RESEARCH ARTICLE

Solute dynamics in a block copolymer: effect of additives and temperature

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ABSTRACT

The solute dynamics in a symmetrical poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) triblock copolymer, P-123 were studied in aqueous and saline solutions as a function of additives, temperature using spectroscopic techniques. We have chosen a hydrophobic solute Coumarin-6 (C-6) as probe to study the photophysics and rotational dynamics in the above systems. We found that the rotational dynamics of C-6 is hindered in saline solution compared to that in aqueous solution of P-123. Therefore, in the saline solution of P-123, C-6 faced more restricted environment compared to the aqueous solution of P-123. In the absence of saline, at a temperature higher than cmt, we observed that addition of 0.02 M n-hexanol decreases the rotational relaxation time compared to that in P-123. Whereas, addition of 0.02 M n-hexylamine the rotational relaxation remains almost same compared to that in P-123. Therefore, n-hexanol affect more on the solute dynamics compared to n-hexylamine because of its preferential partitioning in the micellar phase owing to its greater hydrophobic ranking compared to n-hexylamine. In the saline solution we observed that addition of 0.02 M n-hexanol on P-123 solution affect more on the rotational dynamics of C-6 compared to the addition of 0.02 M n-hexylamine.

1. Introduction

Poloxamers coined by BASF (Badische Anilin-und SodaFabrik) inventor Irving Schmolka, who was granted the patent for its synthesis in 1973, are a class of water soluble, non-ionic, synthetic tri-block copolymers. They consist of hydrophilic poly (ethylene oxide) (PEO) and hydrophobic poly (propylene oxide) (PPO) tri-block units [1,2] arranged in A-B-A fashion thereby giving PEO-PPO-PEO structure. A notable feature of these classes of solutions includes self-assembling and thermogelling behaviour; both of which are temperature dependent. Detailed observations reveal that below the critical micelle temperature (cmt) and critical micelle concentration (cmc) discrete units of block copolymers namely unimers are present in solution. Assemblage of individual unimers occurs above cmc or cmt through a process called micellisation. The assemblage is entropy driven process [3]; occurring as a consequence of dehydration of the polyoxy propylene block that tends towards lower solubility with increasing polymer concentration or temperature. Consequently, the interaction of the PPO block with the solvent decreases, and the micelle takes up a unique structure with PPO chains forming the hydrophobic core and PEO chains forming the hydrophilic shell.

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The resulting self-assembled structure can take varied forms namely spherical, ellipsoidal, cylindrical, worm-like or vesicles depending on the EO/PO ratio, total molecular weight and concentration of block copolymer [3]. As to which structure will form is strongly affected by the presence of additives like electrolytes/non-electrolytes, hydro topes or conventional surfactants [4]. The micelle formation and phase behaviour of Pluronic has been investigated by many workers and extensively reviewed [4–6]. Substantial reduction in the cmc, cmt and cloud-point (cp) have been observed by the addition of salts. Thus, despite temperature change micellisation can be induced in these systems by salts and additives.

The micelle formed by Pluronic being of amphiphilic nature, can fit lipophilic molecules in their central hydrophobic core thus acting as effective drug carrier. The micelle so formed being of larger size with precisely defined structure owns the possibility of being used as nano reactors for carrying out reactions not possible in aqueous and organic solvents. Some important concerns that emerge in this respect is what kind of environment the solute solubilised in these micelle face. How do they move? Curiosity regarding the microenvironment is diminished using techniques like NMR, static and dynamic light scattering, neutron scattering and fluorescent techniques [7–10]. Photophysical studies on aggregate assemblies are beneficial tools to understand the microenvironment around the probe molecule [10–14]. For our study P-123 (Scheme 1) has been used because its micellar phase is distinctively characterised, micellar properties well-documented, and also various photophysical studies were reported [12, 15–17]. The cmt of P123 is concentration dependent in a manner that solution with higher concentration have lower cmt. Usually, the cmt of 2% w/v aqueous solution of P-123 is 19.2°C which suggests that below this temperature monomer units of P-123 are present in solution.

With an increase in temperature, dehydration of water from the PPO blocks [18] take place thereby transfiguring unimers to micelle. Immediately after micelle formation, a structural dependency on the dehydration behaviour of PEO blocks occurs. Thus, temperature becomes a predominant criterion for micellisation. But during actual implementation, it may not be always feasible to vary temperature. In such a case, to obtain desirable micellar properties salts are added. The addition of salt is fascinating as it entropically favours dehydration of water from pluronics [19]. Beside salts, organic additives also exert intriguing effect on micellar behaviour. Elaborate study reveals that addition of short chain alcohols like methanol, ethanol accelerates dimicellisation process of pluronics [20].

![Scheme 1. The structure of Pluronic P-123 where x = 20, y = 70, z = 20.](image1)

![Scheme 2. The structure of Coumarin 6 (C-6).](image2)

Benzo α-pyrones, familiarly known as Coumarins represents an important family of fluorescent compounds. These are characterised by the presence of fused pyrone and benzene rings with the pyrone carbonyl group at position 2 [21]. These group of dyes are highly responsive to the local environment; the degree of responsiveness changing substantially with polarity and viscosity of the medium [22]. Coumarin 6 (C-6) (Scheme 2) have been used as the fluorescence probe in this study.

In this study, we are interested in finding out how the dynamics of probe molecules solubilised in aqueous solutions of P-123 is influenced by the addition of salt (NaCl) as well as organic additives such as alcohols and amines thus, throwing a light on the microenvironment of the micelles formed. To this effect, rotational diffusion of the hydrophobic probe C-6 has been examined in 2% (w/v) aqueous, and saline solution of P-123 over the temperature range 10°C- 50°C in steps of 5°C. We have chosen two additives namely n-hexanol and n-hexylamine which varied hugely in terms of its hydrophobic ranking thus allowing us to understand the effect of additives on probe dynamics more distinctly.

2. Experimental section

2.1. Materials

Coumarin-6 (C-6) was purchased from Sigma Aldrich and used as received. Pluronic P123 (M.W. = 5750, [EO]_{20}[PO]_{69}[EO]_{20}) was purchased from Sigma Aldrich. The employed chemicals such as n-hexanol, n-hexylamine, pyrene (99%), NaCl was purchased from Sigma Aldrich, and used without further purification.
2.2. Sample preparation

For experimental purpose stock solution of coumarin 6 was prepared in methanol. From the prepared stock, required amount of solution was taken in cuvette to make final concentration 2 μM in the solution. 2% (w/v) solution of P123 was prepared in triple distilled water by appropriately weighing in a glass bottle. The bottle was tightly stoppered and refrigerated overnight to ensure complete dissolution of polymer. A clear solution of P123 was obtained after a period of about 12 h. Dynamic light scattering (DLS) measurements were performed using triple water filtered through a 0.2 μm filter obtained from Axiva.

2.3. Instrumentation and methods

The ground state absorption spectra were obtained by a spectrophotometer from Shimadzu (Model No: UV-2550). The fluorescence emission spectra were obtained by a Horiba Jobin Yvon spectrofluorometer (model: Fluoromax-4). A picosecond time correlated single photon counting (TCSPC) setup (Edinburgh Instruments, Model: Life Spec- II, U.K.) was used for lifetime measurements. A diode laser of wavelength 405nm was used to excite the sample. The signals were collected using a Hamamatsu MCP PMT (3809U) detector. The time resolved fluorescence decays were collected at a magic angle of 54.7° polarisation. A quartz cuvette of 1cm path length was used for all spectroscopic measurements. The temperature variation throughout the experiment was conducted by using a Peltier-controlled cuvette holder (Model: TLC-50) from Quantum Northwest. After deconvoluting instrument response function, the fluorescence emission decays were fitted by using the following exponential function:

\[
I(t) = A + \sum_{i=1}^{N} B_i \exp \left( -\frac{t}{\tau_i} \right)
\]  

Here, ‘\(B_i\)’ represent pre-exponential factor, ‘\(\tau_i\)’ represent fluorescence decay time, and ‘\(A\)’ represent background. Using the equation \(\langle \tau \rangle = \sum_{i=1}^{N} (C_i \tau_i)\), we have calculated weight-average lifetime. where, \(C_i = \frac{B_i}{\sum_{i=1}^{N} B_i}\).

When calculating the anisotropic decay \(r(t)\), the following equation was used

\[
r(t) = \frac{I_{||}(t) - GI_{\perp}(t)}{I_{||}(t) + 2GI_{\perp}(t)}
\]  

In this equation ‘\(G\)’ denoted the correction factor for detector sensitivity to the polarisation direction of the emission. We have used F-900 software to analyse all the time-resolved fluorescence emission decays, and anisotropy decays. The particle size distribution of micelle was performed by a Litesizer 500 dynamic light scattering (DLS) instrument from Anton Paar at 298 K.

3. Results and discussion

3.1. Absorbance studies

We measured the steady state absorption spectra of C-6 in aqueous and saline P-123 solution along with additives such as n-hexanol and n-hexylamine. 2% (w/v) of the tri-block copolymer was used to study, with the variation of temperature from 10°C to 50°C maintaining a difference of 5°C in between. C-6 originally has sparing solubility in water [23, 24] but, in the presence of P-123 micelle it increases considerably. Hence, we can safely conclude that C-6 is preferentially solubilised in P-123 micelle. The dye owing to its intermolecular charge transfer (ICT) character is fairly polar in nature [25, 26], and hence its solubility is quite high in polar protic solvents like MeOH, EtOH, hexanol etc. It is thus expected that C-6 in P-123 micelle resides in micellar corona region of intermediate polarity.

The absorption maximum of C-6 in P-123 was observed to be 458 nm at 25°C. The addition of 0.08 M n-hexanol at 25°C causes no appreciable change. Representative absorption spectrum of C-6 in the presence of P-123 and 0.02 M n-hexanol was shown in Figure 1. While, the addition of 0.02 M n-hexylamine at 25°C causes a blue shift. The above findings can be elucidated considering the solubilisation of the 6-membered organic additives in the core region of the micelle. In the presence of 1 M NaCl, no appreciable change was observed at 25°C compared that in the absence of NaCl. With the

![Figure 1](image-url)
addition of 0.02 M n-hexanol and n-hexylamine at this temperature, a small shift to 459 and 461 nm, respectively was observed. The additives get partitioned between the aqueous phase and micellar core region. When concentration of additive is high, the cumulative increase of the hydrodynamic diameter becomes smaller since, after a distinct concentration of additive, growth in micellar size is retarded by distribution in water pseudo phase [20]. Hence, lesser or no deviation results with 0.08 M concentration of n-hexanol or n-hexylamine.

However, the addition of NaCl causes salting out effect that facilitates micellisation by leaching out some water molecules from micellar region thereby inducing hydrophobicity [27]. Also, NaCl prompts screening of the repulsive force thus favouring growth of micelle by decreasing intermicellar interaction.

3.2. Fluorescence emission studies

The coumarin-Pluronic aggregate shows unique temperature dependence. Unlike the routine trend, here fluorescence intensity increases with increase in temperature. Increase of fluorescence intensity with increasing temperature can be ascertained to micellar shape transition. C-6 in P-123 exhibits an emission maximum of 512 nm at 10°C (below cmt) which undergoes a substantial blue-shift to 498 nm at 25°C (higher than cmt). This throws light on the fact that the microenvironment around the probe once bound to the micelle is exceedingly hydrophobic in nature. The above phenomenon can only occur when the probe molecule resides within the micellar core [28]. A more detailed inspection of the fluorescence emission spectra of the investigated systems reveals the following:

(a) In the absence of salt and additive the fluorescence intensity increases uniformly towards the lower temperature range, attaining a maximum at its cmt 19.2°C thereafter, increasing again (Figure 2(a)). The maximum corresponding to cmt is a well-documented fact however, further increase suggests that a micellar transition from spherical to rod like is occurring which corresponds to increasing fluorescence intensity.

(b) Addition of NaCl to P123-C6 aggregate causes no appreciable change of the fluorescence intensity throughout the entire temperature range (Figure 2(b)). The presence of NaCl influences the screening of repulsive forces thereby decreasing intermicellar interactions and favouring growth of micelles by allowing amphiphilic molecules to approach each other more closely. Consequently, a structural conversion from sphere to rod is more favoured in the presence of salt [20]. Hence fluorescence intensity remains same in the presence of salt with increase of temperature compared to that in the absence of salt. The cmt in the presence of salt have been found out and shown in Figure S1.

(c) When 0.02 M and 0.08 M of n-hexanol and n-hexylamine was added to P123-C-6 aggregate in the presence and absence of salt no appreciable variation of fluorescence intensity with temperature was noted.

When each of the system (above cmt) was excited at 465 nm the emission maximum remains unchanged and was observed around 500 nm. However, varying the excitation wavelength from 465 nm to 495 nm caused the fluorescence emission maximum of each system (above cmt) to be shifted towards the longer wavelength of the spectrum thus indicating a prominent red edge excitation.

Figure 2. The fluorescence emission spectra of C-6 (a) in the presence of P-123, (b) in the presence of P-123 and 1 M NaCl with the variation of temperature from 10°C to 50°C, with increment of 5°C. Excitation wavelength: 465 nm.
shift (REES). REES is a well-established phenomenon which provides an insight into the surrounding atmosphere of the polar probe while it’s emitting from the excited state. For an exceeding majority of fluorophores, the emission spectra are independent of the excitation wavelength thus abiding Kasha’s rule. Nonetheless, excitation dependent shifts are observable for polar fluorophores in polar viscous solvents. An increase in the REES magnitude is suggestive of a considerable restraint on the mobility of the solvent molecule surrounding the probe molecule bound within the hydrophobic cavity of the micro heterogeneous system. Our study revealed that the C-6 molecule underwent a small red edge shift in the micellar environment. Probable reason underlying the REES may be intermolecular H-bonding with the media [29].

3.3. Fluorescence lifetime measurements

Any probe dispersed in micellar media can be solvable either in the micellar phase or the aqueous phase or it can segregate between the two phases depending on its chemical constitution. C-6 is used for our study is hydrophobic hence, it’s reasonable to expect that the dye will be located in the micellar phase. C-6 is a neutral, non-polar dye possessing polar functional group and therefore may be sparingly soluble in the aqueous phase. Fluorescence emission spectrum of the aqueous solution of C-6 was recorded which showed poor emission intensity. Hence, we can conclude that there is an ample tendency of the dye to be absent in the aqueous phase. In order to find out how the probe distributes itself in the presence of P-123, fluorescence emission decay of C-6 was measured in water at 25°C, and the average fluorescence lifetime was found to be 1.97 ns. The fluorescence emission decay of C-6 in the aqueous solution was fitted by biexponential functions, with faster and slower decay components of 820 ps and 2.47 ns respectively. The slower component arises owing to the locally excited C-6 molecule while intramolecular charge transfer (ICT) in C-6 gives rise to the faster component. In aqueous solutions, the C-6 microcrystals experience \( \pi-\pi \) interactions thereby giving a fast decay component [23]. Further, the fluorescence decay of C-6 was measured in a non-polar solvent viz n-hexane; the decay was found to be single exponential in nature with a lifetime of 2.40 ns. Hence, we can safely conclude that C-6 resides within the core of the P-123 micelle; the micellar core is a homogeneous medium with properties resembling that of an oil drop [30]. The lifetime value C-6 in water is lesser than the one containing P-123 at the same temperature, which is 2.91 ns.

With the addition of P123 the emission decays were fitted by biexponential function at lower temperatures, while decays are fitted by single exponential function at higher temperatures (Figure 3(a)). Under salt-free condition, addition of 0.08M n-hexanol shows single exponential decay pattern while addition of 0.02M n-hexanol and 0.02 M, 0.08M n-hexylamine caused the decay to change to biexponential at lower temperature and single exponential at higher temperature. Introduction of NaCl to the C6-pluronic system caused the emission decays to be bi-exponential at a lower temperature and single exponential at a higher temperature range (Figure 3(b)). Upon addition of 0.02 M and 0.08 M n-hexanol to the saline system the emission decays changed to single exponential over the entire temperature range. Whereas, addition of 0.02 M n-hexylamine caused the decay to change to multieponential at lower temperature and single exponential at higher temperature but, addition of 0.08 M n-hexylamine to the saline system caused the emission decays to be single-exponential throughout the entire temperature range.

Prior reports on micellisation behaviour of triblock copolymers reveal that the size of the PPO blocks and the ratio of PPO/PEO were crucial for dissolution of organic additives [30, 31]. Variance of n-hexanol and n-hexylamine w.r.t hydrophobic ranking caused n-hexanol to exert more pronounced effect; thereby decreasing hydrophobic effect, and decreasing micellar aggregation. When salt is added, the micelles thus formed are rod-like [20]. Upon addition of n-hexanol or n-hexylamine to the saline solution the micellar shape changes from spherical to rod like or possibly ellipsoidal [20]. The added n-hexanol gets segregated between water phase and micellar phase. The preferential segregation in the micellar core causes enhanced growth thus aiding transfiguration to bigger elongated micelle. Contrarily, addition of n-hexylamine in concentration as low as 0.02 M, induced micellar transformation from sphere to long ellipsoidal even though, extent of micellar growth was less noticeable at higher concentration [20]. In order to understand the above phenomenon, we take into account two contrasting factors: (i) Destabilisation of the micelle caused due to segregation of n-hexylamine in aqueous phase thereby affecting water structure. (ii) Micellar growth due to hydrophobic environment caused by interfacial content of n-hexylamine.

On addition of 0.02 M n-hexanol to C6-P123 system biexponential emission decay is observed at 10°C which changes to single exponential thereafter. The faster and slower components at 10°C are 1.24 ns and 2.70 ns respectively. Addition of 0.08 M n-hexanol to the C6-P123 system shows single exponential decay patterns over the entire investigated temperature range. When NaCl is added to C6-P123 system the decays are single exponential at lower temperature and bi-exponential
Figure 3. The time resolved fluorescence emission spectral profile of C-6 (a) in the presence of P-123, (b) in the presence of P-123 and 1M NaCl. Excitation wavelength: 405 nm.

thereafter. Dehydration of the PEO micellar corona occurs upon addition of the salt while, C6 inserts in the micelle.

In the absence of NaCl, addition of 0.02 M n-hexylamine to C6-P123 system shows a triexponential decay pattern at 10°C, biexponential decay pattern at 15°C and single exponential thereafter. Upon addition of 0.08 M n-hexylamine to the said system the decays are fitted by biexponential functions at 10°C but, single exponential function over the higher temperature range. Addition of 0.02 M n-hexylamine to saline C6-P123 system shows biexponential decay pattern at 5°C and single exponential thereafter whereas, 0.08 M n-hexylamine to saline C6-P123 system shows single exponential decay trend over the entire temperature range. The lifetime data are tabulated in Tables S1–S10 in the supporting information.

3.4. Fluorescence anisotropy measurements

The fluorescence anisotropy decays of C6 in P123 were collected in the presence and absence of NaCl, n-hexanol and n-hexylamine with the variation of temperature from 10°C to 50°C, with increment of 5°C (Figure 5). Anisotropy measurements give detailed account of the rigidity faced by the dye molecule in the media. A closer look reveals that each of the anisotropy decays has started below 0.4 which is due to limited resolution of our TCSPC setup. When only copolymer was added to C6 solution the fluorescence anisotropy decay was found to be biexponential below cmt and single exponential above cmt. On addition of 0.02 M and 0.08 M n-hexanol in the presence and absence of NaCl, the decay pattern was found to be single exponential over the entire temperature range. In the absence of NaCl, addition of 0.02 M and 0.08 M n-hexylamine showed biexponential decay pattern at lower temperature followed by a single exponential decay pattern at higher temperature. However, when NaCl was added to 0.02 M and 0.08 M n-hexylamine a single exponential decay pattern was observed throughout. Besides the temperature-dependent agglomeration behaviour of P123 in water, an understanding of the location of the probe would help us evaluate the observed results lucidly. Henceforth, analysis is based upon the rotational diffusion of the probe below and above cmt.

3.4.1. Rotational diffusion below CMT

When the temperature is below cmt, P-123 retains its monomeric form in water hence; its rotational diffusion should be akin to what’s observed in solvents [32]. From our experiment it emerged that the rotational relaxation of C-6 in water is 0.20 ns at 25°C. But below cmt the rotational diffusion of C-6 is much higher compared to that in neat water. Despite the fact that micelles are not fully formed below cmt yet, the rotational relaxation time increases are indicative of the association of the probe molecule with incompletely aggregated micellar structure which causes lessening of fluorescence depolarisation [33]. When 2 %(w/v) P-123 was added, an increase in rotational relaxation time was observed at 19.2°C. This corresponded to micelle formation. Under the circumstances of micellisation the C-6 molecule faces a more restricted environment resulting in an increase in rotational relaxation time by several folds compared to that in neat water.

3.4.2. Rotational diffusion above CMT

The tri-block P-123 aggregates and forms micelle above cmt thus, the rotational diffusion of the probe will be strikingly different from what’s observed below cmt. The
anisotropy decays are single exponential above cmt in the presence of P-123 or with 0.02 M n-hexanol and n-hexylamine in the presence of NaCl. In such micellar arrangements, the fluorescence depolarisation arises wholly due to the rotational dynamics of a single fluorophore species engulfed in the micelle. From our study it appeared that the rotational dynamics of C-6 above cmt is different indicative of the fact that the microenvironment of the probe molecule must have changed at above cmt, and hence the rotational relaxation time also changes.

Dynamic light scattering (DLS) data were collected at 25°C for P-123 alone, in the presence of 1 M NaCl and in the presence of 1 M NaCl along with 0.02 M n-hexanol and 0.02 M n-hexylamine. (Figure 4). At 25°C, the hydrodynamic diameter of P-123 micelle is found to be 17.63 nm which increases to 30.56 nm in the presence of 1 M NaCl. To account for this behaviour, we presume that the added salt prompts growth of micelle by screening the repulsive forces thus decreasing the intermicellar interactions. Hence, amphiphilic molecule approaches each other more closely thus indicating larger micellar transition and higher $D_r$ value. Upon addition of 0.02 M hexanol in the presence of NaCl the hydrodynamic diameter is found to be 27 nm while addition of 0.02 M n-hexylamine changes the micellar hydrodynamic diameter to 20.94 nm. The core radius ($R_C$) is different systems was already reported in the literature [20].

At 25°C if we compare the rotational relaxation time of C-6 in saline P-123 system with varying concentration of n-hexanol the order turns out to be 1 M NaCl+P-123 > P-123 + 0.02 M n-hexanol > P-123 + 1 M NaCl + 0.02 M n-hexanol (Figure 5(b,i,j)).

In order to explain the above phenomenon, we take into account the screening of repulsive forces by NaCl which decreases the intermicellar interactions thereby favouring micellar growth by allowing the amphiphilic molecule to approach each other more closely leading to a sharp increase in microviscosity, and hence rotational-relaxation time. For the same systems at a lower temperature similar trend is observed.

The microviscosity was calculated using the rotational diffusion coefficient ($D_r$) obtained from the Stokes–Einstein equation and correlation time ($\tau_r$) for a spherical micelle [33]. Using the Edwards method [34], the radius of C-6 molecule was found to be 4.165 Å. In the equation used, $T$ stands for temperature in Kelvin scale, $\eta$ stands for microviscosity, $r$ stands for radius of probe molecule. Though the above equations are applicable only when anisotropy decays are single exponential in nature however, we have extended our calculations even to biexponential anisotropic decays for comparison purposes in few systems. The biexponential decays have been asterisk marked in (Tables 3–5).

$$D_r = \frac{kT}{8\pi\eta r^3}$$
$$\tau_r = (6D_r)^{-1}$$

Considering the non-saline system of at 25°C with varying the concentration of n-hexanol, the rotational relaxation time of C-6 varies as P-123 > P-123+0.02 M n-hexanol > P-123+0.08 M n-hexanol (Figure 5(a,c,d)). The larger value of rotational relaxation time is suggestive of a more hindered environment experienced by C-6 moiety within P-123 micelle than in the presence of n-hexanol. Owing to the rigid environment the movement of the dye gets restricted and rotational relaxation time increases. In non-saline system, at a lower temperature i.e. 10°C the rotational relaxation time of C-6 varies as P-123+0.08 M n-hexanol > P-123+0.02 M n-hexanol > P-123. Here, the temperature being below cmt no micelle formation occurs but as the n-hexanol concentration is increased it distributes itself in the continuous water pseudo phase which causes an increase in microviscosity thereby increasing the rotational relaxation time.

A comparison of the non-saline in the presence of n-hexanol and n-hexylamine at 25°C reveals that the rotational relaxation time of C-6 varies as P-123+ 0.02 M n-hexylamine ≈ P-123 > P-123+ 0.02 M n-hexanol (Figure 5(a,c,g)). The above order can be explained by assuming the fact that n-hexylamine being more hydrophilic than n-hexanol remained notably more soluble in water and less in micelle. This preferential solubility produces a little effect on the micellar structure, and hence a little change in microviscosity. Hence, the

![Figure 4](image-url). DLS result of 2 wt% P-123 in 1 M NaCl and in the presence of cosolvents at 25°C.
The rotational relaxation time of the probe molecule remains same even in the presence of n-hexylamine. The greater rotational relaxation time of the n-hexylamine system was due to greater rigidity faced by the probe than in the n-hexanol system. However, at a lower temperature of 10°C the following trend is observed: P-123+0.02 M n-hexylamine > C-6+P-123+ 0.02 M n-hexanol > C-6+P-123. It is due to the fact that decrease in temperature increased the microviscosity faced by the probe in n-hexylamine and n-hexanol as compared to P-123 alone thereby increasing rotational relaxation time.

In the presence of NaCl, when n-hexylamine in increasing concentration was added to C-6+P123 system at 25°C, the rotational relaxation time trended as C-6+P-123+1 M NaCl+0.02 M n-hexylamine > C-6+P-123+1 M NaCl > C-6+P-123+1 M NaCl+0.08 M n-hexylamine (Figure 5(b,e,f)). The conjoint effect of the presence of salt and n-hexylamine in lower concentration brought about a greater change in micellar microviscosity as compared to only salt and hence a higher rotational relaxation time. As the concentration of n-hexylamine increased, it preferentially partitioned in the continuous water pseudo phase thereby affecting microviscosity and rotational relaxation time the least. At a lower temperature of 10°C, the trend modified to C-6+P-123+1 M NaCl > C-6+P-123+1 M NaCl+0.02 M n-hexylamine > C-6+P-123+1 M NaCl+0.08 M n-hexylamine thus suggesting that in the lower temperature range effect of salt alone on the microviscosity as experienced by the probe is greater than that of salt and n-hexylamine combined together.

Under a salt free condition at 25°C, addition of varying concentration of n-hexylamine to P-123+C-6 system varied rotational relaxation time as C-6+P-123+0.02 M n-hexylamine ≈ C-6+P-123 > C-6+P-123+0.08 M n-hexylamine (Figure 5(a,g,h)). n-hexylamine being more hydrophilic, remained notably more soluble in water and less in micelle. This preferential solubility does not affect micellar structure. Hence, the rotational relaxation time of probe molecule remained same even in the presence of 0.02 M n-hexylamine. In higher concentration preferential partitioning of n-hexylamine in the continuous water pseudo phase affected microviscosity as compared to only salt and rotational relaxation time the least. As the temperature was decreased to 10°C the order of rotational relaxation time altered to C-6+P-123+0.08 M n-hexylamine > C-6+P-123+0.02 M n-hexylamine > C-6+P-123. A decrease in temperature increased the microviscosity faced by the probe thereby increasing rotational relaxation time. The anisotropy decay parameters of C-6 in the presence of saline P-123, additives along with non-saline P-123 and additives have been represented in Tables 1–5. The polymer chains inside a micellar core do not have any orderliness hence, the microenvironment resembles that of a homogeneous non-polar liquid. In our study, we have found out that the anisotropy decays above
Table 1. Anisotropy decays parameters of C-6 in P-123 and in the presence of 1 M NaCl.

| System | $r_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) | System | $r_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) |
|--------|----------------|---------|----------------------|--------|----------------|---------|----------------------|
| P-123+C-6 at 10°C | 1.43 | 0.092 | 18.4 | P-123+C-6+1 M NaCl at 10°C | 13.03 | 1.008 | 168 |
| P-123+C-6 at 15°C | 2.33 | 1.048 | 30.4 | P-123+C-6+1 M NaCl at 15°C | 9.47 | 0.963 | 124.4 |
| P-123+C-6 at 19.2°C | 7.03 | 0.926 | 29.6 | P-123+C-6+1 M NaCl at 19.2°C | 9.12 | 0.959 | 121.4 |
| P-123+C-6 at 25°C | 6.27 | 1.050 | 84.9 | P-123+C-6+1 M NaCl at 25°C | 6.12 | 1.098 | 83.9 |
| P-123+C-6 at 30°C | 5.07 | 0.985 | 69.6 | P-123+C-6+1 M NaCl at 30°C | 4.76 | 1.053 | 65.8 |
| P-123+C-6 at 35°C | 4.35 | 1.052 | 61.6 | P-123+C-6+1 M NaCl at 35°C | 3.97 | 1.100 | 56 |
| P-123+C-6 at 40°C | 3.32 | 1.048 | 47.5 | P-123+C-6+1 M NaCl at 40°C | 3.23 | 0.995 | 45.8 |
| P-123+C-6 at 45°C | 2.78 | 1.130 | 41 | P-123+C-6+1 M NaCl at 45°C | 2.82 | 1.077 | 41 |
| P-123+C-6 at 50°C | 2.19 | 1.142 | 32.3 | P-123+C-6+1 M NaCl at 50°C | 2.36 | 1.075 | 34.6 |

Table 2. Anisotropy decays parameters of C-6 in the presence of P-123 and 0.02 M n-hexanol and 0.08 M n-hexanol.

| System | $r_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) | System | $r_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) |
|--------|----------------|---------|----------------------|--------|----------------|---------|----------------------|
| P-123+C-6+0.02 M n-hexanol at 10°C | 2.24 | 1.008 | 28.9 | P-123+C-6+0.02 M n-hexanol at 10°C | 6.09 | 1.014 | 78.8 |
| P-123+C-6+0.02 M n-hexanol at 15°C | 7.47 | 0.964 | 98.2 | P-123+C-6+0.02 M n-hexanol at 15°C | 5.58 | 1.018 | 73.5 |
| P-123+C-6+0.02 M n-hexanol at 19.2°C | 7.39 | 1.012 | 96.6 | P-123+C-6+0.02 M n-hexanol at 19.2°C | 4.84 | 1.125 | 65 |
| P-123+C-6+0.02 M n-hexanol at 25°C | 5.70 | 1.095 | 77.6 | P-123+C-6+0.02 M n-hexanol at 25°C | 3.67 | 1.028 | 50 |
| P-123+C-6+0.02 M n-hexanol at 30°C | 4.41 | 1.038 | 61 | P-123+C-6+0.02 M n-hexanol at 30°C | 2.94 | 1.032 | 40.6 |
| P-123+C-6+0.02 M n-hexanol at 35°C | 3.60 | 1.031 | 50.6 | P-123+C-6+0.02 M n-hexanol at 35°C | 2.35 | 1.041 | 33 |
| P-123+C-6+0.02 M n-hexanol at 40°C | 2.96 | 1.142 | 42.8 | P-123+C-6+0.02 M n-hexanol at 40°C | 1.88 | 1.052 | 27 |
| P-123+C-6+0.02 M n-hexanol at 45°C | 2.52 | 1.036 | 36.6 | P-123+C-6+0.02 M n-hexanol at 45°C | 1.55 | 1.122 | 22.4 |
| P-123+C-6+0.02 M n-hexanol at 50°C | 1.94 | 1.094 | 29 | P-123+C-6+0.02 M n-hexanol at 50°C | 1.27 | 1.099 | 18.7 |

3.5. Comparison with the previous literature data

At this phase, a comparison of the microenvironment of P-123 with other non-ionic micelles such as F-127 would be intriguing. All of the aforesaid micelles though consist of similar PPO units in their outer shell yet, differ strikingly in the number of PEO units. There is 70% contribution of PEO units in F-127 while 30% PEO units in P-123 [20]. Even though, the probes so used in these micelles are all solubilised into the PEO
region; the microenvironment so experienced varies; F-127 copolymer is more hydrophilic as compared to P-123. A detailed literature survey reveals that when NaCl was added to F-127 solution, a marked reduction in CMT was observed which corresponds to the salting out effect by NaCl thus promoting micellisation [20]. The effect of salt on both the copolymers is thus akin. However, when additives such as n-hexanol and n-hexylamine were added to F-127 solution literature survey revealed that its effect was lesser as against P-123. This can be justified by considering that the greater content of PEO moiety makes F-127 micellar region less hydrophobic [20] thus posing a difficulty in the accumulation of the additives.

Literature survey revealed that the fluorescence emission decay of C-151 and C-153 probes when measured in P-123 and F-127 micelles as a function of temperature, the lifetimes did not show appreciable variation with increasing temperature and was found to fit single exponentially over the entire temperature range [30]. This led the authors to conclude that there occurred no significant change in the microenvironment of the two micelles in the studied temperature range. Anisotropy decays collected for the same system as a function of temperature revealed a bi-exponential nature [30] which is different from our studied system. Dutt et al. have demonstrated the rotational diffusion of two structurally alike hydrophobic probes DMDPP and DPP in aqueous P-123 solution along with variation of temperature [7]. Two widely different results were obtained pertaining to cmt. Below cmt, the life-time and anisotropy decays were single exponential in nature whereas, above cmt bi-exponential decay patterns were obtained [7] for both life-time and anisotropy studies. The life-time

### Table 4. Anisotropy decays parameters of C-6 in the presence of P-123, 1 M NaCl, 0.02 M n-hexanol and 0.08 M n-hexylamine.

| System | $\tau_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) |
|--------|------------------|---------|---------------------|
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 10°C | 9.58 | 0.995 | 123.7 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 15°C | 8.22 | 0.911 | 107.9 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 19.2°C | 6.55 | 1.013 | 87.5 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 25°C | 5.13 | 1.039 | 69.9 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 30°C | 4.07 | 1.034 | 56.2 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 35°C | 3.25 | 1.078 | 45.7 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 40°C | 2.58 | 1.063 | 36.8 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 45°C | 2.11 | 1.064 | 30.7 |
| P-123+C-6+1 M NaCl+0.02 M n-hexanol at 50°C | 1.76 | 1.105 | 25.9 |

*a Error is ±0.5 ps.  
*b Error is ±1 cP.

### Table 5. Anisotropy decays parameters of C-6 in the presence of P-123, 1 M NaCl, 0.02 M n-hexylamine and 0.08 M n-hexylamine.

| System | $\tau_{rot}$ (ns) | $\chi^2$ | $\eta_{micro}$ (cP) |
|--------|------------------|---------|---------------------|
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 10°C | 11.23 | 0.956 | 145.4 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 15°C | 9.86 | 0.908 | 129.6 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 19.2°C | 7.23 | 1.041 | 96.2 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 25°C | 5.09 | 1.062 | 70.8 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 30°C | 4.04 | 1.055 | 55.8 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 35°C | 3.39 | 1.015 | 47.8 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 40°C | 2.71 | 1.049 | 38.7 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 45°C | 2.22 | 1.070 | 32.2 |
| P-123+C-6+0.02 M n-hexylamine+1 M NaCl at 50°C | 1.93 | 1.034 | 28.5 |

*a Error is ±0.5 ps.  
*b Error is ±1 cP.
values for both the probes were lower below cmt and higher above cmt. In our case using C-6 in P-123, we found bi-exponential anisotropy decays patterns below cmt whereas above cmt single exponential decay was observed.

4. Conclusion

The present work studied the dynamics of a hydrophobic molecule C6 within a triblock micellar media of P-123 in the presence and absence of NaCl, n-hexanol and n-hexylamine with a variation of temperature from 10°C to 50°C, with an increment of 5°C. The absorption spectrum of C6 in the presence of P-123 was observed at 458 nm at 25°C. Addition of 1 M NaCl did not show subsequent shift in the absorption spectra whereas, addition of 0.02 M n-hexanol and n-hexylamine to the saline system produced a small shift to 459 and 461 nm. 0.08 M n-hexanol or n-hexylamine to the saline system did not exert any effect on the absorption spectra owing to their preferential solubilisation in the water pseudo-phase. The emission study signified an increase in emission intensity with increasing temperature owing to micellar shape transition (sphere to rod). C6 in P-123 undergoes a blue shifting of the emission maxima above cmt indicating the presence of the probe molecule within the micellar core. Additional of NaCl or n-hexanol and n-hexylamine did not produce appreciable change in the emission spectra. The near equality in the values of fluorescence lifetime of C6 in n-hexanol and P-123 with and without salt and additives confirms the location of the C6 molecule within the core of the micelle. In the presence of P-123 alone, the decays are biexponential below cmt while single exponential above it. Under salt free condition, addition of 0.08 M n-hexanol shows single exponential decay pattern while addition of 0.02 M n-hexylamine caused the decay to change to biexponential at lower temperature and single exponential at higher temperature. Introduction of NaCl to the C6-pluronic system caused the emission decays to be single exponential over the entire temperature range. Upon addition of n-hexanol or n-hexylamine to the aforesaid system the emission decays remained as single exponential. Anisotropy measurements reveal a difference in the rotational dynamics of the probe below and above cmt thus indicating a change in the microenvironment of the probe molecule. When 2 wt% P-123 was added, an increase in rotational relaxation time was observed at 19.2°C. This corresponded to micelle formation. Under the circumstances of micellisation the C-6 molecule faces a more restricted environment resulting in an increase in rotational relaxation time by several folds compared to that in neat water (0.20 ns). The anisotropy decays are single exponential above cmt in the presence of P-123 or with 0.02 M n-hexanol and n-hexylamine in the presence of NaCl.

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Disclosure statement

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