP network is highly inelastic with non-spherical compact cavities (or a small mesh size) where large amplitude polymer motion is almost completely arrested, as expected for a polymer matrix far below the $T_g$.

PVP Films at Intermediate Moisture Contents (an RH of 30%). With an increase in ambient RH to $\sim$30%, SMs exhibit a variety of rotational dynamics ranging from immobility ($\sim$25%) to slow hindered rotation ($\sim$50%) to relatively more facile reorientation ($\sim$25%). Figure 4g–i shows LD($t$) traces for three SMs (Figure 3d–f, M4–M6), which typify the majority

---

Figure 3. Representative time-traces of polarization-resolved SM emission intensities ($I_1$ and $I_p$) for three single representative Rh6G molecules in a PVP matrix at three different moisture contents, symbolically shaded green (RH $\sim$ 10%), yellow (RH $\sim$ 30%), and cyan (RH $\sim$ 50%). Red and green traces refer to intensity in “$s$” and “$p$” detection channels, respectively. These nine SMs (M1–M9) have been used to depict the dominant (dynamical) behaviors of Rh6G probes embedded in a PVP matrix under the three different moisture contents of the polymer film, although statistical analysis was performed on 100 SMs in each ambient RH.
subset of probe behaviors. It is evident that Rh6G molecules can undergo reorientation over $50° - 60°$ (Figure 6b), with varying periodicity ranging between $\sim 0.1$ and $5$ Hz. Closer inspection of LD$(t)$ (Figure 4g–i) reveals that the manner in which SMs reorient in the PVP network is also quite diverse. For instance, large dipolar reorientation ($>30°$) can occur via cumulative angular increments over seconds (M5 $2.5 - 12.5$ s) and M6 ($0 - 5$ s), or large angular changes occur within a few hundred milliseconds (M4, Figure 4g), and may involve intermittent pauses (up to seconds) before subsequent angular jumps (M6: $12.5 - 25$ s, Figure 4i). Furthermore, the rotational dynamics of any SM probe is most often nonuniform at different acquisition time windows, as evidenced from angular velocities (Figure 4j–l), which visibly change with time. In fact, a particular Rh6G molecule generally features all the above mentioned behaviors, and rarely do we find probes which exclusively display certain distinctive dynamics. Although the wide range of mobility observed for different probes suggests that rotational correlation times can span over a few decades for various SMs, the observed temporally irregular, hindered tumbling of probes make it extremely challenging to extract reliable values (or even assign) a characteristic rotational time scale. Similar spatiotemporal nonuniformity in probe reorientation dynamics at comparable time scales ($\sim 0.1 - 10$ Hz) has been reported for SMs incorporated in polymer matrices in the glassy state, and such diverse SM reorientational dynamics in polymer film matrices have been ascribed to be an indicator of spatiotemporal heterogeneities in network cavities.$^9,10,42-45$

It is interesting to note that the nature of both time-averaged SM P(LD$(t)$) and P$(\phi(t))$ (Figure 5b,e) are markedly different (typically wider) as compared to that under dry conditions (Figure 5a,d). Interestingly, we find that P(LD), (and therefore, P$(\phi)$, the time-averaged distribution of SM in-plane angles) is most often unevenly distributed (typically with a maxima). Furthermore, for a lesser fraction (10–15%) of probes P(LD) is clearly bimodal (M6 in Figure 5b), similar to that reported for SM probes in PMA slightly above $T_g$.$^9$ This is a consequence of temporal nonuniformity in preference of certain dipolar orientations within a network cavity, that is, all in-plane orientations cannot be accessed with equal ease and there is an uneven distribution of residence times, dictated by the shape/size and flexibility of the polymer void. Figure 5b,c essentially points out that the irregular (nonspherical) shape of polymer voids can be effectively mapped using angularly restricted SM reorientation propensities.

Figure 4. Temporal evolution of LD$(t)$ and (azimuthal) angular velocities $(\omega(t))$ for the nine SMs (M1–M9) shown in Figure 3, at the three different moisture contents of the PVP film network. The color codes of the three SM trajectories in each ambient RH are based on photobleaching times (or trajectory length), blue (short lived), green (intermediate lived), and red (long lived), exemplifying the range of behaviors exhibited by single Rh6G probes.
We attribute the hindered whole-molecule reorientation (or tumbling) of SMs in seconds time scale to structural reorganization of the polymer cavity in subnanometer length scales, likely to arise from torsional movement involving the pendant pyrrolidone groups (Figure 6b). It is conceivable that the onset of such slow hindered tumbling mark the very initial phase of plasticization, that is, \( T_g \) being lowered to the extent where the slowest components of \( \beta \)-relaxation processes, often referred to as Johari–Goldstein (JG) relaxation, become active. JG relaxation processes, associated with local movements of the polymer backbone or side chain rearrangements, act as precursors to primary (\( \alpha \)) relaxation processes involving larger (\( \sim \)nm) cooperative main chain movements.

Previous studies on SM rotational dynamics in various polymer thin films, performed at temperatures slightly below and up to \( T_g \) reveal that slow time scale (hindered) large-angle reorientation of probes are initiated only above \( \sim 0.85 T_g \) (K), and probe reorientation is expected to be very slow (\( \sim 0.1 \) Hz or less) at a few tens of degrees below the \( T_g \). If these estimates are applicable for the partially hydrated PVP thin-film (plasticized network), given the observed time scale of probe reorientation to be \( \sim 1 \) Hz, it can be inferred that at a RH of \( \sim 30\% \), the effective \( T_g \) of the polymer matrix is dramatically reduced to the range of 330–340 K (from \( >400 \) K). Our ballpark estimate on the drastic lowering of \( T_g \) within such a slight increment of moisture absorbed is somewhat consistent with the increasing dominance of JG relaxation processes at \( \sim T_g - 50 \) K for PVP, and comparable to that reported for thicker (drop cast) PVP films (\( T_g \sim 340 \) K) and powdered samples (\( T_g \sim 350 \) K) at an ambient RH of 30%.

**PVP Films at a Relatively High Moisture Content (an RH of 50%).** With further increasing moisture absorption of the PVP film to \( \sim 50\% \), there is a dramatic reduction in the population of SMs that exhibit either slow dipole reorientation or remain immobile. LD(\( t \)) trajectories (Figure 4m,o,q) reveal that slightly below RH\( Trans \) the rotational dynamics of Rh6G molecules is significantly augmented but is not completely uninhibited. Although for most SMs, the mean value of P(LD(\( t \)) is close to zero as expected of fast reorientation (Figure 5c), the fluctuation (\( \sigma > 0.2 \)) in LD(\( t \)) is substantially

![Figure 5. Distribution of LD(\( t \)) and azimuthal angular velocity (\( \omega(\tau) \)) for each of the nine SM trajectories under various moisture contents of PVP films](image-url)
larger than that observed under dry conditions (Figure 5a) of the network. Such fluctuations are attributed to the time-averaged signal arising from molecular rotation being faster than the time scale of data acquisition (10 Hz). However, the observed LD(t) (Figure 4m,o,q) as well as the P(LD) (Figure 5c) cannot arise from probes rotating significantly faster (>100 Hz) than the time scale of measurement, as that would have led to a zero-centered P(LD) with very narrow widths (σ well within 0.2).11 Whereas very often large (>20°) angular changes are found to occur within few hundreds of a millisecond, the majority of the probes exhibit intermittently hindered rotation, that is, dipoles become momentarily frozen (M8: ∼1 kHz) primary relaxation processes (although may not indicate the actual time scales of relaxation).6,9,27 For moisture induced plasticization of PVP thin films, we find that not far below RH$_{trans}$ probe reorientation time scales can often be tens of milliseconds (∼20–100 Hz). This may come as somewhat of a surprise because a slight (5%) increase in the RH can potentially allow SM tracers to be translationally active, which is likely to be an outcome of much faster (∼1 kHz) primary relaxation processes achieved only when the matrix is well within the rubbery state (∼1.1T$.g$). We note, however, that at an RH$_{trans}$ of 55%, a considerable fraction (60%) of SMs remain translationally static due to spatial heterogeneity in the $T_g$ of the plasticized PVP film, and the vast majority (80%) of probes can undergo translational diffusion only near an RH of 60%.17 It is therefore possible that with an increase in RH of ∼10%, the vast majority of the PVP matrix achieves low enough $T_g$ at RT for translational motion to be initiated. This idea is consistent with previous reports that even a 10 K increment above $T_g$ can augment α-relaxation time scales in certain polymers by a few orders of magnitude which can result in an onset of tracer translation.5,6

The observed SM rotational dynamics at an RH of 50% is a consequence of further lowering the effective $T_g$ and the increasing dominance of primary (α) relaxation processes that involve cooperative segmental motion of the polymer backbone as well as larger amplitude side chain movements. It is interesting to note that the time scales for α-relaxation processes in several other polymer matrices is known to increase by 2–3 orders of magnitude from slightly below the $T_g$ to ∼1.1T$.g$ (51,52) Moreover, for some low $T_g$ amorphous polymers such as PMA, the time scales of α-relaxation (obtained from SM studies) are expected to vary over one to two decades in the range of $T_g$ + 5° to $T_g$ + 15°, and the local fluctuations in the polymer become faster than ∼100 Hz.6,8 While these estimates have been measured in the absence of any absorbents (and may also be polymer specific), SM reorientational time scales related to the local dynamics of the polymer network are expected to scale with the primary relaxation processes (although may not indicate the actual time scales of relaxation).50,51 For moisture induced plasticization of PVP thin films, we find that not far below RH$_{trans}$ probe reorientation time scales can often be tens of milliseconds (∼20–100 Hz). This may come as somewhat of a surprise because a slight (5%) increase in the RH can potentially allow SM tracers to be translationally active, which is likely to be an outcome of much faster (∼1 kHz) primary relaxation processes achieved only when the matrix is well within the rubbery state (∼1.1T$.g$). We note, however, that at an RH$_{trans}$ of 55%, a considerable fraction (60%) of SMs remain translationally static due to spatial heterogeneity in the $T_g$ of the plasticized PVP film, and the vast majority (80%) of probes can undergo translational diffusion only near an RH of 60%.17 It is therefore possible that with an increase in RH of ∼10%, the vast majority of the PVP matrix achieves low enough $T_g$ at RT for translational motion to be initiated. This idea is consistent with previous reports that even a 10 K increment above $T_g$ can augment α-relaxation time scales in certain polymers by a few orders of magnitude which can result in an onset of tracer translation.5,6

It should be noted that for bulk PVP of similar MW, the $T_g$ is lowered only to ∼320 K at an RH of 50%,19,20 and thicker (drop cast) PVP films (or powder) require considerably more moisture (RH ∼70%) to undergo a glassy-to-rubbery transition at RT (295 K).19,24 In contrast, based on prior SM data in various polymer thin films,5,6,47,48 our results suggest that even at an RH of ∼50%, partially hydrated PVP thin films are already plasticized at RT, implying that the extent of moisture induced $T_g$-lowering is enhanced (down to ∼270–290 K) in films as compared to that for the bulk polymer.19,20 Although an
intriguing possibility, it is unlikely that polymer films of a few hundred nanometers thickness will show significant interfacial effects, and are not expected to behave much differently as compared to the bulk polymer material.\textsuperscript{33–35} On the other hand, it is possible that when ambient RH is changed from 20 to 50\%, the partially hydrated polymer matrix still remains in the glassy state, but SM probes can still undergo fast reorientation very close to (below) \( T_g \). Although a bit speculative, this proposition is not totally unfounded either; it has been suggested earlier that molecular mobility of PVP in the presence of moisture can be significant even at 30 K below its bulk \( T_g \).\textsuperscript{26} Besides, SM probes at RT can not only undergo reorientation in glassy poly(methyl methacrylate) (PMMA) or polystyrene (PS) (\( T_g \sim 370 \) K for both),\textsuperscript{32,43,56} but have also been shown to undergo slow translational motion in poly(vinyl butyral) (PVB) thin films (\( T_g \sim 340 \) K) below \( T_g \).\textsuperscript{53,56,57} Furthermore, ensemble electroabsorption measurements on solvatochromic dyes embedded in PMMA matrices have provided evidence that residual (trapped) solvents can allow for probe reorientation to occur even at 70 K below the \( T_g \) and dyes dispersed within polycrylamide (Nylon) fibers (\( T_g \sim 330 \) K) can undergo slow translational mobility at RT, which suggest that primary relaxation processes can be operational at relatively fast time scales well within the glassy state (\( T_g \sim 30 \) K).\textsuperscript{99}

**Heterogeneity at Different Stages of Plasticization.** The diversity in rotational mobility of individual molecules (Figure 4) as well as their frequency distributions (Figure 5a–f) serve as a pointer to the extent of spatiotemporal heterogeneity in the PVP network (at any given RH) in terms of rigidity of nanoscale cavities and relaxation processes operative therein. Under very low moisture contents of the PVP matrix (up to \(~20\%\) RH), we find that both \( P(LD(t)) \) and \( P(o(t)) \) are quite narrow (Figure 5a,d), which indicates that the physical properties of the network cavities are in fact, not dissimilar along spatial dimensions. In contrast, at intermediate to relatively high moisture contents (\(~10–20\%\) by wt, RH 20–50\%), there are rather large differences in the shape of both \( P(LD(t)) \) and \( P(o(t)) \) of individual probes (Figure 5b,c,e,f). This is a consequence of diverse rotational behaviors over space and time, ranging from near immobility (wobbling) to hindered whole molecule reorientation (or tumbling) to nearly facile (intermittently hindered) rotation, the proportions of which systematically change with increasing RH (or decreasing \( T_g \)).

The frequency distributions of \( LD(t) \) and \( o(t) \), obtained by analyzing dynamical data from individual molecules and collating such information over many SMs is depicted in Figure 5g–l. At very low moisture contents (up to \(~20\%\)), the overall \( P(LD) \) is bimodal (Figure 5g), as expected from isotropically oriented dipoles immobilized in a highly rigid environment,\textsuperscript{14,60} indicating that the entire network is in a glassy state, with \( T_g \) being significantly above RT (at least 60–80 K). In the presence of low moisture contents (Figure 5h), \( P(LD) \) remains bimodal, but the fraction of near zero events are substantially higher, pointing out just enough swelling of the PVP network to allow for probe reorientation. We note that despite the qualitative similarity in the shape of \( P(LD) \) under dry conditions and at an RH of 30\% (Figure 5g,h), the former arise from various SMs oriented at fixed random orientations, whereas the latter is a consequence of the diverse reorientational propensity of SMs at different spatial locations, in conjunction with the temporal variability in angular diffusivity of each SM probe. At higher moisture contents of the polymer matrix (Figure 5i), the shape of \( P(LD) \) becomes unimodal (zero centered), which is expected for rotationally labile probes.\textsuperscript{44,49} However, the relatively large width and extended tails for the ensemble \( P(LD) \) (Figure 5i) indicates that even at an RH of 50\%, the fraction of time for which Rh6G probes remain rotationally immobile is not negligible.

So, it is evident that (1) the initiation of plasticization can occur only beyond a certain moisture content (\(~20\%\) RH) of PVP, and that the softening/swelling of network cavities is highly nonuniform along various spatial locations, (2) the partially hydrated PVP matrix is spatiotemporally very inhomogeneous at intermediate moisture contents (RH 30–50\%), and (3) the extent of such heterogeneity decreases with the increase in moisture content of the matrix, but does not disappear even for plasticized (rubbery) PVP films. While it is perhaps not a surprise to observe spatial heterogeneity near the glassy to rubbery transition,\textsuperscript{62,64} it is possible that islands of high-density (low energy) and low-density (high energy) exist even in the glassy state. At low amounts of moisture, water molecules can localize in specific high-affinity binding sites (possibly the low-density areas) and make these local domains more rigid via hydrogen bond formation, sometimes referred to as antiplasticization.\textsuperscript{62–64} With a further increase in the hydration levels, various local regions of the matrix exhibit higher mobility (to different extents) due to differential amounts of water molecules present therein (Figure 6d–f), which can result in observed diversity in probe reorientation.

**Nature of SM Probe Reorientation.** An interesting anecdote is on the qualitative change in shapes of \( P(o(t)) \), the rotational analogue of the van Hove self-correlation function,\textsuperscript{65,66} for an ensemble of SMs under dry conditions of the matrix and in the presence of moisture (Figure 5j–l). When the cavities are compact, which only allows for “wobbling” of SMs (\( \Delta \phi \) within \(~15\%) \), \( P(o(t)) \) nearly follows a Gaussian distribution (dashed line, Figure 5j). In contrast, for the slightly swollen PVP network, \( P(o(t)) \) deviates from being Gaussian (Figure 5k,l). Although the \( P(o(t)) \) (at an RH of 30 and 50\%) could be nicely fit to a Gaussian for small \( \Delta \phi \) (up to \(~12\%/0.1\) s), both these distributions have prominent exponential tails (solid lines, Figure 5k,l). This nature of \( P(o(t)) \) for the partially hydrated polymer matrix is quite intriguing, and is reminiscent of non-Gaussian displacement jump distributions for translationally mobile SMs in hydrated PVP films and hydrogels,\textsuperscript{68} as well as for tracers navigating in cytoskeletal environments\textsuperscript{53} and colloidal supercooled fluids.\textsuperscript{17,62,67,68} In accord with literature on tracer (translational) diffusion dynamics in heterogeneous media,\textsuperscript{62,69,70} it is possible that the non-Gaussian angular jump distributions (Figure 5k–l) can arise due to slow time-varying environmental fluctuations in the partially hydrated PVP network cavities, which in effect can give rise to a (dynamic) distribution of SM angular diffusivities. This is consistent with recent theoretical models as well as simulations on translationally active tracers in time-fluctuating disordered environments,\textsuperscript{70–73} where a spatiotemporal distribution of diffusivity has been invoked to explain anomalous diffusion. Although it is apparent that the van Hove correlation function for translationally diffusive tracers can very often be non-Gaussian in heterogeneous media,\textsuperscript{32,66–71,73} there is no prior experimental evidence on angular jump distributions for single dipoles to behave in a similar fashion. Recently, to understand the local pore structure in heterogeneous media, the rotational diffusion dynamics of spatially segregated dumbbells have been investigated using simulations, near the
pore percolation transition. Here, non-Gaussian angular displacement distributions (with exponential tails) have been observed for an ensemble of dumbbells (similar to our data), while for individual molecules, the \( P(o(t)) \) remains Gaussian (with different rotational diffusivities). It is important to note that these simulations were conducted over more than three decades in time, which is extremely challenging to emulate in SM measurements due to limited survival times of organic fluorophores, and lack of long-time SM (angular) trajectories can also lead to erroneous inferences in terms of the deviation of \( P(o(t)) \) from being Gaussian. At this juncture, due to the unavailability of statistically relevant SM \( P(o(t)) \)s (Figure 5e–f), we refrain from concluding whether the observed shape of ensemble \( P(o(t)) \) (Figure 5k–l) arise from spatial heterogeneity in angular diffusivities for various SMs or is a consequence of slow time-varying environmental fluctuations in network cavities for each SM \( ^{62,69} \) or whether both mechanisms are operational simultaneously. Efforts are currently on the way to extract long-time SM rotational mobility data in partially hydrated PVP networks to elucidate the origin of non-Gaussian van Hove angular correlation function (Figure 5k,l).

**SUMMARY**

We have investigated the rotational mobility of individual fluorescent probe molecules at different ambient humidities to understand the extents of heterogeneity during moisture induced plasticization of PVP thin films at RT. Under dry to very low moisture contents of the matrix (RH \( \sim 0–20\% \)), SMs are only found to undergo a small-angle wobbling motion and whole molecule reorientation is almost completely restricted, indicative of the extremely rigid and compact network cavities expected of the PVP matrix deep within the glassy state. Upon a slight increase in the moisture content (RH \( \sim 30\% \)) of the matrix, the slow tumbling motion of SMs is initiated suggesting slight swelling of the cavities. Interestingly, the vast majority of probes are found to undergo hindered whole-molecule reorientation, often restricted within a certain angular range dictated by the shape of the local network cavity where they are embedded. Such slow hindered tumbling motion is attributed to local (subnanometer) structural reorganization of polymer voids, likely related to the torsional motion of the pyrrolidone side chains, suggesting that the slowest component of the secondary relaxation processes likely become operational even at quite low moisture contents (in the glassy state). Whether the slow, intermittently hindered, restricted large-angle reorientation of probes report for some lower-order transition (such as ductile to brittle transition) \(^{65} \) remains to be seen. Interestingly, at low to intermediate moisture contents (RH \( \sim 30\% \)), we find a very wide variety of probe reorientation behaviors for SMs in different spatial locations as well as for individual probes over different observational time-windows, demonstrating extensive spatio-temporal heterogeneity of the partially hydrated network in the glassy state. Although SM rotational dynamics is considerably faster for a more hydrated PVP matrix (RH \( \sim 50\% \)), the majority of probes exhibit transient pauses during reorientation and their angular velocities fluctuate considerably over time. This implies that spatio-temporal heterogeneity exists well within the rubbery state, very close to network hydration levels that allow for translational mobility of SM probes, where primary relaxation processes are operational at very fast time scales. Such insights into the partially hydrated polymer network behaviors and how moisture induced plasticization is initiated for high-\( T_g \) polymers such as PVP may be useful in understanding navigation mechanisms and slow release of drug molecules embedded in polymer gel networks.

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: arindam@chem.iitb.ac.in.*

**ORCID**

Arindam Chowdhury: 0000-0001-8178-1061

**Present Address**

1Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K. (S.D.).

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

S.B., D.K.S., and S.D. thank CSIR (India) for Ph.D. scholarships. We acknowledge IRCC, IIT Bombay, for the initial funding, and the Departments of Physics, Chemistry, and Chemical Engineering for the usage of central facility instruments. We thank Rajarshi Chakrabarti, Anirban Sain, Amitabha Nandi, and K.L. Sebastian for valuable discussions, Mohan Srinivasaarao and George Zograf for critical comments, and Naresh Patwari for providing Rh6G.

**REFERENCES**

(59) Srinivasarao, M. Georgia University of Technology, Atlanta, GA, Personal communication, 2015.