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# Monitoring thermo-reversible dehydration of pluronic microenvironment using 4-chloro-1-naphthol as ESPT fluorescent molecular probe

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## Abstract

Pluronics are polymeric surfactants which undergo sol-gel phase transition both as a function of temperature and concentration and have high impact in therapeutics. An excited state proton transfer (ESPT) fluorescent molecular probe, 4-chloro-1-naphthol, has been employed to explore the sol-gel transition of pluronics in aqueous media. In aqueous homogeneous media, 4-chloro-1-naphthol shows anionic emission, whereas, in heterogeneous media it offers dual emission of its neutral and anionic form. Moreover, the variation of intensity (I) ratio, $I_{neutral}/I_{anion}$ and the area (A) ratio under the two individual curves, $A_{neutral}/A_{anion}$ faithfully reflect the progressive changes of medium heterogeneity. Both the $I_{neutral}/I_{anion}$ and $A_{neutral}/A_{anion}$ values increase with increasing temperature during sol-gel phase transition and reach maxima at the phase transition temperature. Moreover, the higher value of $I_{neutral}/I_{anion}$ and $A_{neutral}/A_{anion}$ in 10% P123 than that of F127 signify lower micro-polarity of P123 media than F127. Evidence of progressive dehydration comes from the excited state decay dynamics. It shows, proton transfer rate decreases remarkably up to the sol-gel transition temperature and after that it remains almost constant. In the present study, thermo-reversible sol-gel transition of pluronics (P123, F127) along with the dehydration of micelle has been monitored by 4-chloro-1-naphthol.

## Keywords

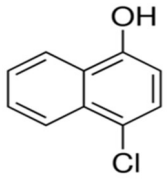
4-chloro-1-naphthol, ESPT, pluronics, sol-gel transition, dehydration.

## Introduction

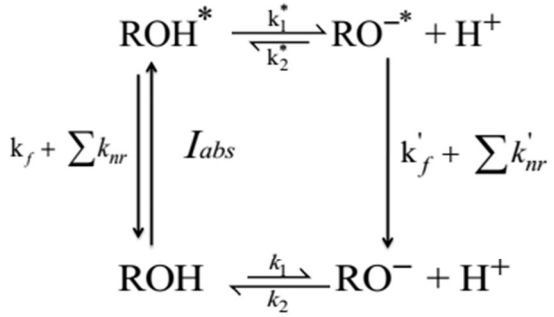
Molecules having higher acidity at the excited state are called, excited sate acids or photoacids[1-5] e.g. naphthols, benzophenones, coumarins, curcumin, falvones etc.[6-12] Re-distribution of charge density in the excited state causes facile removal of proton upon photo-excitation.[1-12] Naphthol based excited state proton transfer (ESPT) fluorescent molecular probes are very useful because of their faster proton transfer rate than fluorescence.[13] As for example, 1-naphthol ($pK_a$ = 9.2, $pK_a^*$ = 0.4) is a well-known ESPT fluorescent molecular probe which has been used frequently in biological and physical studies.[13-21] With the large difference in $pK_a$ (8.75) and $pK_a^*$ (1.73) values, 4-chloro-1-naphthol, a 1-naphthol derivative, can also be used as an effective ESPT fluorescent molecular probe.[22] Here, electron withdrawing inductive effect of chlorine helps in stabilizing the conjugate base of 4-chloro-1-naphthol in excited state.[22] As described by Pappayee et al., 4-chloro-1-naphthol is a potential but rarely used ESPT molecular probe among the naphthol derivatives.[22] Figure 1 shows molecular structure of 4-chloro-1-naphthol and schematic representation of its ESPT process. 4-chloro-1-naphthol is mainly used as a staining agent. In the presence of peroxide, horseradish peroxidase (HRP) catalyzes oxidation of 4-chloro-1-naphthol to 4-chloro-1-naphthon. This provides an easy chromogenic detection which can be photographed easily.[23-28] But unlike 1-naphthol, there are very few photo-physical studies which describe the potential of 4-chloro-1-naphthol as a probe of organized media.[22,29-31]

In water, 4-chloro-1-naphthol remains as ground state neutral form which upon photo-excitation undergoes ESPT process to form anion. Four water molecules make a cluster which acts as a

base to solvate the proton ejected by the photo-acid.[32] As a result, in aqueous medium emission comes from anionic (460 nm) form entirely. With the decrease in polarity and availability of water this anionic peak diminishes along with the increase in neutral peak (360 nm).[16,22] Because of having two distinct emitting states it acts as a multi-domain probe in micro-heterogeneous media.[22] The prototropic equilibrium between anionic and neutral form is highly sensitive towards the micro-environmental change, which is evident from the alteration in emission intensity ratio.[22] The fascinating aspect of this ESPT molecule has been employed here to follow the thermotropic micro-environmental change of pluronic co-polymers, P123 and F127.




(a)




(b)

Figure 1: (a) Molecular structure of 4-chloro-1-naphthol and (b) schematic representation of ESPT process.

Pluronics are water soluble tri-block copolymers ($PEO_x$-$PPO_y$-$PEO_x$, x and y vary for different variety of pluronics) composed of hydrophilic poly(ethyleneoxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) units.[33] These polymers possess amphiphilicity due to the temperature induced preferential solubility of one unit over another.[34,35] As a result, they form spherical micelle and gel both as a function of temperature and concentration.[36,37] Highly hydrophobic anhydrous inner core region of these micelles helps in solubilizing water insoluble drug molecules.[37,38,39] Highly swollen hydrophilic outer surface, corona helps in the water solubility of these polymers.[40-43] Here, two pluronic varieties, hydrophobic P123 ($PEO_{20}$-$PPO_{70}$-$PEO_{20}$) and hydrophilic F127 ($PEO_{100}$-$PPO_{64}$-$PEO_{100}$) have been used to monitor the temperature induced changes. 10% solutions of pluronics have been used over a range of temperature, such that the solutions remain above CMC (Critical Micellar Concentration) or CMT (Critical Micellar Temperature) but below the transparent hydrogel.[37,44,45] It is known that, monomeric units of pluronic are effective for the biological activities.[40] So, in drug composition these polymers are used at higher concentration, so that it dilutes into monomer in blood stream.[40] In our previous work, an aggregate forming coumarin-cholesterol conjugate (Cum-Chl) was used to investigate the micro-polarity difference and sol-gel transition of pluronics.[41] Characteristics fluorescence feature of Cum-Chl, $I_{monomer}/I_{aggregate}$ was used for that purpose. Here, a conceptually different, easily usable, commercially available, ESPT fluorescent molecular probe has been employed in pluronics media to follow the progressive dehydration during sol-gel transition with the aid of its $I_{neutral}/I_{anion}$ and $A_{neutral}/A_{anion}$ values, shift of emission maxima and lifetime of two prototropic forms. Use of such small fluorescent molecular probes is sometimes useful because of their minimum perturbation to the investigating system as compared to the aggregate forming bulky molecular probes.[41] Moreover, systems can be monitored at two different wavelengths

with ESPT probes having largely separated emission spectra. Also, the fluorescence lifetimes of the ESPT probe help to calculate the proton transfer rate which is an indicator of the progressive dehydration.

The specific objectives of this paper are (i) to follow the sol-gel phase transition, (ii) to monitor the sensitivity of 4-chloro-1-naphthol towards the micro-polarity of different pluronics by using $I_{neutral}/I_{anion}$ and $A_{neutral}/A_{anion}$ values and (iii) to probe the dehydration and modulation of ESPT process with sol-gel transition using fluorescence lifetime data.

## Experimental

### Materials

Pluronic P123, F127 and 4-chloro-1-naphthol were purchased from Sigma Chemical Co. (Bangalore, India). Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). Same batch of pluronics have been used for all experiments. Triple distilled water used for all experiments was prepared by using $KMnO_4$ and NaOH. Spectroscopic grade ethanol was used for preparing stock solution of probe.

### Preparation of pluoronic solutions

10% (w/v) solution of pluronic P123 and F127 in pH 7 phosphate buffer were used for the temperature dependent experiments. Temperature was varied from 5-24℃ and 13-34℃, respectively, for P123 and F127 solution. As pluronics are soluble in cold water only, polymeric solutions were kept in fridge to ensure complete dissolution. Then aqueous probe solution was added with the prepared polymeric solutions and kept over-night to ensure homogeneity.

**Steady state and time resolved fluorescence measurements**

Steady state fluorescence measurements were performed by using Fluoromax 4 (Horiba Jobin Yvon) spectrofluorimeter having 150 W Xenon lamp as source of excitation. 3/3 slit width has been used for all experiments. Data acquisition for lifetime experiments was done by Horiba Jobin Yvon TCSPC lifetime instrument in time-correlated single-photon counting arrangement. Nano-LED of 295 nm was used as excitation source. The pulse repetition rate was set at 1 MHz. Instrumental full width at half-maxima of the 295 nm LED, including the detector response was measured to be ~800 ps. The instrument response function was collected by using scattered medium, LUDOX AS40 colloidal silica. IBH software was used for the decay analysis. Decays were fitted to get a symmetric distribution keeping $\chi^2$ value in the range $0.99 \leq \chi^2 \leq 1.4$. Average fluorescence lifetime ($\tau_{aveg}$) was calculated by using the following equation where $\tau_i$ is the lifetime of a component with amplitude $\beta_i$, i signifies number of components present.[46]

$$\tau_{average} = \frac{\sum_{i=1}^{n} \beta_i \tau_i}{\sum_{i=1}^{n} \beta_i}$$

Ethanolic solution of 4-chloro-1-naphthol was used as stock solution for all experiments and finally diluted with water. Ethanol contamination was kept less than 1% to avoid any kind of interference. Final probe concentration was maintained at 2.5 μM for all experiments. The desired temperature was controlled by water circulation through jacketed cuvette holder from a refrigerated bath (Julabo, Germany).

## Results and Discussion:

### Steady state fluorescence spectroscopy data.

Here, the steady state fluorescence response of 4-Cl-1-naphthol has been monitored during sol-gel transition of pluronics. It was found that 10% P123 and F127 undergo sol-gel transition at 15℃ and 21℃, respectively.[41] In aqueous medium, emission of 4-Cl-1-naphthol comes only from the anionic species ($RO^-$*), Supporting Information, Figure S1. This anionic emission decreases slightly with increasing temperature. But, in presence of polymeric heterogeneity both the anionic ($RO^-$*) and neutral form (ROH*) emissions are observed.[16,17,21,22] Figure 2a and b show the fluorescence spectra of 4-Cl-1-naphthol as a function of temperature in presence of 10% P123 and F127, respectively. Neural form intensity has been found to increase along with the concomitant decrease in anionic emission intensity with increasing temperature. An iso-emissive region ~ 415 nm signifies two states equilibrium between the neutral and anionic form. This indicates, that the ratio of neutral to anionic form intensity, $I_{neutral}/I_{anion}$, can be a reliable tool to monitor the thermo-reversible sol-gel transition.

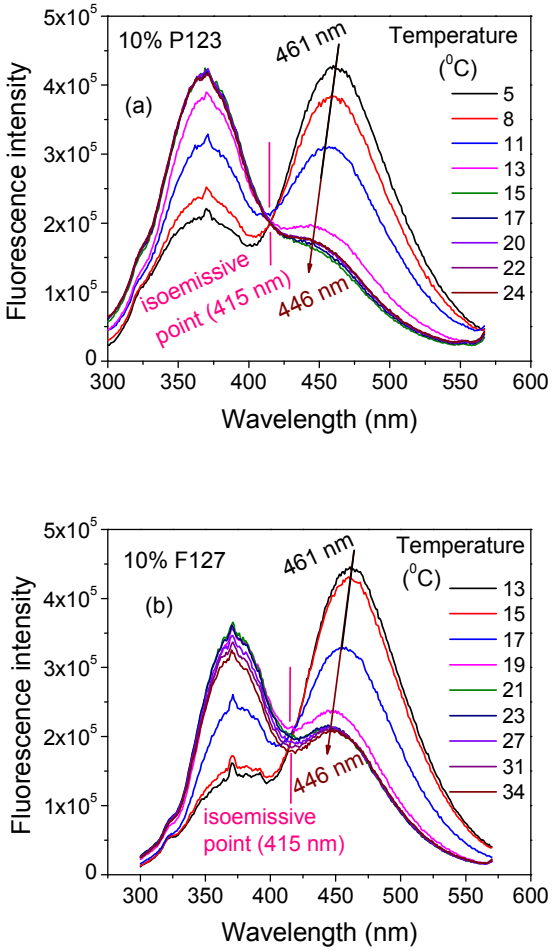
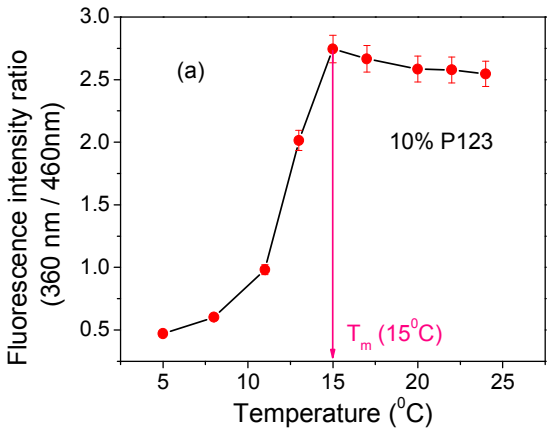
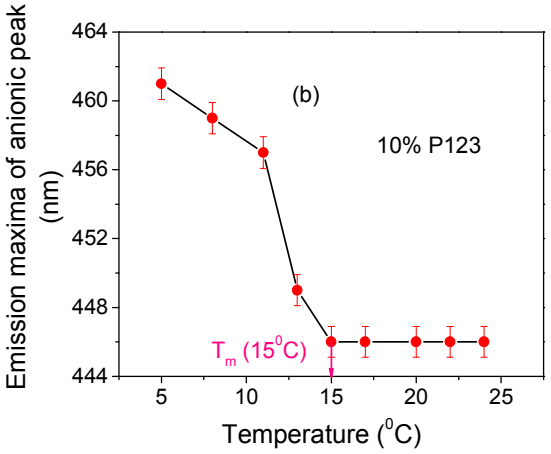


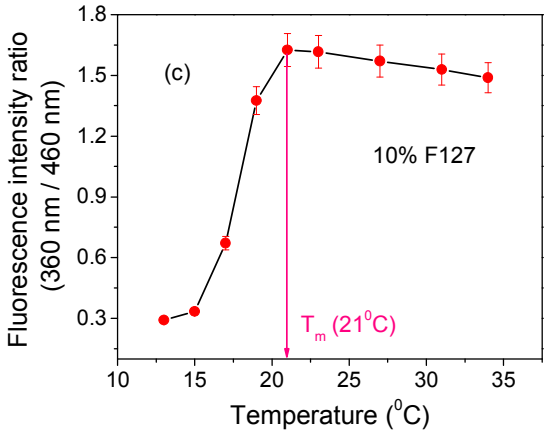

Figure 2: Fluorescence spectra of 4-Cl-1-naphthol in (a) 10% P123 and (b) 10% F127 media as a function of temperature; at $\lambda_{ex}$ 290 nm.

$I_{neutral}/I_{anion}$ values calculated from Figure 2 along with the blue shift of the anionic emission have been used to monitor the change in pluronic micro-environment. Figure 3a and c show the variation of $I_{neutral}/I_{anion}$ values as a function of temperature in P123 and F127 media, respectively. With increasing temperature, $I_{neutral}/I_{anion}$ increases significantly up to the sol-gel transition temperature (Figure 3a and c) and after that it decreases slightly. Individual emission intensities of both the neutral and anionic form also follow the sol-gel transition. Both the increase of neutral (Supporting Information, Figure S2a for 10% P123 and c for 10% F127) and decrease of anionic emission intensity (Supporting Information, Figure S2b for 10% P123 and d

for 10% F127) show onset at the sol-gel transition temperature. In polymeric sol media, anionic emission (461 nm) is blue shifted as compared to the aqueous media (470 nm), due to the increased non-polarity in presence of pluronics (Figure 3b and d). Moreover, the anionic emission maxima have been found to undergo a hypsocromic shift of ~15 nm (from 461 nm in the sol phase to 446 nm in gel phase) with sol-gel transition (Figure 3b and d). The blue shift of anionic emission maxima follows the sol-gel transition of the respective pluronics (Figure 3b and d). The shift of anionic emission maxima had also been found with 1-naphthol in F127 media.[21] In sol state, pluronics are known to be in spherical micellar form which is highly hydrated in the solution. Corona region of PEO units are known to swollen twice of its actual volume in presence of water.[47] As temperature increases, hydrogen bonding of PEO units with water molecules breaks down leading to an increase in hydrophobic interaction. This enhanced hydrophobic interaction induces hydro-gel formation which shrinks the corona region in its cubic gel phase.[48] As a result, hypsochromic shift of emission maxima and the intensity variation of both neutral and anionic from has been observed (Figure 2). This kind of polarity change is more pronounced before sol-gel transition. As a result, all the fluorescence parameters undergo considerable change up to the sol-gel transition temperature.[49,50] The slight decrease in the value of $I_{neutral}/I_{anion}$ after sol-gel transition temperature is due to the expulsion of the small molecular probe from the micellar media into aqueous bulk.[49] Similar response of steady state fluorescence parameters had also been observed for 1-naphthol in pluronic F127 media.[21]



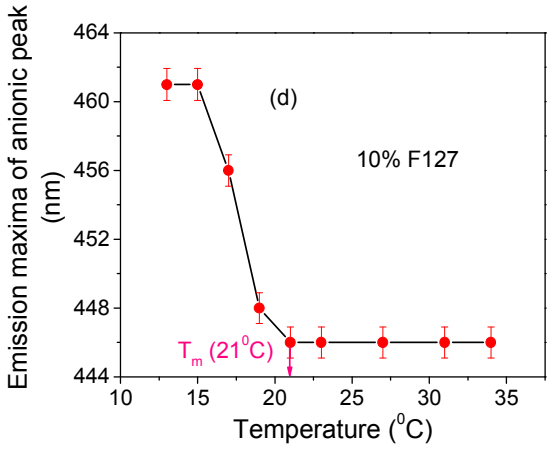



Figure 3: (a) Variation of $I_{neutral}/I_{anion}$ and (b) anionic emission maxima of 4-Cl-1-naphthol in 10% P123 media with temperature. (c) Variation of $I_{neutral}/I_{anion}$ and (d) anionic emission maxima of 4-Cl-1-naphthol in 10% F127 media with temperature; at $\lambda_{ex}$ 290 nm.

Two different prototropic forms (neutral and anionic) of 4-Cl-1-naphthol, report from two different micro-domains of the polymeric micelles.[51] Being neutral in nature, ROH* emission is expected to come from the core region and core-corona interface of micelle, whereas, $RO^-$* emission comes mostly from the interfacial region. This has been further discussed in the next section with the help of lifetime data. From Figure 3a and c it is clear that, $I_{neutral}/I_{anion}$ has higher value in 10% P123 media than F127. This is due to the higher hydrophobicity of P123 (HLB index, 8) that favors more neutral population than the F127 (HLB index, 22).[39] Here, $I_{neutral}/I_{anion}$ not only follows the thermo-reversible sol-gel transition but also reflects the difference in hydrophobicity of the polymeric media. In our previous publication, synthesized probe molecule Cum-Chl had been used to follow the sol-gel phase transition and micro-polarity of pluronics using its $I_{monomer}/I_{aggregate}$ parameter.[41] The polarity driven partitioning of Cum-Chl H-aggregates into the micellar media in form of monomer was the key factor to determine $I_{monomer}/I_{aggregate}$ ratio. Here, in this present investigation the polarity induced proton transfer of 4-Cl-1-naphthol has been used as the principle of analysis.

In connection with this, it is important to note that both the polymers, P123 and F127 have their intrinsic fluorescence which contaminates mostly the neutral form emission and slightly the anionic emission, as evident from Supporting Information, Figure S3a and b. Contribution of intrinsic fluorescence is negligible as compared to the emission of 4-Cl-1-naphthol neutral form as seen in Supporting Information, Figure S3a and b (intrinsic fluorescence has been plotted in the same scale of the probe's emission intensity). Moreover, this intrinsic fluorescence doesn't change with temperature unlike the neutral form fluorescence.[21] Additionally, as the neutral form intensity increases with temperature the contribution of intrinsic fluorescence become progressively insignificant. In Figure S4 (Supporting Information), the intrinsic fluorescence of pluronics (Supporting Information, Figure S3) has been subtracted from the spectra of 4-Cl-1-naphthol in pluronics media (Figure 2) to get more reliable spectra. It shows that the newly obtained spectra (Supporting Information, Figure S4) do not alter the previous conclusion obtained from Figure 2. The subtracted spectra have been fitted with double Gaussian function (Supporting Information, Figure S5 and S6) to visualize the individual spectra. From this, area under the fitted curves has been calculated which indicates the contribution of two different prototropic forms. Like intensity ratio ($I_{neutral}/I_{anion}$), area ratio of the two forms ($A_{neutral}/A_{anion}$) has been plotted in Figure 4 which has been found to follow the sol-gel transition of the respective pluronics. The individual area under the plots also follows the sol-gel transition of 10% P123 and F127 (Figure S7, Supporting Information). The insignificant contribution of the neutral form in 10% F127 media at initial temperatures (13 and 15℃) has been taken as zero as they could not be fitted with double Gaussian function. The higher value of $A_{neutral}/A_{anion}$ in 10% P123 media than that of the F127 indicates higher contribution of neutral form in P123 media. This is due to the lower micro-polarity of P123 micelles than F127.

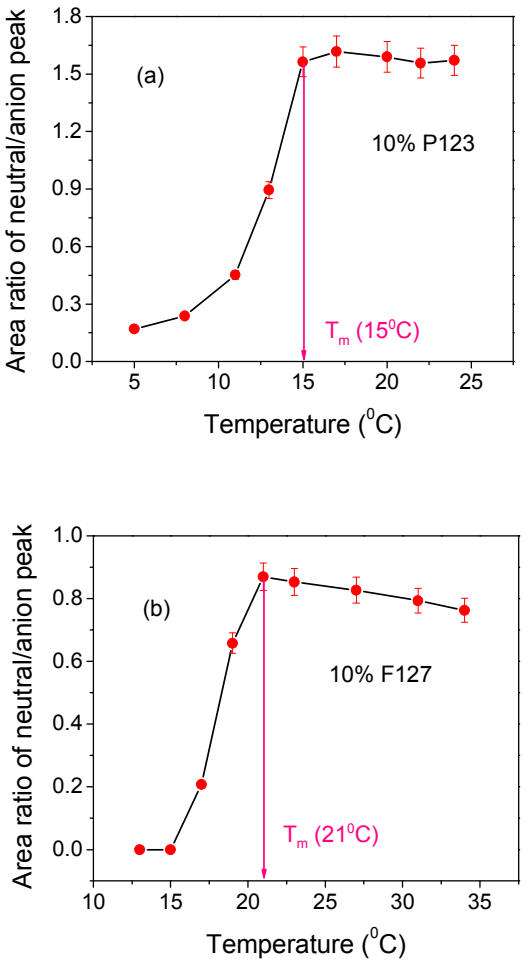



Figure 4: Variation of $A_{neutral}/A_{anion}$ in 10% (a) P123 and (b) F127 media with temperature; at $\lambda_{ex}$ 290 nm.

**Time resolved fluorescence spectroscopy data.**

Excited state decay dynamics has been analyzed after monitoring the steady state fluorescence parameters of 4-Cl-1-naphthol during sol-gel transition of pluronics. Figure 5 represents the fluorescence lifetime decay of 4-Cl-1-naphthol in water with temperature. The lifetime of anionic decay ($RO^-$*, $\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 470 nm) has been fitted with a single exponential function which shows lifetime of ~9 ns, matching closely with the literature value.[22] In addition, this lifetime is found to decrease marginally with increasing temperature, which is consistent with the steady state data. The fluorescence lifetime of 4-Cl-1-naphthol anion in water as a

function of temperature has been enlisted in Table 1. Residue distribution plots for the same have been given in Supporting Information, Figure S8.

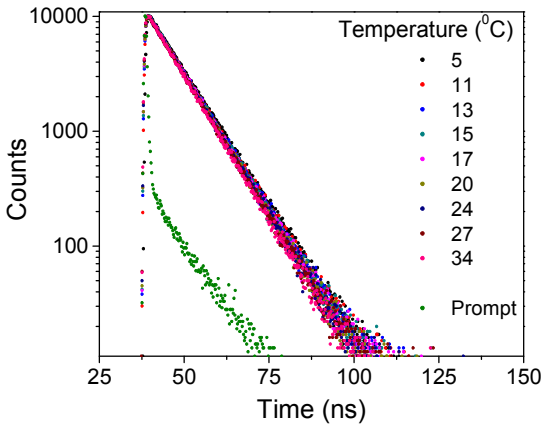



Figure 5: Fluorescence lifetime decay profiles of 4-Cl-1-naphthol anion ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 470 nm) in water as a function of temperature.

Table 1: Fluorescence lifetime data of 4-Cl-1-naphthol anion ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 470 nm) in water as a function of temperature.

| Temperature (°C) | τ (β) | $\chi^2$ |
|---|---|---|
| 5 | 9.47 (1) | 1.09 |
| 11 | 9.33 (1) | 1.05 |
| 13 | 9.30 (1) | 1.28 |
| 15 | 9.19 (1) | 1.21 |
| 17 | 9.15 (1) | 1.24 |
| 20 | 9.11 (1) | 1.17 |
| 24 | 9.06 (1) | 1.19 |
| 27 | 8.99 (1) | 1.26 |
| 34 | 8.83 (1) | 1.19 |

In presence of pluronic P123 and F127, emissions have been monitored for both neutral ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 360 nm) and anionic ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 460 nm) form. Figure 6a and b show the fluorescence lifetime decay profiles of the neutral form emission as a function of temperature in

P123 and F127 media, respectively. As expected from the steady state data, fluorescence lifetime decay of the neutral form is contaminated slightly from the intrinsic fluorescence of pluronics. But, this contamination does not induce any ambiguity in analysis as it is separable after fitting. Fluorescence decay of the neutral form in pluronics media have been fitted with a triple exponential function. The lifetime data of neutral form as a function of temperature has been provided in Table 2 and Table 3 for P123 and F127 media, respectively. Residue distribution plot for the same has been given in Supporting Information, Figure S9 and Figure S10. The longest lifetime component ($\tau_3$, 14-25 ns) with least contribution ($\beta_3$, 0.01-0.03) is coming from the intrinsic fluorescence of pluronics.[21] Similar to the steady-state data, intrinsic fluorescence has a negligible contribution in lifetime and it decreases with increasing temperature.[21] The ~3-4 ns lifetime component ($\tau_2$) is coming from the anhydrous core region, whereas, the shortest lifetime component ($\tau_1$, ~1-2 ns) is originating from the corona and core-corona interfacial region of the micelles. In literature, it has been found that the longer lifetime component of 1-naphthol neutral form comes from the relatively anhydrous and hydrophobic core region.[17,21] The core region is more rigid as compared to the hydrated corona region as a result, non-radiative decay is less in core.[51] This reflects in the higher value of fluorescence lifetime in relatively anhydrous and non-polar core region.[16,17,21] The value of both $\tau_1$ and $\tau_2$ increases from sol state to gel state and is highest at the sol-gel transition temperature of the respective pluronics. As, the change of the pluronic micelles is more pronounced up to the sol-gel transition temperature,[41,49,50] $\tau_1$ and $\tau_2$ show highest value at the phase transition temperature. After that lifetime value decreases slightly due to the increase in non-radiative decay with increasing temperature.[41] Bi-exponential decay of the neutral emission, excluding the intrinsic pluronic decay, signifies its distribution over different sites of pluronics micelle.[51] Similar lifetime distribution had also been found

earlier for both parent 1-naphthol and 4-Cl-1-naphthol neutral form in various micro-heterogeneous media.[16,17,21,22] In liposome media, shorter lifetime (~1 ns) and longer lifetime (~4 ns) component of 4-Cl-1-naphthol neutral form had been assigned for the interfacial and interior population, respectively.[22] Here, higher amplitude of $\beta_1$ over $\beta_2$ indicates greater population of the probe in the interfacial region.

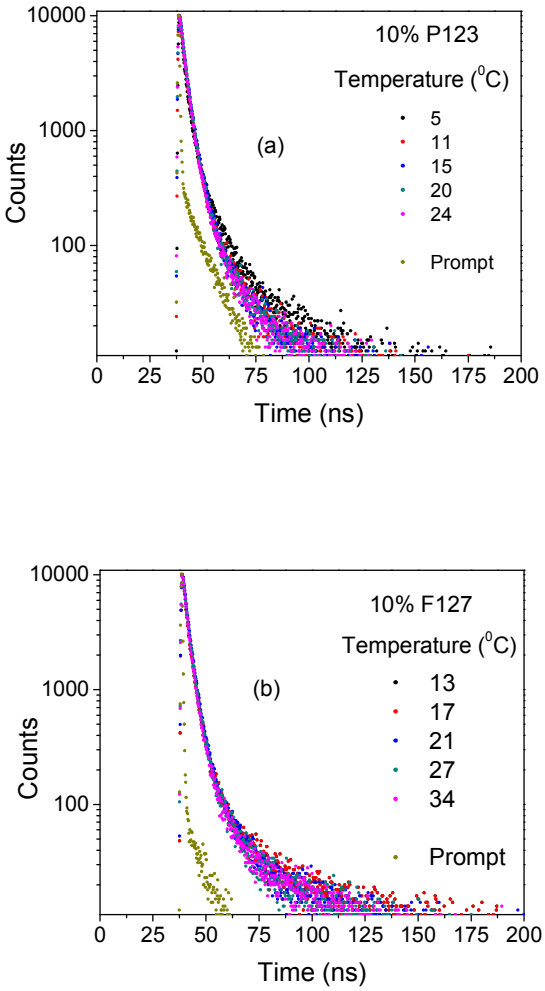



Figure 6: Fluorescence lifetime decay profiles of 4-Cl-1-naphthol neutral form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 360 nm) in (a) 10% P123 and (b) 10% F127 media as a function of temperature.

Table 2: Fluorescence lifetime data of 4-Cl-1-naphthol neutral form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 360 nm) 10% P123 media as a function of temperature.

| Temperature (°C) | $\tau_1$ ($\beta_1$) | $\tau_2$ ($\beta_2$) | $\tau_3$ ($\beta_3$) | $\chi^2$ |
|---|---|---|---|---|
| 5 | 1.10 (0.66) | 3.48 (0.31) | 14.52 (0.03) | 1.22 |
| 11 | 1.67 (0.52) | 3.57 (0.46) | 14.15 (0.02) | 1.18 |
| 15 | 2.21 (0.72) | 4.72 (0.27) | 19.22 (0.01) | 1.02 |
| 20 | 2.09 (0.70) | 4.27 (0.29) | 18.43 (0.01) | 1.18 |
| 24 | 2.11 (0.71) | 4.10 (0.28) | 15.28 (0.01) | 1.09 |

Table 3: Fluorescence lifetime data of 4-Cl-1-naphthol neutral form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 360 nm) in 10% F127 media as a function of temperature.

| Temperature (°C) | $\tau_1$ ($\beta_1$) | $\tau_2$ ($\beta_2$) | $\tau_3$ ($\beta_3$) | $\chi^2$ |
|---|---|---|---|---|
| 13 | 1.09 (0.53) | 3.29 (0.45) | 16.38 (0.02) | 1.22 |
| 17 | 1.41 (0.60) | 3.62 (0.39) | 18.07 (0.01) | 1.14 |
| 21 | 2.37 (0.79) | 4.86 (0.20) | 20.20 (0.01) | 1.01 |
| 27 | 2.07 (0.72) | 4.10 (0.27) | 17.66 (0.01) | 1.23 |
| 34 | 2.10 (0.82) | 4.98 (0.17) | 25.47 (0.01) | 1.16 |

Figure 7a and b show the fluorescence lifetime decay profiles of the anionic emission of 4-Cl-1-naphthol with temperature in P123 and F127 media, respectively. In pluronics, anionic decay has been fitted with a double exponential function. The lifetime data of anionic form as a function of temperature has been given in Table 4 and Table 5 for P123 and F127 media, respectively. Residue distribution plot for the same has been given in Supporting Information, Figure S11 and Figure S12. It has been found, that the anionic emission is slightly contaminated from the intrinsic fluorescence of pluronics due to the presence of its tail towards longer wavelength. The shorter component ($\tau_1$, 3-5 ns) is coming from the intrinsic fluorescence of the pluronics

(Supporting Information, Table S1), whereas, the longer component ($\tau_2$, 13-14 ns) is originating from the anionic form of 4-Cl-1-naphthol in presence of pluronics. Anionic lifetime of 4-Cl-1-naphthol in pluronics is higher as compared to the aqueous media and it does not change with increasing temperature because of its interfacial location. As the probe is bound to the polymer interface the rate of the non-radiative decay decreases leading to an increase in fluorescence lifetime.[52] Unlike 1-naphthol, 4-Cl-1-naphthol anionic form does not show any distributive nature because of its higher polarity due to the presence of the chloro group. The non-distributive nature of 4-Cl-1-naphthol anionic form has also been found earlier in liposome media.[22] In contrast to the anionic form neutral form has been found to undergo multi-domain distribution which can be attributed to the less polar character of the neutral form.[22] For 1-naphthol the observation of rise time (3-4 ns) of anionic fluorescence implies that in the hydrophobic pluronic environment the ionization rate constant of 1-naphthol was significantly reduced.[21] This has not been observed here with 4-Cl-1-naphthol. This is possibly due to the faster rate of proton transfer from 4-Cl-1-naphthol due to the electron withdrawing stabilizing effect of the chloro group at its para position.

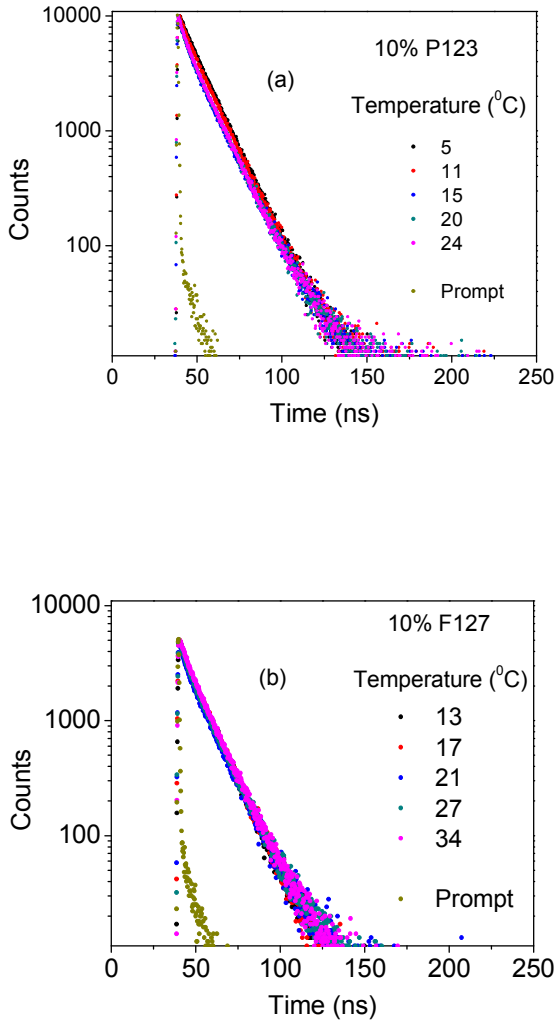



Figure 7: Fluorescence lifetime decay profiles of 4-Cl-1-naphthol anionic form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 460 nm) in (a) 10% P123 and (b) 10% F127 media as a function of temperature.

Table 4: Fluorescence lifetime data of 4-Cl-1-naphthol anionic form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 460 nm) in 10% P123 media as a function of temperature.

| Temperature (°C) | $\tau_1$ ($\beta_1$) | $\tau_2$ ($\beta_2$) | $\chi^2$ |
|---|---|---|---|
| 5 | 5.58 (0.23) | 14.34 (0.77) | 1.07 |
| 11 | 5.04 (0.19) | 14.34 (0.81) | 1.16 |
| 15 | 4.82 (0.34) | 14.77 (0.66) | 1.07 |
| 20 | 4.39 (0.27) | 14.40 (0.73) | 1.13 |
| 24 | 4.04 (0.29) | 14.15 (0.71) | 1.07 |

Table 5: Fluorescence lifetime data of 4-Cl-1-naphthol anionic form ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 460 nm) in 10% F127 media as a function of temperature.

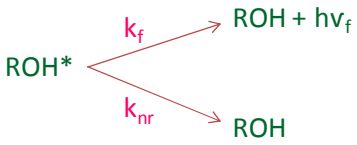
| Temperature (°C) | $\tau_1$ ($\beta_1$) | $\tau_2$ ($\beta_2$) | $\chi^2$ |
|---|---|---|---|
| 13 | 3.88 (0.23) | 13.35 (0.77) | 1.22 |
| 17 | 3.77 (0.22) | 13.42 (0.78) | 1.16 |
| 21 | 3.59 (0.34) | 13.99 (0.66) | 1.29 |
| 27 | 3.89 (0.30) | 13.97 (0.70) | 1.12 |
| 34 | 4.41 (0.20) | 13.81 (0.80) | 1.12 |

In heterogeneous media, the origin of lifetime components of such ESPT molecular probes can be assigned easily. But, this is very complicated with the aggregate forming probe molecules.[41] Along with that, aggregate causes scattering of light due to their larger size which causes difficulty in analysis. This is a significant advantage of using small molecular probes for sensing micro-environmental changes in organized media.

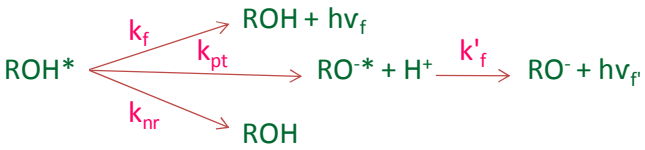
**Determination of ESPT rate.**

Proton transfer rate of ESPT molecule is directly related to the degree of hydration.[16,21] Accessibility of water molecules regulate the polarity of heterogeneous media which reflects in the variation of lifetime data. So, from the lifetime values of 4-Cl-1-naphthol in different

domains rate of ESPT process can be calculated. For this purpose a simple model has been adopted which assumes, in the dry core region ESPT is completely restricted but allowed in the interfacial region.[21] Accordingly, the photo-processes involved after excitation at the core (1) and the interfacial region (2) can be expressed as follows,




(1)




(2)

Scheme 1: Photo-processes of 4-Cl-1-naphthol in (1) core and (2) interfacial region after excitation.

here, $k_f$, $k_f'$ = rate constant of radiative process,

$k_{nr}$ = rate constant of non-radiative process,

$k_{pt}$ = rate constant of proton transfer.

The corresponding lifetimes of the two domains can be expressed as follows,

$$\tau_{core} = 1 / (k_f + k_{nr}) \quad (3)$$

$$\tau_{interface} = 1 / (k_f + k_{nr} + k_{pt}) \quad (4)$$

So, it is obvious that proton transfer rate ($k_{pt}$) can be determined from the difference of two lifetime reciprocals,

$$k_{pt} = (1 / \tau_{interface}) - (1 / \tau_{core}) \quad (5)$$

Figure 8a and b show the modulation of proton transfer rate as a function of temperature, in P123 and F127 media, respectively. There is a sharp decrease in the proton transfer rate with increasing temperature in the sol phase and after sol-gel transition it remains almost constant. This signifies the progressive dehydration of pluronic micelle during the sol-gel transition. This kind of dehydration process can be visualized easily with ESPT molecular probes but not with aggregate forming probes .[41] Reports show that, 1-naphthol had already been used to monitor the level of hydration of different media (e.g. pluronics, lipid bilayer membrane in presence of sub-micellar bile salts, polyvinyl alcohol (PVA) films etc.).[21,16,18]

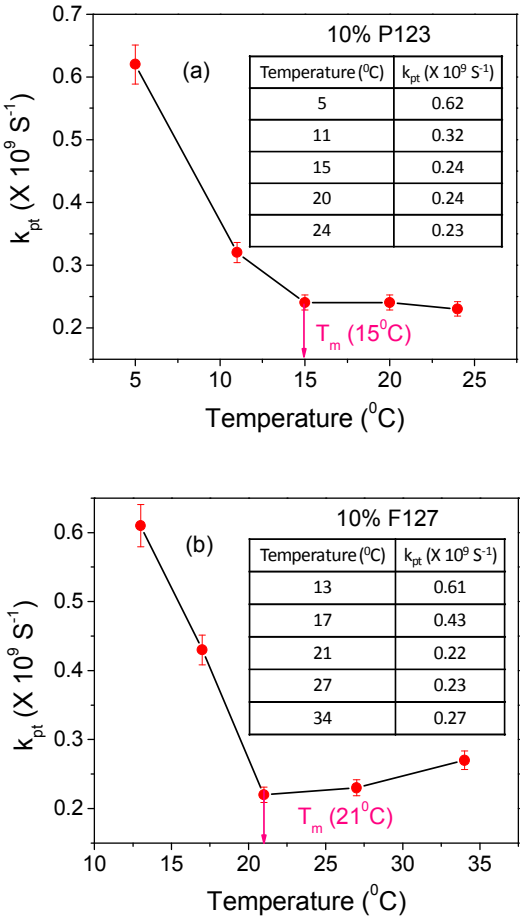



Figure 8: Variation of the proton transfer rate in (a) 10% P123 and (b) 10% F127 media with temperature.

## Conclusions:

Sensitivity of excited state proton transfer (ESPT) fluorescent molecular probe, 4-chloro-1-naphthol, has been employed to investigate the thermo-reversible sol-gel transition of pluronics. Being present in two different prototropic forms of different polarity this molecule serves as a multi-domain probe distributing over various possible micro-domains of pluronics (e.g. core, corona, interface etc.). The multi-domain distribution of 4-chloro-1-naphthol is advantageous over various known fluorophores. Lifetime components with different order of magnitudes also support its distributive nature. Neutral form emission, which comes from the micellar region, increases with increasing temperature. Anionic emission, coming from the interfacial region, decreases with increasing temperature with a blue shift. This makes $I_{neutral}/I_{anion}$ a sensitive parameter for sol-gel transition. Sensitivity of all the fluorescence parameters ($I_{neutral}/I_{anion}$, $A_{neutral}/A_{anion}$, shift of emission maxima, lifetime) indicates the thermo-reversible sol-gel transition of pluronics (P123, F127). Additionally, $I_{neutral}/I_{anion}$ and $A_{neutral}/A_{anion}$ parameters show their sensitivity towards the micro-polarity of different pluronics. Moreover, with increasing temperature, proton transfer rate has been found to decrease remarkably up to the sol gel transition followed by a labeling effect. The rate of proton transfer depends on the availability of water molecules inside the pluronic micelles. So the decrease in proton transfer rate actually signifies the expulsion of water molecules, during sol-gel transition. Finally, from this study 4-chloro-1-naphthol, a staining agent emerges as a potent excited state proton transfer (ESPT) fluorescent molecular probe for studying the thermotropic sol-gel transition, thermo-reversible dehydration and micro-polarity of pluronics.

## Associated Content:

**Supporting Information.** Fluorescence spectra of 4-Cl-1-naphthol in water with temperature, Variation in the fluorescence intensity of neutral form and anionic form of 4-Cl-1-naphthol in 10% P123 and 10% F127 media with increasing temperature, Intrinsic fluorescence of 10% P123 and 10% F127, Subtracted fluorescence spectra of 4-Cl-1-naphthol in (a) 10% P123 and (b) 10% F127 media as a function of temperature; at $\lambda_{ex}$ 290 nm, Double Gaussian fitting of the subtracted spectra of 4-Cl-1-naphthol in 10% P123 media with temperature, Double Gaussian fitting of the subtracted spectra of 4-Cl-1-naphthol in 10% F127 media with temperature, Area under the two curves, in 10% P123 media, (a) neutral and (b) anionic form and in 10% F127 media, (c) neutral and (d) anionic form. Residue distribution plots of 4-Cl-1-naphthol in water at different temperatures, Residue distribution plots of 4-Cl-1-naphthol neutral form in 10% P123 media at different temperatures, Residue distribution plots of 4-Cl-1-naphthol neutral form in 10% F127 media at different temperatures, Residue distribution plots of 4-Cl-1-naphthol anionic form in 10% P123 media at different temperatures, Residue distribution plots of 4-Cl-1-naphthol anionic form in 10% F127 media at different temperatures, Intrinsic fluorescence lifetime data of pluronics at 20°C ($\lambda_{ex}$ = 295 nm, $\lambda_{em}$ = 460 nm).

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# Monitoring thermo-reversible dehydration of pluronic microenvironment using 4-chloro-1-naphthol as ESPT fluorescent molecular probe

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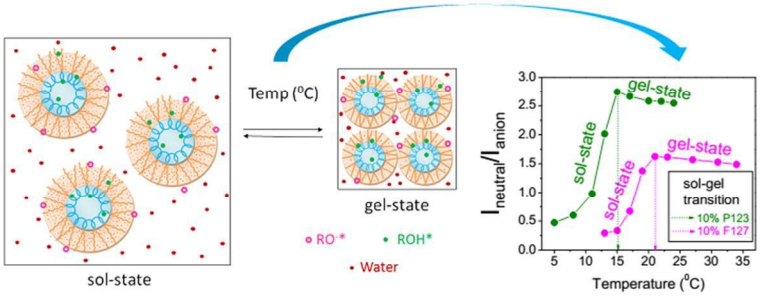
## Graphical abstract




An ESPT fluorescent molecular probe, 4-chloro-1-naphthol has been employed to study thermo-reversible sol-gel transition, dehydration and micro-polarity of pluronics.