CHAPTER - 6
SUMMARY AND CONCLUSION

Summary and Conclusion

In Chapter 1, a general introduction covering the description of various organised molecular assembly, viz., micelles, microemulsions, vesicles, lipid bilayers etc. and their importance are presented. The physico-chemical properties of the organised assemblies are generally studied by introducing a probe molecule (e.g., pH indicators) and monitoring the change in the properties of the probe in the new environment. For example, investigations of acid-base equilibria of amino acids, their interaction with metal ions in media of varied ionic strength, temperature and dielectric constant throw light on the mechanism of enzymes catalyzed reaction. Although it is known that the polarity of the active site cavities in proteins is lower than that of the bulk, a direct measurement of dielectric constant is not possible. A method wherein comparison of formation constants obtained from acid-base and/or metal complex equilibria with the corresponding values observed at the biological centre offers a way to estimate an effective dielectric constant for the cavity has been invoked.

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Chapter 2 describes the scope and object of the present investigation. The importance of interfacial region between aqueous and non-polar part of the self-assembled lipid phase is very well recognized in biological membranes. For the measurement of the electrical potential at the surface of a charged membrane or a similar interface one requires a probe of molecular size which does not disturb the system itself. It has been suggested that the use of pH indicators can act as probes for charged micelles and attributed the "apparent" shift of pK_a detectable in the micellar solution, as compared to pure aqueous solutions, to a change of the "local interfacial" proton activity at the surface of charged micelles as compared to that in bulk water. The equilibrium of an indicator, bound to a surface, may be affected not only by an electrostatic

potential, but in addition by a different local environment, e.g., by a lower dielectric constant as compared to bulk water and also by specific interactions of the indicator at the surface, if any. Although different types of organic indicator molecules have been applied as spectroscopic probes for studying above properties, surprisingly, two important uv active aromatic amino acids e.g., tyrosine and tryptophan have not been used so far. However, there are certain class of molecules which are biologically quite important but their spectral properties are quite insensitive to the surroundings. It has been found that while the protonation-deprotonation equilibrium of hydroxy group of tyrosine and 5-Hydroxytryptophan influence their electronic spectra to a great extent, the spectral profile of tryptophan is insensitive to acid or base. Thus sometimes, the spectral characteristics, specially fluorescence spectra, are very sensitive to the environments of the systems. Because of this, fluorescence spectroscopy has become one of the fundamental methods for the study of the structure and dynamics of the microheterogeneous systems. The photophysics of probe molecules, therefore, are studied in those systems. Physical gelation of surfactant micelles and their stimuli responsive behaviour are interesting from biology as well as chemical technology points of view. It is particularly interesting that while a wide variety of worm-like ionic micellar solution display identical rheological responses, a common element in all of these systems is the presence of salt anions like sodium salicylate. This limitation has perplexed the scenario to some extent and impeded the development of an acceptable theory which may explain micellar shape transition under dilute condition. It has been found in the present work that molecules like 1- and 2-Naphthols with a strong hydrophobic aromatic ring and a polar hydroxy group support shape transitions of surfactant micelles very efficiently.

Keeping the above aspects in view, a number of biologically important organic molecules and their hydroxy derivatives and also 1- and 2-Naphthols are chosen as indicator (probe) molecules for studying the protonation-deprotonation equilibria at the micellar surface of different ionic and non-ionic

micelles as well as in aqueous-organic medium. These indicator molecules are, 5-Hydroxyindole, 5-Hydroxy-L-tryptophan, L-Tyrosine, L-Tyrosine-methylester, 1-Naphthol and 2-Naphthol. A detail study on the protonation-deprotonation equilibrium of these probes in micellar media has been undertaken. Fluorescence spectroscopic properties of these molecules have been studied in different solvents to understand the photo-physics of the systems.

Moreover, the role of 1- and 2-Naphthols in developing stimuli sensitive properties of surfactant solutions has been studied. The physicochemical aspects of micellar shape transition, rheological behaviour have been investigated and the microstructure of the system is proposed.

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In chapter 3, results of the study on protonation-deprotonation equilibrium of OH groups of biologically important indicator molecules in micellar media as stated above have been presented. The surfactants which have been employed in the present study are Cetyltrimethylammonium bromide (CTAB; cationic surfactant), Sodium dodecylsulfate (SDS; anionic surfactant), Sodium bis (2-ethylhexylsulfo-succinate) (Aerosol-OT or AOT; double tailed anionic surfactant), Polyoxy-ethylenesorbitan monopalmitate (Tween – 40; non-ionic surfactant) and Polyoxyethylenelauryl ether (Brij-35; non-ionic surfactant).

In section 3.1, an introduction and review of previous works is given. Organic compounds are generally soluble in micellar media although they may or may not be soluble in water or organic solvent alone. This solubilization leads to a change in the acid-base equilibrium of the solubilisate organic acid. Two established models, viz., thermodynamic model and pseudophase ion exchange model explain the observed pK_a shifts of the acid-base equilibrium. A brief discussion on both of the models are included. In theoretical discussion on

acid-base equilibrium of a indicator, which has its prototropic moiety residing within the interfacial region of a self-assembled surfactant can be represented as

$$HIN \xrightleftharpoons{K_a} H^+ + IN^-$$
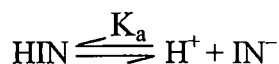
where HIN, IN, H^+ denote the protonated (acid), deprotonated (base) forms of the solute organic molecule and the proton respectively.

For the organic indicators in aqueous micellar solution, the apparent pK_a values were obtained from the change in the ultraviolet absorption spectrum of each indicator with bulk aqueous pH by means of the expression

$$pK_a^{obs} = pH - \log \frac{[IN]}{[HIN]}$$

with

$$\frac{[IN]}{[HIN]} = \frac{\alpha}{1-\alpha}$$

and

$$1 - \alpha = \frac{A_{IN} - A}{A_{IN} - A_{HIN}}$$

where A represents the uv absorbance at the uv wave length band maximum of the deprotonated form of the indicator, λ_{max}, at a given pH, A_{HIN} the absorbance at λ_{max} when all the indicator molecules are protonated and A_{IN} the absorbance at λ_{max} when all the indicator molecules are deprotonated. Representative ultraviolet absorption spectra for 5-Hydroxyindole, 5-Hydroxy-L-tryptophan, L-Tyrosine, L-Tyrosinemethylester, 1-Naphthol and 2-Naphthol, as a function of bulk aqueous pH are presented.

The thermodynamic acid-base equilibrium constant for the reaction in 1,4-dioxane-water mixtures is given by :

$$pK_a^m = B + \log U_H^o - \log \frac{[IN]}{[HIN]} - \log \frac{\gamma_{IN}^m}{\gamma_{HIN}^m}$$

where γ_{IN}^m and γ_{HIN}^m denote the activity co-efficient of the base and acid forms of the indicators, respectively, referred to the particular 1, 4 dioxane-water mixture at infinite dilution. It is not completely clear how one should approximate the activity co-efficients of large complex organic ions such as the present indicator molecules. Consequently, the pK_a^m values given in this work neglect the activity co-efficient term.

In the present work following points have been considered during the study of the acid-base equilibrium of interfacially located indicator molecules:

(i)   Variations of pK_a's, which appear in the study of an interfacial acid-base equilibrium, are characterized.

(ii)   Titration of all indicators (molecular probe) in charged and uncharged micelles is described.

(iii)   The observable shifts of "apparent" pK_a which is partitioned explicitly into a component due to the electrical potential and a component caused by a change of polarity for a charged micelle is described.

(iv)   By comparing the shift of the interfacial intrinsic pK_a^i to the pK_a shift measured in non-polar non-aqueous solvents, an attempt has been made to estimate the effective interfacial dielectric constant wherever possible, exhibiting the indicators as probes of interfacial polarity in same systems.

The theoretical background to the forthcoming analysis of the interfacial protonation-deprotonation equilibrium of the present indicator (probe) molecules has been given in this chapter. Provided there is no significant contribution to the apparent acid-base equilibrium constant of an interfacially located organic molecules from specific solute-solvent interactions, the following relationship hold:

$$pK_a^i = pK_a^m - \log m\gamma_H^+$$

$$pK_a^0 = pK_a^i + \log \frac{\gamma_{IN}^i}{\gamma_{HIN}^i}$$

$$pK_a^0 = pK_a^{obs} + \frac{F\psi_0}{2.303RT}$$

where $m\gamma_H^+$ is the medium effect on the proton, γ_{IN}^i and γ_{HIN}^i denote the activity co-efficients of the deprotonated and protonated forms of the indicator molecules respectively referred to the interfacial phase at infinite dilution, and F, R, T, Ψ_0 represent the Faraday constant, the universal gas constant, the absolute temperature and the electrostatic surface potential respectively.

In addition, it is convenient to define:

$$\Delta pK_a^m = pK_a^m - pK_a^w$$

$$\Delta pK_a^i = pK_a^i - pK_a^w$$

$$\Delta pK_a^0 = pK_a^0 - pK_a^w$$

The nature of variation of ΔpK_a^m and ΔpK_a^i as a function of the dielectric constant of 1,4 dioxane-water mixtures are studied. In calculating pK_a^0 for charged micelles, surface potential ψ of CTAB, SDS and AOT micelles were taken as +141 mv, -140 mv and, -140 mv respectively.

In 3.2, experimental aspects have been presented.

In section 3.3.1, ultraviolet spectral profile of 5-Hydroxyindole and 5-Hydroxy-L-tryptophan at different pH have been presented. This spectra have been taken in water as well as in various concentrations of CTAB, SDS, AOT,

Tween-40 and Brij-35 in order to study protonation-deprotonation equilibria in micellar microenvironment of above surfactants. Protonation-deprotonation equilibria of hydroxy groups of the two indicators are also studied in 1,4 Dioxane-water mixture. pK_a^{obs}, pK_a^0, ΔpK_a^0 values are calculated for the indicators solubilized in interfacial region of the micelles and ΔpK_a^m and ΔpK_a^i of the indicators are measured in 1,4 Dioxane-water mixture of different compositions. Result shows that ΔpK_a^0 is a function of concentration of the surfactants and both the indicator molecules perhaps undergo specific interaction with the surfactant headgroups of anionic surfactant, viz., SDS and AOT. On the other hand, most probably 5-Hydroxyindole and 5-Hydroxy-L-tryptophan are protonated weakly in non-ionic micelles and values of ΔpK_a^0 is small. However, these indicators are partitioned in the interfacial position of CTAB to a greater extent and D_{eff} value of 38.8 to 52.2 is obtained for 5-Hydroxyindole depending on the concentration of CTAB. This value of interfacial polarity, D_{eff} is measured by comparing ΔpK_a^0 of the indicators in the interfacial region of the micelle with ΔpK_a^i's of the same in organic-water mixtures. On the other hand, a value of ~ 55 is obtained for 5-Hydroxy-L-tryptophan for the same micelle and indicates that both the molecules are partitioned in the interfacial region and distributed around upto certain distance from the micellar surface.

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uv spectral profile of L-Tyrosine and L-Tyrosinemethylester have been presented in section 3.3.2. Like 5-Hydroxyindole and 5-Hydroxy-L-tryptophan, spectra of these indicator probe molecules were taken in water and in various concentrations of different surfactants including organic-water medium as mentioned in section 3.3.1 to study the protonation-deprotonation equilibria in these systems. However, the spectral profiles of these indicator molecules become complicated by giving more than one isobestic points. The values of λ_{max} of deprotonated form of L-Tyrosine is found to undergo a red shift in 1,4-

Dioxane-water mixture of low dielectric constant. Due to the existence of an isoelectric point of L-Tyrosine near 5.66, the protonated form remain closer to SDS micellar interphase than those of deprotonated form due to electrostatic repulsion at high pH. The spectral properties do not show any observable effect of the easterfication of carboxylic acid group in L-Tyrosinemethylester. From the pK_a^{obs} values of both the indicators in Tween-40 and Brij-35 micelles, it is apparent that the deprotonated forms of the indicators are stabilized more in micelles than in water.

Values of ΔpK_a^i and ΔpK_a^m for both the indicators are consistently positive in 1,4- Dioxane-water mixtures. On the other hand, ΔpK_a^0 values are positive for both the non-ionic micelles but negative in anionic micelles. The results once again show that while the indicator molecules are weakly partitioned by non-ionic micelles, there is specific interaction between indicator molecules and anionic micelles. The effective dielectric constant (D_{eff}) values for CTAB micellar interface observed from the acid-base equilibrium of both the indicators are more or less same, i.e., 49 ± 1. The result once again shows that the indicator molecules are distributed in the interfacial region of the micellar surface upto a certain distance.

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Section 3.3.3 describes uv spectral study of protonation-deprotonation equilibrium of 1- and 2- Napthols. While spectral profile 1-Napthol in different media under investigation provides interesting result, the uv spectra of 2-Napthol is insensitive to solution pH. Therefore, attempt has not been made to study the acid-base equilibrium of 2-Napthol in micellar media by the present spectroscopic technique. From the results of foregoing investigation for 1-Napthol shows that the deprotonated form of indicator is stabilized in interfacial region of the micelle. The deprotonated form of 1-Napthol probably interacts with SDS and AOT headgroups and not efficiently partitioned in the micelles. However, 1-Napthol is probably partitioned more efficiently than all

other indicators under investigation, as is evident from high ΔpK_a^0 values. The interfacial polarity of CTAB micelle (D_{eff}) observed from the present study yield a value of ~ 45, indicating a distribution of indicator molecules surrounding the interfacial region.

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Chapter 4 deals with the study in photophysical properties of indicator organic molecules when they are in a variety of organic solvent of different dielectric constant, viz., different alkanols and 1,4-dioxane-water mixtures. An introduction and a brief review have been illustrated in section 4.1. Mainly, there are two types of solvent effects, one is the general solvent effect and the other is the specific solvent effect. All of these were discussed in this section.

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In section 4.2, the experimental procedure involved in the study was illustrated.

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In section 4.3, i.e., in the results and discussion section, all of the spectral shifts are tabulated. The high frequency polarizability, which is a function of refractive index of the solvent and low frequency polarizability, a function of dielectric constant of the medium are considered to probe the effect of solvent on solutes. The orientation polarizability or the polarity index function, which is a difference of the above two functions are also taken into account. Variation of Stokes' shift with Kosower-Z values, orientation polarizability etc. are studied and interpreted. It was also found that beside the general solvent-fluorophore interaction, there is also the specific solvent-fluorophore interaction, which arises due to hydrogen bonding.

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Chapter 5 of the thesis deals with the study on the role of 1- and 2-Napthol in promoting micellar shape transition under salt free condition. In section 5.1, an introduction and a brief review of the previous works have been presented. Physical gelation of surfactant micelles and their stimuli responsive behaviour are interesting from biology as well as chemical technology points of view. Unlike simple halides, sodium salicylate promotes sphere to worm like micellar transition at the cmc of CTAB. The flexible and elongated worm like micelles show complex and unusual rheological phenomena. Although it is generally believed that micellar entanglement and transient network formation are responsible for developing shear induced viscoelasticity, precise knowledge regarding the nature of interaction is still lacking. It is particularly interesting that while a wide variety of worm like micellar solution display identical rheological responses, a common element of all these systems is the presence of salt anions like salicylate. This limitation has perplexed the scenario to some extent an impeded the development of an acceptable theory for micellar shape transition. In the present work we have demonstrated for the first time that due to the presence of strong hydrophobic aromatic rings and polar hydroxy groups in their molecular architectures 1- and 2-Napthols can promote micellar shape transition in CTAB and CPB (Cetylpyridinium bromide) efficiently.

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In section 5.2, i.e., in the experimental section of this chapter, detail experimental procedure has been presented.

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Section 5.3 deals with the results and discussion of the present study. It is observed that at low concentration of CTAB-Napthol and CPB-Napthol systems, shear thinning property is displayed, which is common for a non-Newtonian fluid. On the other hand, at high concentration the system shows interesting rheological properties. Upto the applied shear of 30 rpm shear thinning is observed above which shear thickening takes lace. The effect of

temperature on the viscoelasticity of the present system is intriguing. Typically when a worm like micellar solution is heated the micellar contour length decays exponentially with temperature. At higher temperature surfactant unimers can hop more rapidly between the cylindrical body and the hemispherical end cap of the worm (the end cap is energetically unfavourable over the body by a factor equal to the end cap energy). Thus, because the end cap constraint is less severe at higher temperature the worms grow to a lesser extent. However, CTAB-Napthol and CPB-Napthol systems display an opposite trend. In this case hydrophobic interaction between the micellar core and the aromatic rings of the Napthol molecules are important. As the temperature is increased Napthol molecules are more and more soluble and partitioned more strongly in the micellar hydrocarbon core. This favours the formation of longer worm like micelle upto the critical temperature above which the increased kinetic energy allowing surfactant unimers to hop more frequently between the body and the end cap of the worm results in the breaking of the micelles. NMR and FTIR spectral study shows that Napthol molecules are embedded inside the surfactant micelles near the surface whereas OH groups are protruded above the surface of the micelles and screen the surfactant headgroup charges. Spectral study shows that the Napthol molecules which are embedded in different micelles bridge between the micelles via H-bonding resulting in the entanglement. The micro-structural diversity associated with intermolecular H-bonding in Napthols, which results in the diffused and broad energy states of the excited normal modes, no longer exists in gel. A comparatively more well-defined and specific H-bond is present in gel, which bridge worm like micelle together to form the network structure.

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