Steady-State and Time-Resolved Fluorescence Spectroscopic Studies to Probe Physicochemical Processes in Various Self-Assembled Molecular Systems

Rishika Aggrawal

Abstract

Different self-assembled molecular systems have been systematically investigated to understand the various phenomenon and processes such as solvation dynamics and rotational relaxation in reverse micelles and mixed micelles, transformation of micelle to a bilayer over gold nanoparticles, refolding of bioconjugated protein by catanions and DNA compaction in presence of SiO_2 NPs using gemini surfactants using several fluorescence spectroscopic techniques and other spectroscopic tools. The present research work uses gemini surfactants *(12-s-12,2Br$^-$, s: spacer),* a special class of surfactants which has two monomeric surfactants joined together at the headgroup with a spacer group to study the various self-assembled structures. Biological water also known as bound water differs from the free bulk water on time scale measurements. A study of dynamic exchange between these water molecules in confined systems such as lipids, micelles, reverse micelles, etc. is crucial to understand various biological processes. The role of four states of water *(bulklike, counterion bound, headgroup bound and free water),* solubilized in the reverse micelles of gemini surfactants/cyclohexane/n-propanol/H_2O along with the effect of spacer chain length of the gemini surfactant on the solvation dynamics and rotational relaxation of Coumarin 490 (C-490) has been investigated. The bulklike water (responsible for faster solvation dynamics) increases with addition of water to the reverse micelles. This makes the microenvironment around C-490 more flexible leading to faster rotational relaxation. The counterion dissociation increases with increasing spacer chain length and causes clustering of water molecules. The counterion dissociation controls the number of counterion-bound water per surfactant molecule responsible for slow component of the solvation dynamics. The rate of rotational relaxation decreases with increasing spacer chain length. The increased clustering of water molecules and decrease in the size of the water pool with increasing spacer chain length enhances the rigidity around the probe molecule which delays the rotational relaxation process. Further, solvation dynamics and rotational relaxation studies were also carried out in the micelles of gemini surfactant 12-s-12,2Br$^-$ (s = 4, 6, 8) in the presence of an ionic liquid, C_{12}mimBr to understand the role of dual behaviour of ionic liquid in these processes. The rates of solvation and rotational relaxation increase with increasing concentrations of ionic liquid due to increased size of the Stern layer of mixed micelles as a result of more water penetration in it. But, the effect of the dual behaviour of ionic liquid as a co-surfactant in lower concentrations and co-solvent at higher concentrations is observed in the weightages of the fast and slow solvation components. The rotational diffusion process is also controlled by the dual nature of the ionic liquid in the micelles. The rotational motion of the mixed micelle and the lateral diffusion coefficient (DL) are controlled by the dual nature of C_{12}mimBr. The size of mixed micelles first increases and then decreases with the increasing amounts of the ionic liquid due to its dual nature. The solvation dynamics gets slower with increased spacer chain length due to enhanced counterion dissociation whereas, the rotational relaxation gets faster due to decreased microviscosity with spacer chain length. The solvation dynamics helps to understand the microenvironment around a probe in a confined system which can prove to be highly helpful to understand various biological and chemical processes. Another investigation carried out included various studies performed to understand the transformation of micelle to a bilayer of gemini surfactants over in-situ synthesized gold nanoparticles (AuNPs) by precise tuning of the location of three polarity sensitive dyes:

Coumarin 480 (C-480), Rhodamine 6G (Rh6G) and Coumarin 153 (C-153). C-480 and C-153 were found to residing in the hydrophobic part of the micelles and bilayer. However, C-480 resides in the most hydrophobic and rigid environment amongst the three dyes. Rh6G is found to be present near the headgroups of the surfactant, so some of the dye molecules are on the surface of the bilayer exposed to the aqueous medium while other are near the headgroups adsorbed on the nanoparticle surface. Advantage of the location of Rh6G was taken by performing an excitation-wavelength dependent rotational relaxation experiment. Bilayer system shows different rotational co-relation times at different excitation wavelength while micellar system shows nearly the same values. This is only possible if the shape of the structure is non-spherical as in the case of bilayer over the gold nanoparticle. The gemini capped gold nanoparticles can be further altered and improvised to form a drug-delivery system with tunable properties. Furthermore, the gemini surfactants were utilized to carry out unfolding of AuNPs-conjugated BSA. The refolding studies of this unfolded AuNPs-conjugated BSA was then executed in the presence of sodium dodecyl sulphate (SDS) by the formation of catanions with the already present gemini surfactant in the solution. Negatively charged SDS extracts cationic gemini surfactants from the protein chain, thus prompting its refolding back. The refolding has been probed by Förster's resonance energy transfer (FRET) and nanoparticle surface energy transfer (NSET) phenomena occurring between Trp residues of BSA and AuNPs. Other spectroscopic techniques like circular dichroism (CD), Fourier-transform infrared spectroscopy (FT-IR), Dynamic light scattering (DLS) and Field emission scanning electron microscopy (FESEM) measurements have also been carried out to understand the refolding mechanism. The presence of gemini surfactant with a longer spacer enhances the refolding due to added hydrophobic effect of the spacer. The refolding of proteins in such step by step manner is very essential to understand to the reason behind various neurodegenerative diseases. Lastly, the compaction of ct-DNA using gemini surfactants, in the absence and presence of SiO_2 NPs has been investigated and various biological studies using these formulations have been carried out. The gemini surfactants in this chapter vary in the number of hydroxyl group substitution in the spacer group as 12-4$(OH)_n$-12,2Br^- (n=1, 2). The gemini surfactant with more hydroxyl groups is more effective in compacting DNA both in absence and presence of the SiO_2 NPs. The added effect of SiO_2 NPs in enhancing DNA compaction is due to adsorption of cationic gemini surfactants over negatively charged silica nanoparticles, which allows to aggregate the surfactant molecules at a lower concentration. The aggregation of surfactants induced by nanoparticles through the electrostatic interaction enhances the co-operative binding between the surfactant molecules and DNA leading to improved efficiency of DNA compaction. 50% DNA compaction was calculated from measurements of the structures obtained by the fluorescence microscopic analysis, where it can be seen that the elongated DNA structure changes to globular shape with increasing concentration of the surfactant. The biological studies, cytotoxicity assay, cell internalization, cellular uptake have been carried out on 4T1 cell line. Also the tumor accumulation studies were carried out by capturing the Near-Infrared Fluorescence images using a real-time *in-vivo* imaging system after intravenous injection of the samples into 4T1-tumor bearing mice. The formulations of gemini surfactant with more hydroxyl group in the presence of SiO_2 NPs delivered the highest amount of ct-DNA in cells and the tumor in a time dependent manner. Thus, the use of a gemini surfactant with a spacer with more hydroxyl groups and SiO_2 NPs in compacting and delivering ct-DNA to the tumor is proven, warranting its further exploration in nucleic acid therapy for cancer treatment. Overall, the research detailed can be used to perform various investigations to explore the basic knowledge further as well as can be used for certain applications.