

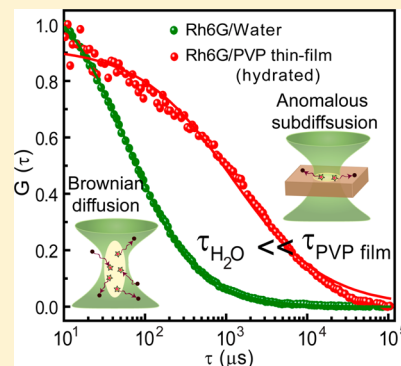
Probing Differential Hydration of Poly(vinylpyrrolidone) Thin Films Using Tracer Mobility: An Insight from Fluorescence Correlation Spectroscopy

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S Supporting Information

ABSTRACT: Dynamics of small probe molecules have been routinely used to unravel the intrinsic details of charged ion transport in polymer brushes and polyelectrolyte multilayer (PEM) thin films. However, corresponding morphological properties affected with absorption of moisture have been hardly dealt with despite numerous applications of isotropic thin films in material chemistry and medical purposes. We have explored the overall structural changes associated with plasticization of PVP thin films by probing dynamics of small reporter (rhodamine 6G, Rh6G) molecules using fluorescence correlation spectroscopy (FCS). It was observed that under lesser amounts of absorbed moisture, the rigidity of the film matrix was high enough to inhibit appreciable molecular mobility. Nonetheless, with gradual increase in the moisture level within the film, molecular movement became extremely facile, so much so that it almost attained close to a solution like state. Molecular mobility was found to be dependent on both the method of preparation and the thickness of the thin films. The diffusivities mostly followed anomalous subdiffusive behaviors, reminiscent of dynamics of tracers in crowded cellular environments. The mobility was found to be independent of any electrostatic interaction between probe and polymer thin film. Hence, the tracer dynamics was attributed most likely to the viscoelasticity of the thin film matrix.



1. INTRODUCTION

Polymer thin films have been known to embrace a wide variety of applications in various fields ranging from material sciences to medicines since long ago.^{1–3} They are regularly used in the manufacture of coatings, paints, semiconductor devices, medical equipment, and construction materials. Polymer thin films are known to exhibit high degrees of spatial and dynamical inhomogeneity by virtue of their nonexponential and non-identical structural relaxation processes.^{4–6} Below a certain thickness range, they often exhibit physical properties viz. phase separation temperature, elasticity, viscosity, and so forth that differ substantially from intrinsic bulk behavior.^{7–9} Single molecule spectroscopy (SMS) is known to be highly proficient in exploration of heterogeneity in such systems by virtue of its competence to probe the fluctuations in local nanoscopic domains around individual molecules.^{2,10} Thus, polymer thin films serve as an excellent substrate to be looked upon from the viewpoint of SM-based detection.

Among the other SM techniques, fluorescence correlation spectroscopy (FCS) has been routinely used in the fields of biochemistry, biotechnology, and biomedical research to measure cellular events in signaling pathways, cell division, freely diffusing molecular events in vitro and in vivo, molecular interaction, and chemical kinetics since its first development by Elson and Webb.^{11–23} However, its usage is relatively limited in the fields of chemistry and chemical interactions to investigate mobility of molecules immobilized in soft materials. In the recent past, a number of studies had been carried out for the

investigation of diffusion dynamics of fluorophores on and inside polymer thin film (polyelectrolyte) brushes and polyelectrolyte multilayer (PEM) films using both SM polarization microscopy and FCS.^{24–30} Polyelectrolyte brushes are films that essentially are tethered (grafted) from one end to a substrate by certain electrostatic or mechanical forces.^{31,32} On the other hand, PEMs are formed using layer-by-layer (LbL) deposition of alternating layers of polycations/polyanions generally over a charged substrate.³³ It is well-known that the physicochemical properties of polyelectrolyte brushes and PEMs are in stark contrast to those of polymer thin films.^{31,32,34} The driving force for diffusion of small molecules within both polyelectrolyte brushes and PEMs is the inherent charge present in them. As a result, strong Coulombic (electrostatic) forces of interaction are experienced by molecules diffusing within polyelectrolyte brushes and PEMs. Nevertheless, there are very few reports on the dynamic behaviors of molecular probes within inorganic/organic polymer thin films, devoid of any inherent charge.³⁵ Detailed investigation of such thin film properties could thus help us envisage the nanoscopic inhomogeneities present therein with respect to bulk.

In a recent study, we have reported translational diffusion of SM probes inside poly(vinylpyrrolidone) (PVP) thin films by

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single molecule fluorescence microscopy (SMFM) by varying the amount of moisture absorbed by the film matrix.³⁶ PVP (amorphous) is often used as coatings to pharmaceuticals for controlled drug delivery due to its high solubility in water and biocompatibility as a consequence of considerable amount of moisture adsorption from the atmosphere (up to ~40% of its weight).^{37–39} It is known that on increasing ambient humidity (RH) in the surrounding environment, the extent of water uptake by the film increases and thereby leads to a dramatic lowering of the glass transition temperature (T_g) of the thin film and thus serves as a model system to study plasticization.^{40–42} Molecular reorientations were observed (rotational mobility) up to a RH of 50%, and beyond a critical (threshold) RH of ~55%, molecules were shown to become translationally active. SM diffusivities could be reported for an ambient humidity level up to 75%, beyond which quantification for translational mobility could not be done accurately using SM tracking (SMT). Under such RH levels, molecules were found to undergo non-Brownian (anomalous) subdiffusion predominantly, resembling mobility of tracers in crowded and confined molecular environments in cells and cytoplasmic systems.^{43–45} We attributed such a type of subdiffusion to arise due to the viscoelasticity of the PVP thin film network. Nonetheless, at this point it is necessary to note that SMT imposed a restriction on molecular mobility to a RH level of 75%, beyond which the molecules underwent exceedingly rapid motion, unable to be tracked by SMT. Therefore, at this juncture it was extremely important to account for the changes taking place inside polymer thin films undergoing plasticization by probing the tracer translational dynamics even for high ambient humidity levels.

Here, we report the differential rates of diffusion of SMs inside polymer thin film matrix with change in the RH from a relatively low (~70%) to very high level (>95%) by utilizing FCS. We found that a gradual increase in molecular diffusivity is attained with increase in the ambient moisture absorbed by the film. With exceedingly high moisture contents (saturation humidity), molecules are rendered to move tremendously fast within the thin film and attain a state much in resemblance to that in a bulk solution. We found that tracers exhibit predominantly subdiffusive behavior up to moderate RH regime, until a state with significantly higher levels of absorbed moisture, wherein Brownian motion takes over anomalous subdiffusion irrespective of the procedure employed for preparation of the thin film. Furthermore, the extent of molecular mobility was found to be greatly affected with changes in the film thickness and was observed to be inversely related to the thickness of the film. It was shown that molecular subdiffusion arises most likely due to viscoelasticity of the PVP matrix and is independent of any electrostatic interaction between polymer and probe molecules unlike in the case of charged polymer brushes and PEMs. Using FCS such a description of subdiffusion in isotropic polymer thin films is probably the first of its kind to extract the intrinsic details of dynamics of individual molecules during the course of plasticization on varying the RH from a relatively low to very high regimes.

2. EXPERIMENTAL SECTION

2.1. Materials. Poly(vinylpyrrolidone) (MW 10 000 and 40 000, Sigma-Aldrich) was used as received. Rhodamine 6G (Radiant Dyes, Germany) was used as the fluorescent probe and No. 1 glass coverslips (Corning, U.S.A.) were used as the

substrate to deposit the polymer. Alexa Fluor-555 (Alexa-555, Molecular Probes/Invitrogen, U.S.A.) and perylenebisimide polyether (PPE, synthesized by Dr. Ping Yan at Columbia University, U.S.A.) were used as other probes (structures shown in Supporting Information in Section S1) for comparison of tracer diffusivities. Milli Q water was used as the solvent for both polymer and probe molecule. Electronic grade ethylene glycol (EG, Sigma-Aldrich) and glycerol (G, Sigma-Aldrich) were used as received to perform control experiments.

2.2. Sample Preparation. Samples were cast on precleaned glass coverslips (sonicated in Piranha solution and 2 M NaOH, followed by several washings with Milli Q water and methanol). The coverslips were dried by blowing N_2 gas, followed by burning in an oxidizing flame to remove any residual fluorescent impurity. FCS experiments were carried out in three different types of samples. First, we carried out experiments on solutions of polymer premixed with dye in Milli Q water to determine the diffusivity of probes in bulk solution. The concentration of the dye was maintained at ~200 pM to ensure near SM sensitivity. Second, sandwich type thin film samples were used to compare the diffusivities with respect to bulk solution. To prepare the sandwich thin film structure of ~200 nm thickness, one drop of 2 mg/mL PVP solution was spin-coated at 2000 rpm (for 1 min) on freshly cleaned glass coverslips, followed by spin-coating a dilute solution (~200 pM) of Rh6G/Alexa-555/PPE premixed with PVP and eventually casting another capping layer of PVP on top.^{36,46} To prepare thicker (~500 nm) films the last step was repeated 4–5 times. In addition, samples were also prepared involving film preparation methods based on drop casting with thickness ~500 nm. To form the drop cast samples, ~7 drops of a mixture of PVP/Rh6G (or PVP/Alexa-555 or PVP/PPE) were cast on top of a clean glass coverslip and annealed to dry up the excess solvent present. The thickness of the films was determined by both atomic force microscopy (AFM) and ellipsometry. The samples were annealed at ~120 °C for 2 h under evacuated conditions for removal of residual solvent and generation of relaxed films. All the samples were mounted over the FCS stage and subjected to ambient moisture for ~30 min until the first data was collected. The RH was varied from ~70 to 95% for all the data acquisition. All the measurements were carried out at 295 K.

2.3. Fluorescence Correlation Spectroscopy Set up. FCS studies were carried out on a home-built set up (Figure S1 in Section S2 of Supporting Information). A 532 nm DPSS laser (Shanghai Dream Lasers Technologies Co. Ltd.) was used to illuminate the sample placed on the stage. The excitation light was allowed to pass through a beam expander, followed by a dichroic mirror to the back focal plane of the objective lens (60 \times , 1.2 NA, water immersion) and finally to the sample placed over the stage. The $1/e^2$ beam radius (transverse) (ω_{xy}) and the one-half height (l) of the excitation volume were calculated to be ~300 nm and ~2 μ m, respectively. The emitted light is reflected back thorough the same objective lens to the emission filter, thereby reaching a concavo convex achromat lens (Thor Laboratories). The lens then focused the light to the pinhole, attached to the optical cable ultimately to be detected by an APD detector (PerkinElmer) that transformed the fluorescence intensity into electrical signal. The signal was then fed into the correlator card (Model No.: FLEX 99 OEM-12D, NJ, U.S.A.) connected to the PC to generate the autocorrelation curve.

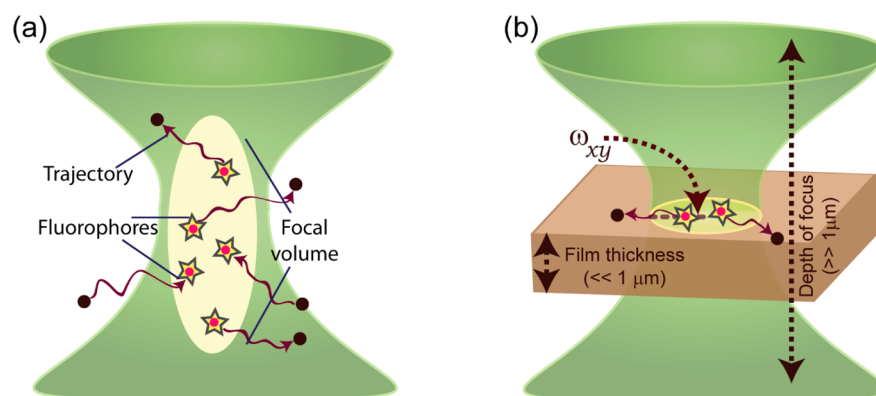


Figure 1. (a) A schematic of the movement of the molecules (bulk) in and out of the focal volume. Red color shows intensity burst for molecules inside the observation volume. The black ends denote nonfluorescent molecules outside the focal volume. The wavy arrows (brown) show the direction of motion of the individual molecules. (b) A schematic showing the position of thin film within the focal volume in the FCS setup facilitating only lateral movement of the probe molecules.

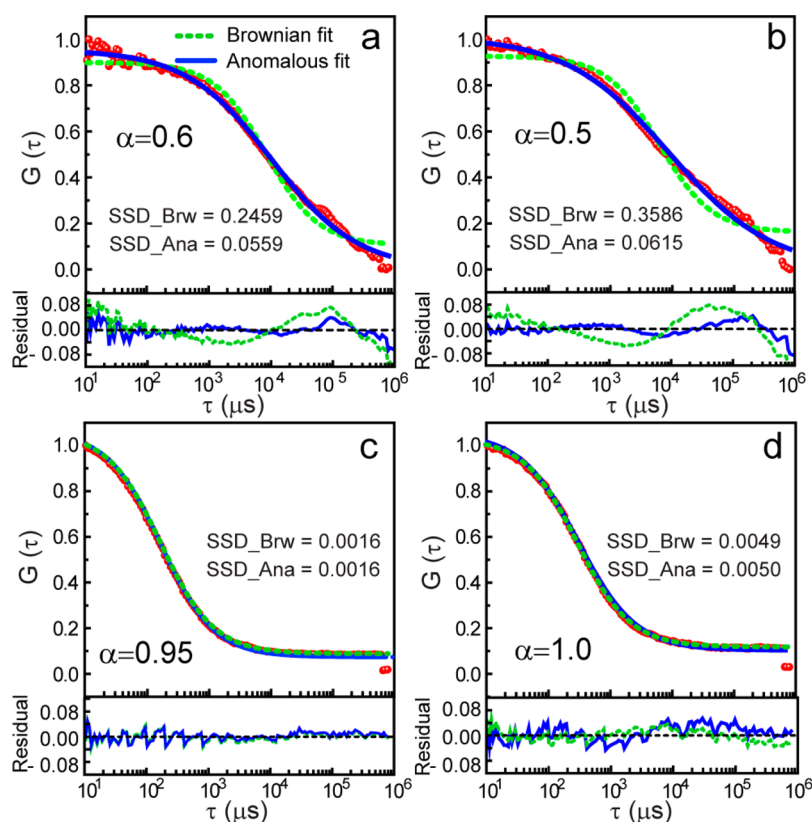


Figure 2. (a–d) Representative autocorrelation plots (red) depicting diffusion of probes inside PVP thin films hydrated to various extents. The consequent fittings along with the residuals for Brownian diffusion and anomalous subdiffusion are shown in green (dotted) and blue (bold), respectively. The sums of square of difference (SSD) values have been shown for all the fits (Brownian, Brw; Anomalous, Ana).

2.4. Data Analysis. A molecule entering into the detection volume is associated with fluorescent bursts in terms of intensity fluctuations and this persists until the molecule remains within the detection volume by virtue of translational mobility of the molecule. A sudden drop in the fluorescence intensity is associated with the moment the molecule goes out of the detection volume. Figure 1a is a diagrammatic representation of the behavior of fluorescent probe molecules in the observation volume in the FCS set up. The autocorrelation $G(\tau)$ of the intensity fluctuation is given by the relation (eq 1).^{11,12,14,15,18,22,23}

$$G(\tau) = \frac{\langle \delta F(t + \tau) \delta F(t) \rangle}{\langle F(t) \rangle^2} \quad (1)$$

where, $\langle F(t) \rangle$ denotes the time-averaged fluorescence intensity and $\delta F(t)$ is the difference in signal at any time t and the averaged intensity $\langle F(t) \rangle$. With increasing τ , the correlation decreases and finally falls off to zero. Generally, in bulk solutions the fluctuations inside the focal volume are random, suggestive of Brownian diffusion of the molecules. Under such conditions, for a three-dimensional (3D) Gaussian-shaped observation volume (ellipsoidal) with radial (ω_{xy}) and axial length (l), $G(\tau)$ is given by eq 2.^{15,22}

$$G(\tau) = \frac{1}{N} \left(\frac{1}{1 + \left(\frac{\tau}{\tau_D}\right)} \right) \left(\frac{1}{1 + \left(\frac{\omega_{xy}}{l}\right)^2 \left(\frac{\tau}{\tau_D}\right)} \right)^{1/2} \quad (2)$$

where, N is the average number of molecules in the observation volume and τ_D is the time taken by a molecule to diffuse across the volume.

The system was calibrated using Rh6G with a known diffusion coefficient in bulk solution.⁴⁷ The τ_D value of 55 ± 1 μ s (inset of Figure S1 in Supporting Information) was obtained for Rh6G in water (eq 2). However, here we have extended our analysis for determination of the diffusivities of molecules inside thin film matrices. The primary difference between conventional FCS and inside thin films is that the dimensions of the thin films are much smaller than the depth of the exciting field, which reduces the focal volume considerably (Figure 1b). Being focused within the film is affirmed by a high intensity that gradually drops on either moving the stage closer or away from the objective, thereby losing the actual focus. Hence, for film on a substrate the fitting eq 2 no longer can fit the data appropriately. Therefore, we specifically probe lateral (two-dimensional) motion of Rh6G molecules within the polymer matrix. For such motions, the following fitting eq 3 has been employed assuming that the molecules undergo random Brownian motion.

$$G(\tau) = \frac{1}{N} \left(\frac{1}{1 + \left(\frac{\tau}{\tau_D}\right)} \right) \quad (3)$$

However, for molecules diffusing within films, the motion necessarily need not be random (Brownian) due to viscoelasticity of the matrix. Hence, for such diffusion fitting the experimental data with eq 3 gives a poor fit and the τ_D obtained is not reliable. Consequently, a new term α ($\alpha < 1$) was introduced in the fitting program (eq 4), which is a measure of the observed extent of deviation from Brownian motion^{43,44}

$$G(\tau) = \frac{1}{N} \left(\frac{1}{1 + \left(\frac{\tau}{\tau_D}\right)^\alpha} \right) \quad (4)$$

The diffusion time is related to the translational diffusion coefficient D_τ by the following relation (eq 5)^{15,22}

$$\tau_D = \frac{\omega_{xy}^2}{4D_\tau} \quad (5)$$

where, ω_{xy} is the transverse radius of the excitation volume. The effective viscosity (η) of the matrix can be calculated from D_τ using the Stokes–Einstein relation (eq 6)^{15,22}

$$D_\tau = \frac{k_B T}{6\pi\eta R_h} \quad (6)$$

where, $k_B T$ is the thermal energy and R_h is the hydrodynamic radius of the tracer molecule.

3. RESULTS AND DISCUSSION

3.1. Effect of Ambient Humidity on Tracer Dynamics inside PVP Thin Films.

Figure 2 depicts representative

autocorrelation plots for translational mobility of Rh6G molecules in PVP thin films with varying amounts of moisture absorbed. The data shown in Figure 2a,b and c,d typically represent probe mobility under lower (~ 75 – 80%) and significantly high RH ($>90\%$) regimes, respectively. In order to account for the nature of diffusion of Rh6G molecules within PVP thin films at different RH, autocorrelation plots were fit independently for Brownian diffusion (eq 3) and anomalous subdiffusion (eq 4) as shown in green (dotted) and blue (bold), respectively, in Figure 2a–d. The corresponding τ_D obtained by fitting the autocorrelation curves (Figure 2a,b) with eq 3 is found to be unreliable to extract diffusivities (D_τ) indicative of the molecular mobility being not purely Brownian under low to moderate RH levels. Therefore, for accurate determination of τ_D and the subsequent D_τ , the autocorrelation curves were fit with eq 4 for anomalous subdiffusion (also see section S3, Figure S2 in Supporting Information) of molecules diffusing within PVP thin films. The goodness of the fits (depicted from residuals) thus clearly depicts the mobility of Rh6G molecules being anomalous subdiffusive in nature. The corresponding α values were found to be 0.6 and 0.5 for plots shown in Figure 2a,b, respectively. However, under significantly high ambient humidity it is observed (Figure 2c,d) that the corresponding τ_D obtained by fitting the autocorrelation curves with eq 3 are slightly in better agreement to extract reliable D_τ than those with eq 4 (also see section S3, Figure S3 in Supporting Information) suggestive of Brownian mobility of the tracers. The α values were found to be 0.95 and 1.0 for the two plots shown in Figure 2c,d, respectively. Therefore, these results clearly show that the nature of diffusion of tracers within a polymer thin film varies to a great extent with the change of humidity content over the thin film.

Experiments were also carried out in bulk polymer solutions (see section S4 in Supporting Information) in order to compare the nature of diffusivities in solution to that in a thin film. The results show that the diffusion in a bulk polymer solution varies to a great extent from that within a polymer thin film. In solution, molecules undergo completely random (stochastic) motion, whereas, inside a film, the molecules might move under constraints offered by the polymer matrix. The molecules in their course of motion are likely subjected to multiple hindrances either due to entanglement within the polymeric chains or movement inside voids within the hydrated polymer.^{48,49} For subdiffusion in PVP thin films, α was found to typically vary from ~ 0.5 – 0.9 with moisture level varying up to moderate levels. High viscoelasticity and excessive crowding within the polymer network are likely to be responsible for such subdiffusive behaviors. However, under exceedingly high amounts of moisture absorbed by the film, the tracers undergo transformation from anomalous subdiffusion to Brownian diffusion suggestive of significant swelling and softening of the polymer matrix to facilitate free diffusion.

Therefore, FCS essentially reveals that the dynamics inside thin films exhibit predominantly anomalous subdiffusive behavior, irrespective of the amount of vapor absorbed, that is, from relatively low (RH $\sim 70\%$) to moderate (RH $\sim 85\%$) ambient humidity. Additionally, under significantly higher levels of RH (RH $> 95\%$) close to that of a solution-like state, Brownian motion takes over anomalous subdiffusive behaviors.

3.2. Effect of Sample Preparation on Probe Diffusion inside PVP Thin Films at Different RH Levels. Translational mobility of a probe in a medium is strongly dependent on the resistance offered by the medium, that is, the viscosity of

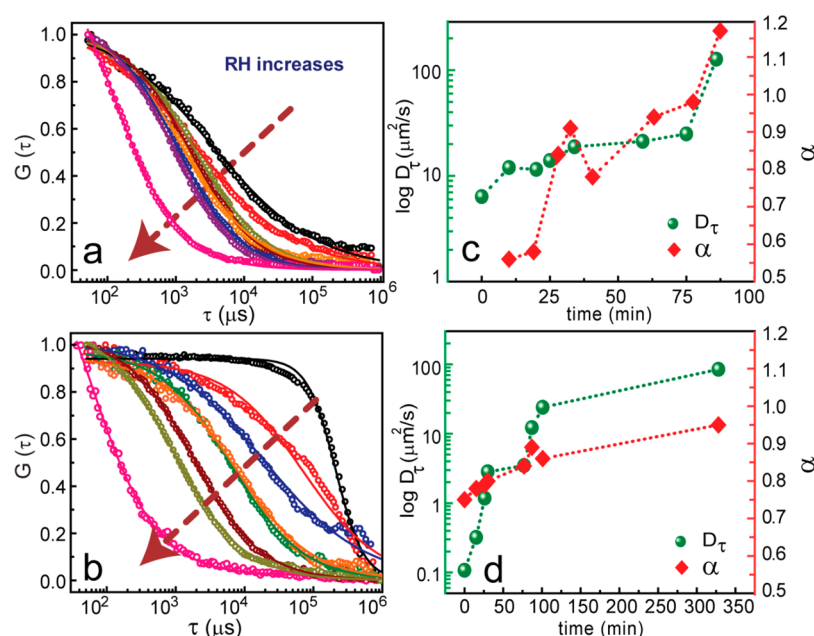


Figure 3. Fitted autocorrelation plots for molecular diffusivities under gradually increasing ambient moisture absorbed in (a) drop cast and (b) spin-coated PVP films, respectively. Arrows (dark red) designate increase in ambient humidity (RH \sim 70–95%). Subsequent diffusivities (D_τ) and extents of deviation from Brownian diffusion (α) in (c) drop cast and (d) spin-coated films, respectively. The left and right-hand side scales for D_τ and α in (c,d) are marked in green and red, respectively.

the surrounding environment toward diffusion of the dispersed solute.^{50–52} In order to gain insight on the effect of matrix viscosity on diffusivity of probe molecules, two different methods viz. drop casting and spin-coating for preparation of thin films were employed. Figure 3a,b shows a series of autocorrelation plots for diffusion of Rh6G molecules in drop cast and spin-coated films, respectively, with varying levels of ambient humidity (\sim 70–95%) over both the samples (for residuals see Figure S5 in Supporting Information). The arrows (dark red) indicate the gradual increase in the ambient moisture absorbed by the film matrix. It was found that under sufficiently lesser amounts (RH \sim 70%) of absorbed moisture (black plot in Figure 3a,b), both the types of films provided enough rigidity where probe diffusion is extremely slow (faster diffusion in drop cast film than in spin-cast film). However, on gradual exposure to ambient moisture the rigidity of both the matrices lessened progressively, thereby allowing larger diffusivities of the probe molecules (pink plot in Figure 3a,b). Overall, it was observed for both the type of samples that, the correlation curves under high moisture (\sim 95%) decayed faster (i.e., had lower τ_D) in comparison to the curves under low RH levels (RH \sim 70%) due to subsequent lowering in the viscosity of the polymer matrix with increase in ambient moisture (from a RH \sim 70% to a RH of \sim 95%) around the vicinity of the molecules as a result of considerable plasticization of the entire polymer matrix.

Interestingly, the decay of the autocorrelation curves shown in Figure 3a depict that the diffusion was much faster in the case of drop cast films than in comparison to the films which were spin-coated, as shown in Figure 3b. The respective diffusivities plotted in logarithmic scale against time of data acquisition (green) are shown in Figure 3c,d and the corresponding τ_D and η for both types of films are shown in Table 1. It is found that the drop cast film (Figure 3c) achieved moderate flexibility (in a rubbery state) in \sim 90 min, whereas, the spin-coated film (Figure 3d) took more than \sim 330 min to

Table 1. Time-Evolved Diffusion Times (τ_D), Diffusivities (D_τ) and Effective Viscosities (η) for the (a) Drop Cast and (b) Spin-Cast Film, Respectively

(a)			
time	τ_D	D_τ	η
(min)	(μ s)	(μ m ² /s)	(Pa·s)
0	3533.03	6.37	0.058
10	1874.59	12.00	0.031
20	1951.63	11.53	0.032
25	1613.01	13.95	0.026
34	1192.95	18.86	0.019
59	1061.10	21.20	0.017
75	902.24	24.94	0.015
86	143.18	157.14	0.002
(b)			
0	209652.89	0.11	3.418
15	70047.87	0.32	1.142
26	19048.91	1.18	0.311
30	7943.01	2.83	0.129
77	6492.92	3.47	0.106
87	1835.56	12.26	0.030
101	937.59	23.99	0.015
328	129.29	174.03	0.002

achieve a similar state. This could probably be attributed to the presence of much more residual solvent in drop cast films in comparison to spin-coated films even after annealing.^{53–58} The τ_D values imply that molecule translates at a faster rate in a medium with less viscosity than in a reasonably more viscous environment, as a direct consequence of the hindrances offered by the media toward molecular mobility. Thus, the spin-coated film behaves as a much rigid and viscous medium in comparison to the drop cast film under all the similar conditions of absorbed moisture (RH). The trend in diffusivities, however, suggest that both the drop cast and

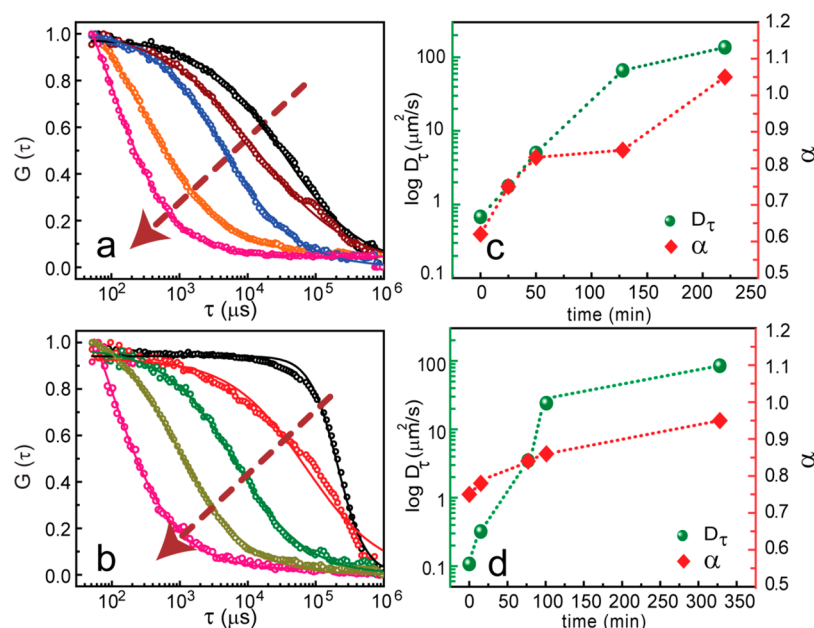


Figure 4. Fitted autocorrelation plots for diffusivities of probe molecules under increasing ambient moisture over spin-coated films of thickness (a) ~ 200 and (b) ~ 500 nm, respectively. Arrows (dark red) designate increasing humidity levels (RH ~ 70 – 95%). The corresponding diffusivities (D_τ) and extents of deviation from Brownian diffusion (α) are shown in films with thickness (c) ~ 200 and (d) ~ 500 nm, respectively. The left- and right-hand side scales for D_τ and α in (c,d) are depicted in green and red, respectively.

spin-coated PVP thin films take a colossal amount of time to reach a state comparable to a bulk solution. It should be mentioned that mobility inside a drop cast film gets closer to Brownian diffusion at a much faster rate (shorter time) than in a spin-coated film as observed from the variation of α (red) in Figure 3c,d. Thus, we can conclude that variation in the amounts of moisture absorbed and present inherently, affect the extent of probe diffusion, that is, diffusivities increase with increase in moisture content or presence of residual solvent. Moreover, the nature of diffusion could also be affected in both the types of films with varying amounts of solvent present, that is, transition from anomalous to Brownian diffusion takes place with increasing humidity.

3.3. Effect of Thickness of PVP Thin Films on Probe Diffusion at Different RH. As already known, diffusivity is directly related to the viscosity of the surrounding media; therefore, it became important to investigate the effect of thickness of films prepared in a similar way with different effective viscosities on probe diffusion. In order to do so, thin films were prepared with different thicknesses using spin-coating. Figure 4a,b shows the series of autocorrelation curves in films having thickness ~ 200 and ~ 500 nm, respectively, with varying amounts of absorbed moisture (for residuals see Figure S6 in Supporting Information). The extracted diffusivities obtained are plotted in logarithmic scale against time of data acquisition (green) as shown in Figure 4c,d and the corresponding values of τ_D and η for different films are shown in Table 2. It is found that the thinner film takes less time (~ 220 min) to hydrate (i.e., rate of percolation of moisture is faster) and achieves a more flexible and softer state in comparison to the thicker film (~ 330 min). At lower levels of RH, for both the films, the decay of the autocorrelation function $G(\tau)$ is sluggish, suggesting restricted mobility of the Rh6G molecules present within the focal volume. The obtained values of D_τ are several orders of magnitude smaller as compared to that of Rh6G in water (Supporting Information

Table 2. Time-Evolved Diffusion Times (τ_D), Diffusivities (D_τ), and Effective Viscosities (η) for Spin-Coated Films with Thickness (a) ~ 200 and (b) ~ 500 nm, respectively

(a)			
time (min)	τ_D (μ s)	D_τ (μ m ² /s)	η (Pa.s)
0	33074.53	0.68	0.539
25	12679.93	1.78	0.207
50	4508.65	4.99	0.074
128	338.59	66.45	0.006
220	101.79	221.04	0.002
(b)			
0	209652.89	0.11	3.418
15	70047.87	0.32	1.142
77	6492.92	3.47	0.106
101	937.59	23.99	0.015
328	129.29	174.03	0.002

inset Figure S1), consistent with a medium of very high viscosity. However, with increasing RH, due to higher diffusivities of the probe molecules, autocorrelation curves decay considerably faster. On further increasing the RH up to saturation levels, the decay of the autocorrelation curves become even faster and the D_τ values obtained approach closer to that for tracer diffusion in bulk polymer solution. We find that the lower bound of D_τ obtained from FCS measurements are consistent with the results in our recent report using translational mobility of SM probes, occurring at higher ends of humidity (~ 70 – 75%).³⁶ The D_τ values obtained by FCS vary from ~ 0.6 – $220 \mu\text{m}^2/\text{s}$ and ~ 0.1 – $170 \mu\text{m}^2/\text{s}$ for a 200 and 500 nm film, respectively. On the other hand, near saturation RH levels, a dramatic increase in the translational diffusivities (upper bound) is observed with lowering of effective viscosity (η) of the polymer matrix. Therefore, this signifies that under such moisture levels, enough water is absorbed by PVP for its

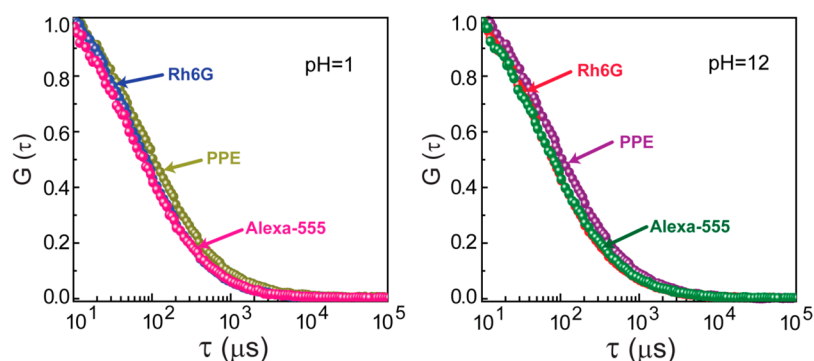


Figure 5. Autocorrelation plots for diffusivity of different tracers (Alexa-555, Rh6G, and PPE) in PVP solution (10 mg/mL) at (a) pH = 1 and (b) pH = 12, respectively.

T_g to be lowered much below room temperature to allow for the primary (α) relaxation of the polymer chains to take place to facilitate larger molecular (probe) diffusion (very high D_r). Additionally, the corresponding reduction in the rigidity of the polymer matrix is attributed to substantial swelling of the thin film network. It is important to note that the rate or the extent of softening of the polymer matrix does not remain constant for the entire RH regime depicted by difference in slopes for variation of α (red) with time (Figure 4c,d). Thus, these results are indicative of the existence of a dynamically heterogeneous environment of the matrix during the entire process of plasticization.

3.4. Effect of Polymer Molecular Weight on Probe Diffusion. It is already established in the previous two sections that the effective viscosity offered by the polymer matrix plays a key role toward tracer diffusion. In the present section, the effect due to steric properties of the PVP matrix (with different extents of entanglement) by virtue of different molecular weight (10 000) has also been investigated (shown in Table T2 in Supporting Information). For a particular type of film (drop-cast/spin-cast) the rate of change in viscosity from highest (at time = 0) to the lowest (at longest time) is achieved faster in lower molecular weight (10,000) PVP, i.e., there is a quantitative difference in the rate of change of viscosity for different molecular weights of PVP. For lower molecular weight (10,000) PVP, changes happen within 50 min (or 177 min) for drop-cast (or spin-cast) film in comparison to 86 min (or 328 min) for drop-cast (or spin-cast) film of higher molecular weight (40,000). This affirmed the existence of more sterically hindered rates of diffusion in polymer with higher molecular weight (levels of entanglement). Nevertheless, it is worth mentioning that the trends in diffusivities, i.e. transition from anomalous subdiffusion to Brownian diffusion were existent in all the above cases, irrespective of the polymer molecular weight (see Section S7 in Supporting Information).

3.5. Effect of Electrostatic Interaction on Diffusivities of Probe Molecules. PVP being an electrostatically uncharged polymer exhibits nearly negligible Coulombic forces of interaction toward of mobility of tracers irrespective of their inherent charge (positively charged Rh6G, negatively charged Alexa-555, and neutral PPE). Figure 5 shows the dynamics of tracers inside PVP (avg. MW 10 000) for a range of pH from 1 to 12. Being neutral, PVP does not ionize in the above-mentioned pH regime even in the presence of excess amounts of H^+ and OH^- ions. Hence, within the entire pH range of 1–12, molecular mobility inside PVP was found to be independent of the surrounding pH. On the other hand, polymer thin film

brushes and PEMs undergo considerable ionization and thus are highly affected by the change in pH in the surrounding environment. Therefore, differential molecular mobility inside PVP thin films could be attributed entirely to the viscoelasticity of the matrix and is independent of the interaction between polymer and probe molecule, unlike in polymer thin film brushes and PEMs having strong electrostatic charges.^{24,25}

3.6. Effect of Tracer Size on Diffusion inside PVP Thin Films. As stated in the previous section that PVP being electrostatically noninteracting with tracers during their course of diffusion, the effect of size of tracers has also been investigated (shown in Table T3 in Supporting Information) in PVP matrices with average molecular weight of 10 000. It was found that Alexa-555 ($R_h \sim 0.58$ nm) and Rh6G ($R_h \sim 0.59$ nm) having similar sizes exhibited similar quantitative rates of diffusion inside PVP matrices (both spin-coated and drop-cast films). However, PPE exhibited slightly slower diffusion due to somewhat greater dimension ($R_h \sim 0.79$ nm) than both Alexa-555 and Rh6G. Thus, it can be evinced that tracer diffusion depends directly on the size of the tracer molecules. Therefore, these results help in better generalization of the diffusion rates of molecular tracers in gradually plasticizing polymer matrices.

4. CONCLUSION

The dynamic behaviors of Rh6G (and Alexa-555/PPE) molecules inside PVP thin films have been studied as a function of moisture absorption using fluorescence correlation spectroscopy. Extensive morphological/structural changes (in terms of swelling of the thin film network) have been found to be associated with increasing levels of moisture absorption that induce translational mobility of probe molecules within the films. It is found that molecular mobility in thin films prepared by two different techniques (viz. drop cast and spin-coating) exhibit similar dynamic behaviors with change in RH in the surrounding environment. Under relatively low humidity conditions, both the films offer resistance to a large extent to inhibit appreciable molecular mobility. It is observed that the drop cast films plasticize more easily and rapidly in comparison to spin-coated film, attributed to the presence of more residual solvents in the former. The nature of mobility of the probe molecules in both the type of films is essentially found to follow non-Brownian (anomalous) subdiffusion under relatively moderate RH regimes (RH up to $\sim 80\%$). Furthermore, it is also observed that molecular mobility can be directly related to the thickness of the thin films. The thinner films (~ 200 nm) are found to plasticize more rapidly thereby offering faster

diffusivity of Rh6G molecules as compared to a thicker film (~ 500 nm), attributed to the faster rate of moisture penetration in the thinner film than in the thicker film. The trend, that is, transition from anomalous subdiffusion to Brownian mobility was found to be unaltered with variation in molecular weight of polymer, however, the quantitative diffusivities were found to be affected by molecular weight of the polymer. Similar trends in diffusion behavior were observed with tracers with different molecular dimensions. Moreover, tracer diffusivities are found to be independent of the pH of the surrounding media implying the absence of any electrostatic interaction between the polymer and the probe molecules.

The deviation from Brownian motion is most likely to be attributed to the high viscoelasticity of the PVP matrix. Such a description for tracer mobility in crowded cellular environments have already been proposed, however, analogous dynamic behaviors in polymer thin films by variation of absorbed moisture using FCS has not been reported earlier. With attainment of the bulk like state (associated with severe drop in the effective viscosity of the matrix) at very high RH levels (RH > 90%), the diffusivities are found to transform from anomalous subdiffusion to Brownian type, plausibly due to the complete plasticization of the films, irrespective of their method of preparation. With increasing amounts of moisture absorbed (RH), such a transition of diffusivity has not been previously described for plasticizing thin films wherein simultaneous probing of both a highly rigid to a bulk solution-like state have been possible in the same sample. Furthermore, with the use of moisture as the plasticizer, a drop in the glass transition temperature (T_g) of a high T_g polymer can be brought about thereby enabling us to monitor a much wider range of temperature that would otherwise not be achieved on conventional alteration of temperature of the system. Therefore, we show that in principle, FCS can be used as an effective tool to probe the changes in viscoelastic behaviors associated with changing ambient moisture even for isotropic polymer thin films devoid of any intrinsic electrostatic charge.

■ ASSOCIATED CONTENT

■ Supporting Information

Figures, tables, and discussions of molecular structures of poly(vinylpyrrolidone) (PVP), rhodamine 6G (Rh6G), Alexa Fluor-555 (Alexa-555), and perylenebisimide polyether (PPE), illustration of home-built FCS set up and calibration, effect of ambient humidity on diffusivities of probes in PVP thin films, diffusivities of probe molecules in solvents with very high viscosity, residual plots for drop cast and spin-coated films as a function of increasing RH, comparison of diffusion data for Rh6G in different polymer with lower average molecular weight, and comparison of tracer mobility in drop-cast and spin-coated samples of PVP (avg. mol wt of 10 000) with different tracers having dissimilar sizes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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