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Microheterogeneity and Microviscosity of F127 Micelle: The Counter Effects of Urea and Temperature

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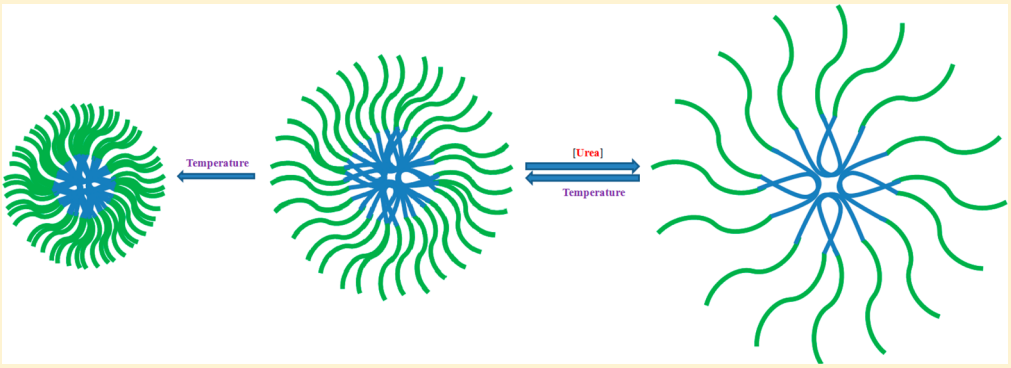
Supporting Information



ABSTRACT: F127 is the most widely studied triblock copolymer and due to the presence of very long polypropylene oxide (PPO) and polyethylene oxide (PEO) groups, F127 micelle has different microenvironments clearly separated into core, corona, and peripheral regions. Urea has been known to have adverse effects on the micellar properties and causes demicellization and solvation; on the other hand, rise in temperature causes micellization and solvent evacuation from the core and corona regions. In the present study, we have investigated the microheterogeneity of the core, corona, and peripheral regions of the F127 micelle using red edge excitation shift (REES) at different temperatures and urea concentrations and correlated the effect of both on the micellar system. It was found that the temperature counteracts the effect of urea and also that the counteraction is more prominent in the core region with respect to corona, and the peripheral region is least affected. Also, the core and corona regions are very much heterogeneous, while the peripheral region is more of a homogeneous nature. Using time-resolved fluorescence anisotropy, we found that the microviscosity within the micelles vary in the order of core > corona > peripheral region, and urea has a general tendency to reduce the microviscosity, especially for core and corona regions. On the other hand, rise in temperature initially increases and then decreases the microviscosity throughout, and at elevated temperatures the effect of urea is being dominated by the effect of temperature, thereby establishing the counter effects of temperature and urea on the F127 micellar system.

INTRODUCTION

Pluronics are amphiphilic triblock copolymers (ABA type) comprising polypropylene oxide (PPO) groups as the central block unit and polyethylene oxide (PEO) groups as the outer blocks and are one of the most well-known high-molecular-weight nonionic surfactants.[1] The block lengths can be adjusted to produce Pluronics of various PPO and PEO chain lengths so that these materials are widely used in various fields, such as detergency, wetting, emulsification, lubrication, as well as cosmetics, bioprocessing, and pharmaceuticals.[2] At high temperatures, the central PPO block becomes hydrophobic, while the PEO blocks remain hydrophilic.[3] Because of this amphiphilic nature, Pluronic molecules above a critical temperature and concentration, self-aggregate in aqueous solutions to form spherical micelles having hydrophobic PPO cores surrounded by hydrophilic PEO coronas. The micellization process and the phase behavioral properties of pure Pluronics in aqueous media depend upon solution temperature, copolymer concentration, and molecular architecture which have been studied using differential scanning calorimetry, static and dynamic light scattering (DLS), small-angle neutron scattering, and surface tension measurements.[3−6] The process of self-association can be induced by increasing the block copolymer concentration to be above the critical micellization concentration (CMC) and/or adjusting the temperature to exceed the critical micellization temperature (CMT).[7,8] Among various Pluronic species, F127 is perhaps the most widely studied copolymer, owing to its high stability, bioadhesive characteristics, thermoreversible gelling ability at room temperature, and nontoxic properties, which all make it a suitable

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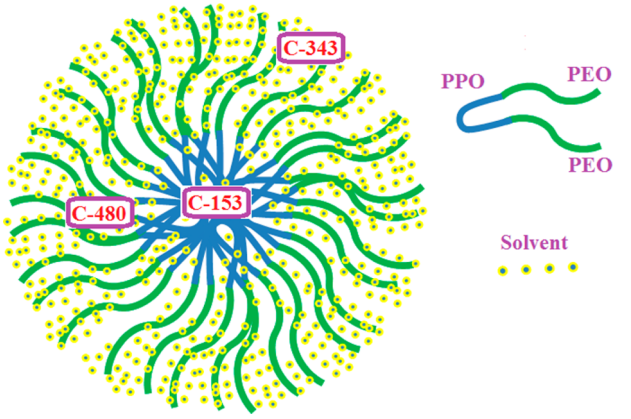
vehicle for drug formulations.[9,10] The inclusion of various additives, such as salts,[11−13] short-chain alcohols,[14−16] and formamide[17] have a strong effect on the aggregation behavior of PEO-PPO-PEO triblock copolymers in the aqueous phase. Among these various additives, urea has been proved to be efficient in altering the micellar properties of PEO-PPO-PEO solutions.[18] Urea and its derivatives are known to be very effective denaturants of proteins because of their abilities to weaken hydrophobic interactions in aqueous solutions.[19,20] Therefore, it can also affect micellar properties by modifying the delicate balance between hydrophobic and hydrophilic interactions of surfactants within themselves and with water molecules.[21] In fact, it has been reported that addition of urea increases the CMC of nonionic[18,22] and ionic surfactants,[23] and at the same time decreases the hydrodynamic radii of ionic micelles[24] and raises the cloud point temperatures of nonionic surfactant solutions.[22,25] However, unlike other micellar systems, there are no reports that confirm structural/morphological changes brought in by urea for F127 micelles. Bhattacharyya and co-workers have used urea to observe its effect on the micelle,[26] protein,[27] and *p*-Nitrophenol (PNP).[28] They opined that urea displaces water molecules from the micellar interface[26] and causes denaturation of a protein, which leads to faster solvation dynamics.[27] They also concluded that urea reduces the population of PNP molecules at the water surface both in the presence and in the absence of surfactant.[28] Two different mechanisms have been proposed to explain the action of urea in aqueous solutions: an indirect mechanism, according to which urea acts as a "structure breaker" thereby facilitating the hydration of nonpolar solutes,[29,30] and a direct mechanism, in which urea has almost no effect on the water structure, replaces some of the water molecules from the hydration shell of the solute and finally self-associates with the nonpolar solutes.[31,32] In a very seminal work, Breslow and Guo[33] used surface tension measurements to show that chaotropic salting-in denaturating agents, like urea, are not just water "structure breakers", contrary to belief. They also suggested that urea must be directly involved in the solvation of a solute by direct interaction and not through cavity formation in water.[33] Thus the increased solubility of hydrocarbons in water in the presence of urea in not due to cavity formation, and urea does not serve as a water "structure breaker".[34] The effect of temperature on the micellar properties of Pluronics has been widely documented.[3,35−39] It has been reported that a rise in temperature causes dehydration of the PPO units[37] (which is the core of the micelles) and, hence, initiates the micellization process, thereby decreasing the CMC.[35,36] The aggregation number[36,39] of the micelles also increases while the PEO units (which is the corona of the micelles) acquire some crystalline structure[3,37] with a rise in temperature. Interestingly, very recent reports reveal that for F127, the micellar size decreases with a rise in temperature.[38,39] Basak and Bandopadhyay encapsulated three drugs inside the spherical F127 micelles and using DLS experiments concluded that the mean hydrodynamic radii of the micelles increase with decrease in temperature and also due to the presence of drug molecules.[38] Hatton and co-workers opined that the CMC and CMT values of the Pluronic micelles also have been reported to increase while the microviscosity to decrease with increasing concentrations of urea.[18] They used intramolecular excimer fluorescence to establish that the microviscosity of another Pluronic copolymer (P105) decreases slightly with a rise in urea concentration.[18] Also, it has been shown that the effect of urea on CMT is more pronounced when the PEO content is more in a Pluronic.[40]

A bathochromic shift in fluorescence emission caused by a shift in the excitation wavelength toward the red edge of the absorption band is termed as red edge excitation shift (REES).[41−50] REES has been used to study the micro-heterogeneity of membranes, micelles, polymers, and proteins, using different fluorescent probes.[42−46,48−50] The phenomena of REES thus serves as an excellent tool to monitor the immediate environment around a fluorophore. In addition to REES, time-resolved anisotropy measurements have been widely used to probe the molecular dynamics of a fluorophore within a restricted environment.[51−56] Studies based on rotational dynamics are extremely helpful in understanding the nature of solvent friction around the fluorophore.

In the present study, we have probed the microheterogeneity (by REES) and microviscosity (by time-resolved fluorescence anisotropy) of the different regions of F127 micelles. Since the polarities of the various regions of the micelles vary considerably,[35,37] we have used three different coumarin dyes (namely, C-153, C-480, and C-343), owing to their preferential binding sites for exploring the effect of temperature and urea on F127 micelles (Scheme 1). Using cyanine dyes, Shiraishi et al.[57] also probed the viscosities of three regions of the polymer F127. They correlated the fluorescence quantum yields of the cyanine dyes to the microviscosities of the different regions of the micelles and concluded that the viscosity of the core is greater than that of the corona and also the corona viscosity increases toward the center of the micelle.[57] It has been reported that C-153 binds to the core region of F127 micelles, while C-343 preferentially gets attached to the peripheral regions. The neutral C-480 resides in the corona region, which has an intermediate polarity as compared to the core and the peripheral regions.[58−60] To the best of our knowledge, this is the first report on the effect of urea and temperature on the microheterogeneity and microviscosity present within the micellar system. Using REES and fluorescence anisotropy experiments, we have conclusively proved that the effect of urea and temperature are opposite in nature.

Scheme 1. Schematic Representation of F127 Micelle and Locations of Three Coumarin Dyes



EXPERIMENTAL SECTION

Materials and Sample Preparation. The triblock copolymer F127 and urea were purchased from Sigma Aldrich and were used as received. Laser grade coumarin 153 (C-153), coumarin 480 (C-480),

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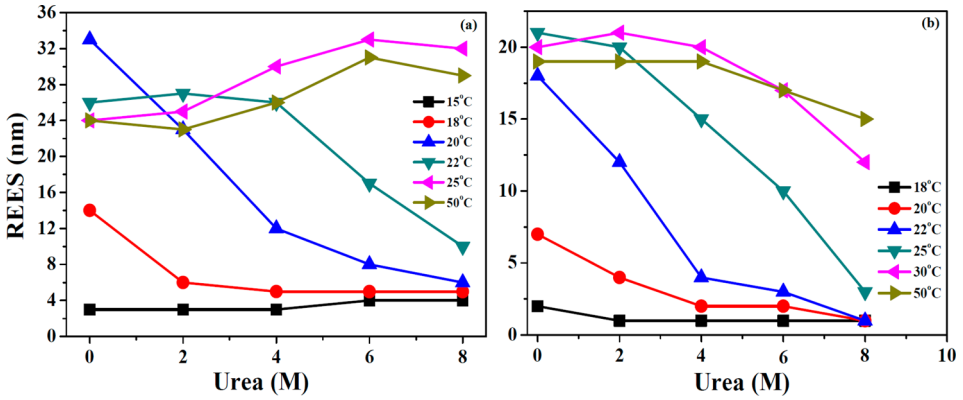

Figure 1. Plot of REES values against urea concentrations at some specific temperatures (as mentioned in the figure) for (a) core and (b) corona regions.

and coumarin 343 (C-343) were also purchased from Sigma Aldrich. Polymer solutions were prepared dissolving 1 g of Pluronic F127 in 10 mL of nanopure water (having a fixed concentration of dissolved urea in order to nullify the volume enhancement brought in by added urea), for 10 w/v% solutions (CMC of F127 is 0.7 w/v% in water),[1] which were magnetically stirred at room temperature in a sealed container for 12−24 h to fully dissolve the Pluronic F127. The solution was kept overnight under refrigeration for stabilization. Next, a calculated amount of stock methanolic solution of the dye was taken in a volumetric flask so that the final concentration becomes 5 μM for a specific volume of F127 micellar solution. After removing methanol completely, specific volumes of stock micellar solution were added into the flask, and the solution was kept for a few hours for the encapsulation of dye into the microenvironment of micelles. For steady-state and time-resolved fluorescence measurements, the temperature was controlled using a Thermo Electron Corporation chiller with a precision of <0.1 °C.

Steady-State Measurements. Steady-state absorption measurements were carried out in a Perkin-Elmer UV−vis spectrophotometer, Lambda-25. All the steady-state fluorescence measurements were recorded on a Horiba Jobin Yvon, Fluorolog 3-111. The fluorescence spectra were measured with a 10 mm path length quartz cuvette.

Time-Resolved Anisotropy Measurements. For anisotropy measurements, the samples were excited at 407 nm using a picosecond diode (IBH-NanoLED source N-405L). The anisotropy decay measurements were carried out by collecting parallel and perpendicular decay components with respect to the polarization of the excitation laser using a Hamamatsu MCP Photomultiplier (Model R-3809U-50). The two decay components were acquired for at least for 1000 s each, such that a good signal-to-noise ratio was obtained. The time-correlated single photon counting (TCSPC) set up consists of an Ortec 9327 pico-timing amplifier. The data were collected with a PCI-6602 interface card as a multichannel analyzer. The typical full-width at half maximum (fwhm) of the system response was about 120 ps. The rotational anisotropy decays were analyzed using IBH DAS-6 decay analysis software.

RESULTS AND DISCUSSION

At 25 °C, C-153 exhibits an absorption peak at 420 nm and an emission peak at 512 nm in F127 micellar solution. Upon the addition of 8 M urea, both the absorption and emission peaks are marginally red-shifted by 2 nm, signifying that addition of urea increases the polarity of the core region of the micelles (Figure S1a of the Supporting Information). In the absence of F127, C-153 has an emission peak at 549 nm in water and when bound to the micellar core it shows a dramatic blue shift of 37 nm. This proves the fact that the microenvironment around C-153 once bound to the micelles is extremely hydrophobic in nature and this can only happen when the fluorophore gets attached in the core region of the micelles. Upon increasing the temperature to 50 °C followed by the addition of urea, both the absorption and emission profiles of C-153 remain the same.

C-480 exhibits an emission maximum at 490 nm in aqueous solution and when bound to the micelles it gets blue shifted to 466 nm. The smaller blue shift in the case of C-480 as compared to C-153 proves that the region of binding of C-480 is more hydrophilic as compared to C-153. At 25 °C, the absorption and emission peaks of C-480 are centered at 383 and 466 nm, respectively, in F127 micelles (Figure S1b of the Supporting Information). Upon the addition of 8 M urea, these peaks are red shifted to 388 and 479 nm, respectively, indicating that urea brings in more polarity in the microenvironment of the fluorophore. When the temperature was increased to 50 °C, the absorption peak still remains the same; however, the emission peak is blue shifted by 3 nm, which is primarily due to the desolvation of the microenvironment around C-480. Similar observations are made when the temperature of the solution is gradually increased to 50 °C in the presence of 8 M urea; the emission peak is blue shifted from 479 to 473 nm (Figure S1b of the Supporting Information).

It has been reported that C-343 binds to the peripheral regions of the Pluronic micelles[58] and hence the probe will experience an environment which is more hydrophilic as compared to C-153 and C-480. In aqueous solution, C-343 shows an emission peak at 495 nm, which is blue-shifted to 488 nm in F127 micelles. These gradations in decreasing blue shifts categorically prove the fact that the C-153, C-480, and C-343 binds selectively in the core, corona, and peripheral regions of the micelles. At 25 °C, the absorption and emission maxima of C-343 in F127 micelles are centered at 430 and 488 nm, respectively (Figure S1c of the Supporting Information). Upon the addition of 8 M urea, the absorption and the emission maxima are red-shifted by 7 and 3 nm, respectively, corroborating the fact that the addition of urea brings in more water molecules in and around the C-343. It has also been observed that a rise in temperature (up to 50 °C) has almost no effect on the spectral properties of C-343. However, on increasing the temperature to 50 °C in the presence of 8 M urea, the emission peak is again blue shifted by 4 nm, indicating the desolvating effect of temperature (Figure S1c of the Supporting Information).

Red Edge Excitation Shift (REES). REES is a well-known phenomenon which provides a clearer picture of the

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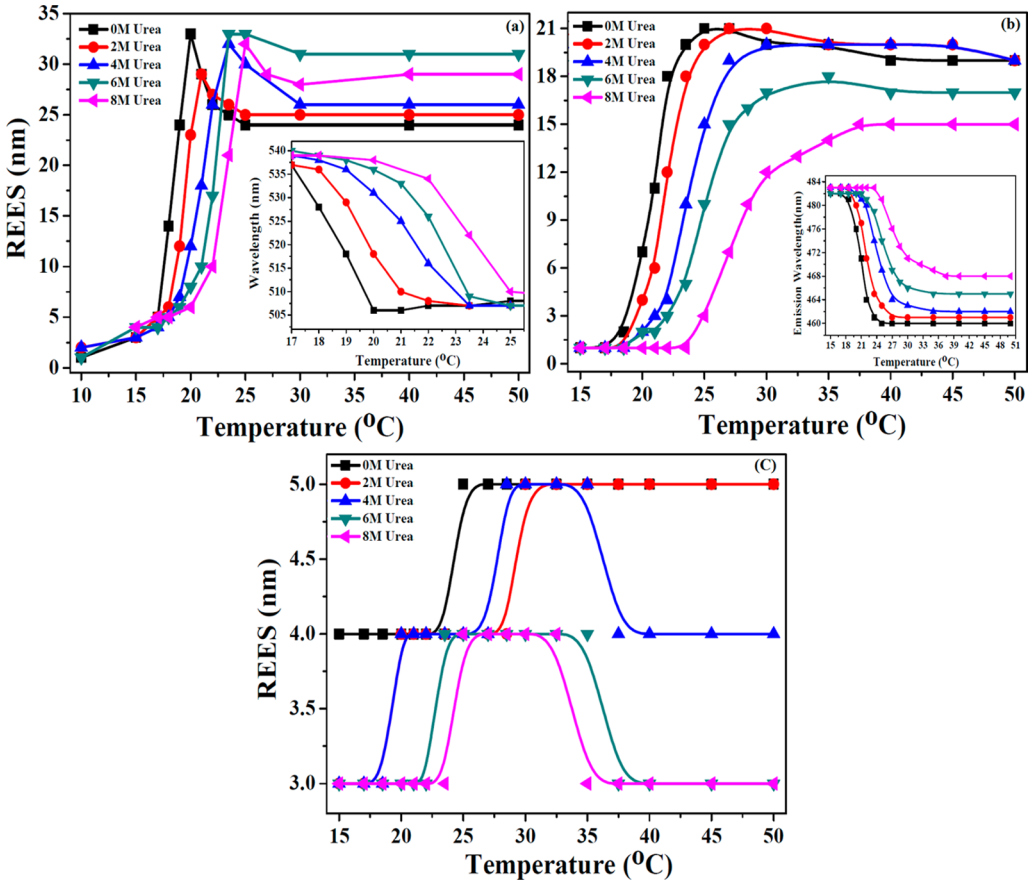

Figure 2. Plot of REES values against temperature for different urea concentrations (as mentioned in the figure) for (a) core, (b) corona, and (c) peripheral regions. The insets of (a and b) represent the emission wavelengths, excited at the very blue end.

atmosphere surrounding a polar probe while emitting from the excited state. The shifting of the emission maxima of the probe toward the longer wavelength upon red-shifting the excitation wavelength is termed as the red edge excitation shift or REES.[41−50] An increase in REES values indicates greater restriction on the mobility of the solvent molecules surrounding the probe molecule bound within the hydrophobic cavity of the microheterogeneous system.[50] Here in this report, we have used three different fluorescent probes to monitor the microheterogeneity of the core, corona, and peripheral regions of the F127 micelle at different urea concentrations and varying temperatures. For monitoring REES, the micellar systems having different fluorophores bound at specific regions were excited at different wavelengths. For all of the three fluorophores, the excitation wavelengths were tuned from 350 to 450 nm, and the corresponding emissions were recorded. At 25 °C and in the absence of urea, C-153 (monitoring the core), C-480 (monitoring the corona), and C-343 (monitoring the peripheral regions) show a REES of 24, 21, and 4 nm, respectively. Hence, the confinement of solvent or the different restricted environments of the fluorescent probe molecules at three different places of a polymeric micelle can easily be inferred. With increasing concentration of urea at 25 °C, the REES values for core region changes to 25, 30, 33, and 32 nm for 2, 4, 6, and 8 M urea, respectively (Figure 1). It must be stated that the core diameter of the F127 micelles has been estimated[1] to be around 8 nm and, hence, the C-153 probe molecules may bind to different regions within the core. The high REES values of C-153 are a clear signature of the greater extent of microheterogeneity the probe experiences. The addition of urea, thus in all likelihood, is not affecting the orientation of the already restricted water molecules within the core of the micelles and thereby the heterogeneity still remains, particularly for those probe molecules which are deep-seated. As stated earlier, there will be a distribution of C-153 molecules within the core region, hence those probe molecules which are located at the interface of the core−corona are likely to be more affected by the addition of urea. Consequently, these C-153 molecules will exhibit a red shift in their emission maxima in the presence of higher concentrations of urea, while those located at interior regions of the core will not be affected (Figure 2a). Similar observations were made when the temperature was increased in small installments up to 50 °C. However, upon decreasing the temperature below 25 °C, water molecules seep in and make the interior regions of the core more hydrated. This is exemplified by the fact that at lower temperatures (less than 25 °C), the observed REES is of a smaller magnitude (Figure 1a). This variation of REES with temperature is in good agreement with reported literature.[40] At elevated temperatures (25 °C and above), urea affects the PPO core by hydrating the same through the PEO groups by an indirect mechanism. However, at lower temperatures (below 25 °C), the mechanism is direct; urea hydrates the PPO core and consequently makes that region more homogeneous.

At 25 °C, the corona (monitored by C-480) shows a sharp fall in the REES values to 20, 15, 10, and 3 nm for 2, 4, 6, and 8 M urea, respectively (Figure 1b). This dramatic decrease in REES values at higher urea concentrations can be attributed to

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the hydration of the corona[40] (PEO group) by urea. Even when exciting at the blue end of the absorption spectrum, C-480 shows a red shift in its emission maximum unlike what was encountered with C-153. The peripheral regions of the F127 micelles are monitored by C-343 and as expected, in this case we observed a REES of 4 nm at 25 °C in the absence of urea. Since C-343 already experiences a more hydrophilic and homogeneous environment as compared to C-153 and C-480, this value of REES is not unusual, and at higher concentrations of urea the REES values remain almost constant (Figure 2c). The interaction of urea at the peripheral regions makes the solvation of C-343 molecules in a more uniform manner. Hence, excitation at the blue and red ends of the absorption spectrum has similar red shifts in their emission maxima. Figure 1 represents the variation of REES for the core and corona regions with urea at some specific temperatures. At 15 °C, only 3 nm REES is observed in the core region and can be attributed to monomeric/premicellar forms, which seem to be unaffected by urea concentration (except for the 1−2 nm red shift throughout due to direct interaction of PPO with urea[40]) and we can compare the microheterogeneity of the core at this temperature to the microheterogeneity of the peripheral region at higher temperatures (postmicellar forms). The REES values show a decreasing trend with the increase in urea concentrations for temperatures below 25 °C, and above that, the trend is opposite for the core region (Figure 1a). Unlike the core, the effect of temperature on the corona region is more direct; the REES values decrease with an increase in urea concentrations at all temperatures, and the trend here is almost similar to that encountered for the core region at temperatures less than 25 °C. As it has already been established, urea has maximum effect on PEO groups,[40] hence, higher urea concentrations solvate all the corona region and the environment changes from being more heterogeneous to less heterogeneous for temperatures above 25 °C. For temperatures below 25 °C, the REES gets almost nullified, and we can infer that the environment becomes almost homogeneous (Figure 1a). From Figure 1 (panels a and b), it is evident that the core and the corona regions become almost homogeneous at 15 and 18 °C, respectively. Upon slowly increasing the temperature for the system, the core starts to show REES at temperatures above 15 °C. This observation signifies that it is the core region (PPO) that initiates the micellar organization followed by the corona (PEO).

To have a better understanding of the effect of temperature on the F127 micellar system, we plotted the REES values as a function of temperature for different urea concentrations (Figure 2). Figure 2a represents the change in REES values for the core with an increase of temperature, and it is evident from the figure that the heterogeneity starts from 17 °C, reaches a maximum at 20 °C, again decreases up to 25 °C, and thereafter remains constant for F127 solution in the absence of urea. As already discussed in the previous section, an increase in temperature causes dehydration of the core and produces a more heterogeneous environment; hence with increasing temperature, we observe an increase in microheterogeneity. But as the temperature increases, the core−corona interface also get dehydrated and as a result, excitation at the extreme red end leads to a small blue-shifted emission and hence is the decrease in REES values. The trends of increase and decrease in REES values are similar for all the F127 solutions with different urea concentrations, except for the temperature at which it starts increasing, reaches the maxima, and decreases to saturation. With increasing urea concentration, the same REES values shift to higher temperatures. The shift of starting point for microheterogeneity at higher temperature represents the shift of onset of micellization and can be assumed as the CMT for the particular F127 sample. To dehydrate the core, greater temperature is required when urea is present and this value is augmented at higher concentrations of urea. The extent of solvation brought in by different concentrations of urea varies; consequently, a direct linear dependence of heterogeneity with temperature is not observed. This trend is more prominent in the case of the corona region where 8 M urea gets its maximum heterogeneity at 40 °C compared to 0 M urea which only takes 25 °C (Figure 2b). Hence, the dehydration is not a direct function of temperature for different urea concentrations and also the temperature effect is diminished in the corona region with respect to the core region. The peripheral region probed by C-343 is not much affected by temperature and both the emission at the blue and red end exhibits hypsochromic shifts of almost equal extents due to an increase in temperature and the REES remains almost the same (Figure 2c). These shifts of total emission spectra (at higher temperature and higher urea concentrations) can be ascribed to dehydration and excessive hydration caused by temperature and urea, respectively.

Time-Resolved Fluorescence Anisotropy. Most of the F127 solutions (with and without different concentrations of urea) are in micellar form at 25 °C and almost remain as micelles up to 50 °C, hence all the anisotropy experiments have been done in the range of 20−50 °C. Rotational anisotropy decays [$r(t)$] are monoexponential functions in pure solvents but generally become biexponential in the case of micellar systems.[52] If the rotational decay is a single exponential in a micellar system then fluorescence depolarization is solely due to the rotational dynamics of a single fluorophore species encapsulated within the micelle. In our case, it was observed that a single-exponential function for $r(t)$ gave a poor fit to the decay; however, a biexponential function for $r(t)$ was adequate to fit the data. Figure 3 shows representative anisotropy decays

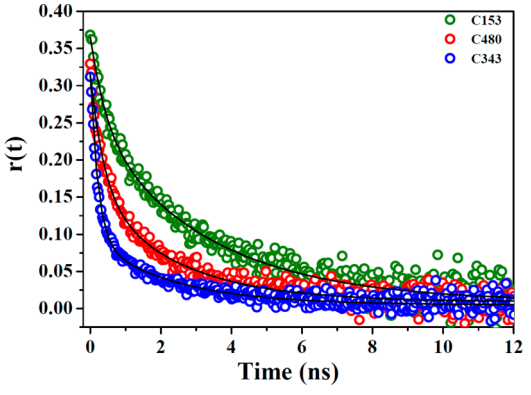

Figure 3. A representative plot for fluorescence anisotropy decays of C-153, C-480, and C-343 in the F127 micelle without urea and at 25 °C.

of C-153, C-480, and C-343 in the F127 micelle at 25 °C. All the anisotropy data were fitted to biexponential decays with very good χ^2 values. There are several models to explain the slow [$\tau_{r(slow)}$] and fast [$\tau_{r(fast)}$] reorientation times of fluorophore in the micellar system. Out of the different models, the one which best fits to our system and explains the variation

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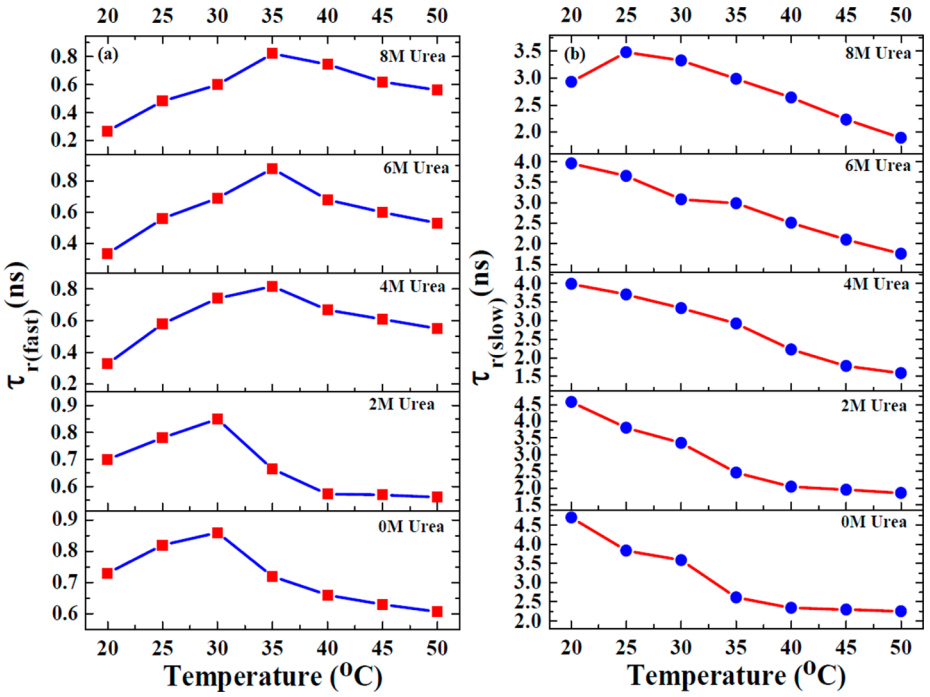

Figure 4. A plot of rotational lifetime (a) $\tau_{r(fast)}$ and (b) $\tau_{r(slow)}$ against temperature for different urea concentrations as mentioned in the respective panels for C-153.

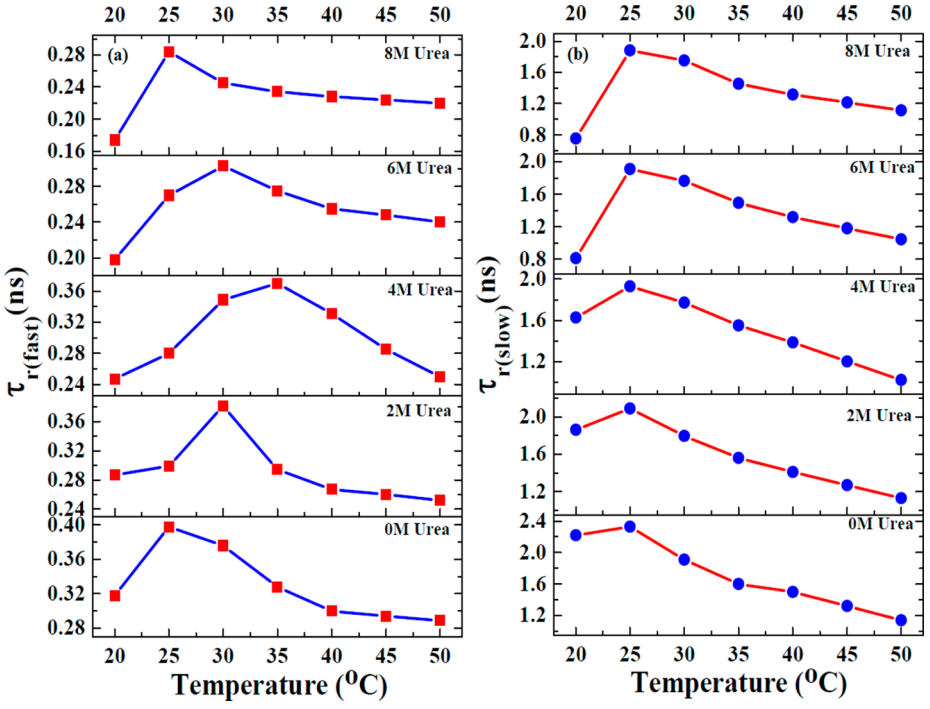

Figure 5. A plot of rotational lifetime (a) $\tau_{r(fast)}$ and (b) $\tau_{r(slow)}$ against temperature for different urea concentrations, as mentioned in the respective panels for C-480.

due to urea and temperature is the one in which the contribution to the anisotropy decay comes from three independent motions: wobbling of the dye molecule in a restricted region (τ_R) (assumed for simplicity as a cone),[52−54] translational diffusion of the dye along the spherical surface (τ_D), and rotation of the micelle as a whole (τ_M).

Effect of Urea on the Core of the F127 Micelles. At lower temperatures, C-153 experiences the micellar core environment which is not fully dehydrated and at higher urea concentrations the core becomes more hydrated and, hence, less friction is experienced by the probe. Therefore, $\tau_{r(fast)}$ decreases from 730 ps (comparatively dehydrated micellar core) to 268 ps (comparatively hydrated core) for 0 and 8 M urea at 20 °C and from 860 to 600 ps for the same system at 30 °C. Hence, it is clear that urea decreases the friction in the micellar core, and faster rotational time constants gradually decrease in the

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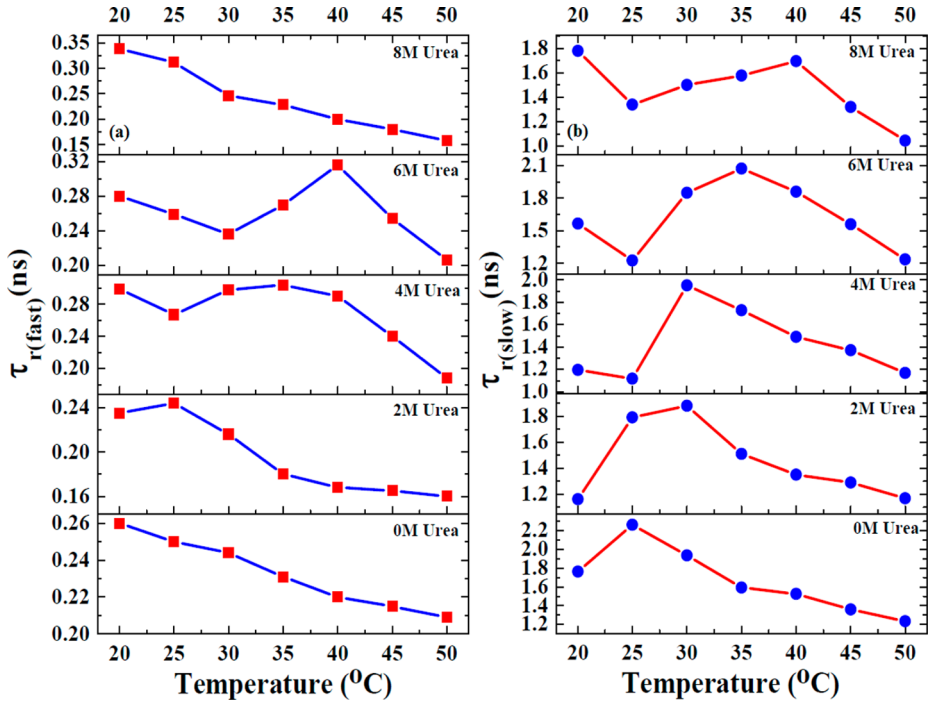

Figure 6. A plot of rotational lifetime (a) $\tau_{r(fast)}$ and (b) $\tau_{r(slow)}$ against temperature for different urea concentrations, as mentioned in the respective panels for C-343.

presence of increasing concentrations of urea. At higher temperatures (greater than 30 °C), the core becomes desolvated and the effect of urea is diminished. Simultaneously, a rise in temperature provides the kinetic energy to the probe, rendering it to be more mobile. Hence, the $\tau_{r(fast)}$ decreases for the solutions having low (2 M) or no urea content and increases until total desolvation is achieved in the case of high urea content samples (Figure 4a). For 20, 25, and 30 °C, $\tau_{r(fast)}$ decreases with an increase in urea concentration, but beyond 30 °C, this particular trend of $\tau_{r(fast)}$ is not seen. Once the $\tau_{r(fast)}$ reaches the maxima, it starts decreasing and at 50 °C, $\tau_{r(fast)}$ values become almost the same for all concentrations of urea (550 ± 20 ps) (See Table 1 of the Supporting Information).

The slower rotational lifetime component $\tau_{r(slow)}$ shows a similar monotonic decreasing trend for all urea with rise in temperature, except for 8 M urea where $\tau_{r(slow)}$ shows a rise at 25 °C (Figure 4b). This small rise in $\tau_{r(slow)}$ at 8 M urea is most probably due to the monomer-to-micelle transformation at 25 °C. It is already established that the increase in temperature causes reduction in micellar size,[38] and solvent removal will not affect the translational diffusion of the probe as much as the wobbling motion will be affected. Hence, we see the changes in the $\tau_{r(slow)}$ value for different urea concentrations. The $\tau_{r(slow)}$ decreases from 4.70 to 2.90 ns at 20 °C and from 3.83 to 3.48 ns at 25 °C for 0 and 8 M urea, respectively. Thereafter (beyond 25 °C), the $\tau_{r(slow)}$ decreases for low urea content (up to 4 M urea) and increases for higher urea content (6 and 8 M urea), and at higher temperatures both urea and temperature have profound effects on $\tau_{r(slow)}$ (see Table 1 of the Supporting Information).

Effect of Urea on the Corona of F127 Micelles. The effect of urea on the corona region was far more interesting. It was found that the $\tau_{r(fast)}$ values have almost no proper trend with respect to urea concentrations, and it may be due to the fact that in the corona region urea has a maximum solvating effect and even local hydrogen bonding with the water molecules, hence, we get different $\tau_{r(fast)}$ at different urea concentrations. For 0, 2, 4, 6, and 8 M urea, the maximum value of $\tau_{r(fast)}$ is obtained at 25, 30, 35, 30, and 25 °C, respectively (Figure 5a). As seen from Figure 5a, the peak of the curves for $\tau_{r(fast)}$ shifts toward the higher temperatures and then rolls back at 25 °C for 8 M urea. This shift in $\tau_{r(fast)}$ maxima values can be explained on the basis of competition between the solvation and the temperature-dependent motions of the probe molecule. From the lower panel of Figure 5a, the rise of $\tau_{r(fast)}$ at 25 °C is due to the local increase in friction as a result of removal of solvent molecules at this temperature and further decrease is due to the pronounced effect of temperature on the probe motion. At higher urea concentrations, there will be more solvation brought in by the added urea; however, elevated temperatures will try and nullify this solvation effect. These two processes run parallel at elevated temperatures and higher urea concentrations and a balance between them controls the overall $\tau_{r(fast)}$ magnitude. At 2 and 4 M urea concentrations, the effect of temperature is both on the solvent removal as well as on probe motion. As removal of solvent causes more friction than the mobility brought in by the elevated temperature, the $\tau_{r(fast)}$ maxima values shift to higher temperature for higher urea content up to 4 . Beyond 4 M urea, the solvent removal needs more thermal energy, and at that high temperature the probe has more mobility also. The friction caused by solvent removal is diminished at higher temperatures, and the maxima is thus shifted to lower temperature where solvent removal is the predominant factor as compared to probe motion.

The slower rotational lifetime component $\tau_{r(slow)}$ shows a decrease with increase in urea concentration at 20 °C, and its value changes from 2.22 ns to 755 ps. Similarly, at 25 °C, it decreases from 2.33 to 1.88 ns for 0 and 8 M urea, respectively (see Table 2 of the Supporting Information). However, with increasing temperature, the trend is not linear and there are

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variations in lifetime at higher urea concentrations. One of the prominent reasons may be the extent of hydration of the corona region with higher concentration of urea and their dehydration by increased temperature. This will affect the translational diffusion motion of the probe as well as the micellar size differently. At 25 °C, all the samples show the maximum value (which also decreases with increasing content of urea) of $\tau_{r(slow)}$ and thereafter display a continuous decrease (Figure 5b). As already stated, $\tau_{r(slow)}$ is more of a function of translational diffusion and micellar size (which decreases with temperature), and hence, it is not exactly what we obtain for the faster lifetime component (which experiences local motion and local friction in a cone). Increase of $\tau_{r(slow)}$ up to 25 °C and then its decreasing trend is different from the values of C-153 in the core (where only 8 M urea containing sample shows a similar trend) and is mainly due to the higher extent of hydration of the corona than the core. At 50 °C, all the samples have almost similar $\tau_{r(slow)}$ values (1.10 ± 0.1 ns), implying similar diffusion and size of the micelle (See Table 2 of the Supporting Information).

Effect of Urea on the Peripheral Regions of F127 Micelles. For probing the peripheral region of the F127 micelles, C-343 has been used and $\tau_{r(fast)}$ values do not have any particular trend. Also, at a fixed temperature for varying concentrations of urea, the variation of $\tau_{r(fast)}$ is not that dramatic. The reason behind it is the bulk solvent-like nature of the peripheral region, which is all the more augmented when urea is present. Nevertheless, $\tau_{r(fast)}$ decreases from 260 to ∼210 ps for 0 M urea and from 340 to 160 ps for 8 M urea. But as we go to higher urea concentrations, the maxima shifts to higher temperatures preceded by a dip in the $\tau_{r(fast)}$ value (Figure 6a). As already stated, urea causes solvation of the PEO group extensively and hence the exterior parts of the F127 will be maximum solvated. Even at low concentrations of urea, it will resemble a bulk solvent-like environment and as the temperature is increased it will be dehydrated but very slowly and less efficiently. Hence, at lower temperature, F127 samples with urea experience friction due to hydrogen-bonded urea, and at higher temperatures, they experience the friction due to presence of PEO groups. Therefore, the maximum of the rotational anisotropy values goes from 20 °C for 0 M urea to 40 °C for 6 M urea (see Table 3 of the Supporting Information).

Figure 6b represents the variation of $\tau_{r(slow)}$ with varying concentrations of urea at different temperatures. The maxima are shifting to higher temperatures with higher content of urea. The difference in temperature for $\tau_{r(slow)}$ and $\tau_{r(fast)}$ maxima values is due to the different extent of effects of urea and temperature on local and translational diffusions as well as change in size of the micelles. Here we can observe that for $\tau_{r(slow)}$ values of core, corona, and peripheral regions of the micelle show a significant deviation with temperature and urea. For the core region, the $\tau_{r(slow)}$ values are dominated by effect of the temperature and, hence, we see a gradual decrease with temperature for all urea contents, while in the corona region, there is a competition between urea and temperature and urea dominates up to 4 M urea up to 35 °C; thereafter temperature becomes the dominant parameter. For the peripheral region, the effect of urea seems to be dominant throughout and the temperature role is not that conspicuous in terms of $\tau_{r(slow)}$.

CONCLUSION

In the present study, we have investigated the microheterogeneity of the core, corona, and peripheral regions of the F127 micelles at different temperatures and urea concentrations and correlated the effect of urea and temperature on it. We found that the temperature counteracts the effect of urea and also that the counteraction is more prominent in the core region with respect to corona, and that the peripheral region is least affected (even urea has lesser impact on the microheterogeneity of the peripheral region). Also higher concentrations of urea lead to a lesser heterogeneity of the corona region up to higher temperatures. Hence, from REES experiments we found that both PPO and PEO regions can be hydrated to a great extent by addition of urea, and the PPO region is easily dehydrated while PEO dehydration is a slow process. Subsequently, time-resolved fluorescence anisotropy experiments have been performed to get an insight of local friction or microviscosity of the three different regions of F127 micelles and to monitor the effect of urea and temperature on them. We found that the microviscosity is on the order of core > corona > peripheral region, and urea has a general tendency to reduce the viscosity, especially for core and corona regions. At elevated temperatures, the effect of urea is dominated by the effect of temperature and also temperature increases the viscosity by removing solvent molecules and at the same time promotes mobility of the probe molecules. Hence, we see a mixture of effect of urea and temperature at lower temperatures and urea concentrations, and at higher temperatures, fluidity dominates. Hence in both cases, the action of urea and temperature are totally opposite, which is reflected in microheterogeneity as well as in microviscosity of the F127 micelles. The knowledge of effect of temperature and urea on polymeric micellar microheterogeneity and microviscosity will not only give an insight of the mechanism of micelle formation and role of solvent and cosolvents but also be useful for formulation of drug delivery systems where such polymeric micelles are being heavily used.

ASSOCIATED CONTENT

Supporting Information

Various absorption spectra, fluorescence spectra and tables showing the anisotropy parameters for different systems are available in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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