CHAPTER – 1
INTRODUCTION

Introduction

The importance of interfacial region between aqueous and non-polar part of the self-assembled lipid phase is very well recognized in biological membranes. It is also well known that the characteristics of this region are different from those of bulk aqueous phase and interior of the lipid or micellar phase, which can mimic the properties of the lipid. Absorption and fluorescence spectroscopic probes are used to estimate the effective dielectric constants (D_{eff}),[1-9] microviscosities,[10-15] surface potentials[1,17-21] etc. One of the techniques involved in finding the above properties is to observe pK_a values of weak acid-base indicators. Such an indicator equilibrium provides the first example of the micellar induced effects upon chemical reactions in aqueous solution. The examples of micellar effects upon reactivity are the observations that the cationic micelles sometimes increase and anionic micelles decrease the deprotonation of the simple weak acids. Although Fernandez and Fromherz,[1] have shown that (using 4-Heptadecyl-7-hydroxycoumrin and 4-Octacycloxy-1-naphthoic acid as acid-base indicators) D_{eff} of ionic micelles can be equated to the D_{eff} of the interface of the nonionic micelles of surfactants with poly (ethylene oxide) head groups, but, more recent researchers,[11,13-16] have shown that, in many cases pK_a^{obs} of weak acid-base equilibrium in ionic micelles can be explained on the basis of the factors as mentioned above viz., dielectric constants, microviscosities, surface potentials etc.

Recently, it has also been reported that there is an effect of ionic and non-ionic detergents on the acid-base properties of some groups like –NH,[24,25] $-NH_2$[26-28] and –OH. The fluorescence spectra of the mono cations formed by the molecules containing the above groups viz., benzimidazols, have shown the tendency to form premicellar aggregates with sodiumdodecylsulfate (SDS) molecules,[29] indicating some kind of interaction with the sulfate group of SDS detergent molecules and proton of the cation of benzimidazols.[30]

Since a number of biologically important organic molecules exhibit either weak acid or weak base behaviour, there is an added incentive to investigate the acid base equilibria of weak acid and weak base at the lipid-water interface. For example, the apparent pK_a values of fatty acids, bile acids, an uncouplers of oxidative phosphorylation, phosphatidylserine, phosphate-dylethanolamine and some local anaesthetics located in the rudimentary model biological membranes, are all shifted negative to their pK_a values in pure water.

It is also well known that the spectral characteristics,[31] specially fluorescence spectra ,[32] are very sensitive to the environments around the systems. Due to this fact, the fluorescence spectroscopy[33] has become one of the fundamental methods for the study of the structure and dynamics of the microheterogeneous systems, e.g., micelles, reverse micelles, membranes, polymers and biological macromolecules. Large numbers of fluorophore molecules are available, where fluorescence spectra are very sensitive to the environments and can be used as probes. Although, there are certain class of molecules which are biologically important, but their spectral properties are not quite sensitive to the surroundings.[34-37] The other useful properties of the molecules which also depends on the nature of the solvents is the acid-base equilibrium as has already been mentioned. Thus, pK_a values for the different acid-base equilibria of indicators are found to be very useful to explore the characteristics of the microheterogeneous systems.[38,39,52,53]

Fluorescence characteristics of various fluorophores have been utilized to find out the polarity, dielectric constants, viscosities, etc. of the micelles, reverse micelles and other biologically active organic mcromolecules.[40-49] The quenching of the fluorophore present in the micelles has find its use to the determination of aggregation number of the micelles,[50] and confirmation of the Menger's model[51] that water molecules can penetrate to some extent into the core of the micelles.

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 or equivalent solution dielectric constant for the cavity has been invoked.

Solvent polarity and the local environment have profound effects on the emission spectra of polar fluorophores. These polar effects are the origin of the Stokes' shift. This is one of the earliest observations in fluorescence. Emission spectra are easily measurable, and as a result, there are numerous publications on emission spectra of fluorophores in different solvents and when bound to proteins, membranes, and different nucleic acids. One common use of solvent effects is to determine the polarity of the probe-binding sites on the macromolecule. This is accomplished by comparison of the emission spectra and/or quantum yields of the fluorophore when it is bound to the macromolecule and when it is dissolved in solvents of different polarity. However, there are many additional instances where the solvent effects are used extensively. Suppose a fluorescent ligand binds to a protein. Binding is usually accompanied by a spectral shift due to nature of the different environment for the bound ligand. Alternatively, fluorophores often display spectral shifts when they bind to membranes.

The effects of solvent and environment on fluorescence spectra are complex, and there is no single theory that can account for all these effects. Spectral shifts result from the general effects of solvent polarity whereby the energy of the excited state decreases with increasing solvent polarity. This effect can be accounted for by the Lippert equation. However spectral shifts

also occur due to the specific fluorophore-solvent interactions and also due to the charge separation in the excited state.

While fluorescence spectral shifts can be interpreted in terms of general solvent effects, this theory is often insufficient for explaining the detailed behaviour of fluorophores in a variety of environment. This is because the fluorophores often undergo specific interactions with the local environment which arises due to hydrogen bonding and also due to the formation of an internal charge transfer state or twisted internal charge transfer state.

The phenomenon of micellar growth as the preferred surface curvature decrease is quite general. Any change in the system that reduces the effective head group area will produce the effect, for example, as well as electrolyte addition, addition of a surfactant with a compact head group, changing the counter ion, changing the anion for cationic surfactants, changing the hydrophilicity of non-ionic head groups and changing the degree of protonation for zwitterionic surfactants.

When the average length of the micelles exceeds the mean distance between them, a very large increase in viscosity occurs. The overlapping micelles interact extensively and a transient network of randomly oriented rods is formed. When such a system is sheared, the fluid flow orients the rods and reduces the interaction between them. This orientation results in a reduction of the effective viscosity, that is, a shear thinning behaviour. This simple technology was used in some of the early formulations of shampoos, which contained an anionic surfactant and enough salt to produce the 'thickness' in the product. The addition of small amount of perfume would complete the very simple formula. Without the salt, the surfactant solution, even at 25% concentration, would be too watery to apply conveniently to the hair, to say nothing of its lack of aesthetic appeal.

A mixture of alkyl ethoxy sulfate and alkyl dimethyl amine oxide, that is, $R–(OCH_2CH_2)_nOSO_3Na$ and $R'–(CH_3)_2N\rightarrow O$, is typical of a mixed

surfactant system that has been used to provide shear thinning viscosity via long cylindrical micelles. The anionic surfactant has a bulky head group and the amine oxide a comparatively compact one. The viscosity, which is a function of micellar length, can be controlled by changing the ratio of the two surfactants, by choosing different alkyl chain lengths, R and R', or by changing the number of ethylene oxide groups in the anionic surfactant. This type of viscosity control works well in products at such extremes of pH and the surfactants provide the products with additional desirable properties such as surface wetting and detergency.

The study of bilayer membranes in the form of liposomes and vesicles has taken on a much wider perspective with the development of chemical routes to a greater range of artificial membrane-forming lipids.[54,55] Surfactant aggregates, either in the simplest form of monolayers or in the form of micelles, vesicles, liposomes and microemulsions, all provides unique opportunities to bring other molecules closer together, to orient them in specific ways and to alter their reactivities. This field, which has acquired the name 'membrane mimetic chemistry'[55] has understandably been the subject of considerable research interest.

Some applications of liposome dispersions are already established and others are in the process of being put into practice, such as the drug carriers. When dispersions of liposomes are injected intravenously, they travel around the circulatory system, have a long lifetime and can be shown to be taken up preferentially by certain organ in our body, such as the liver and spleen.[56] Knowledge of this behaviour has prompted many attempts to devise drug-carrier systems based on liposomes.[57] The notion is to dissolve the water-soluble or insoluble drug in the liposomes, inject intravenously and allow the drug to be carried to the target organ.

Liposomes can also be used as medium for enzyme reactions. Enzymes trapped within the liposomes occupy the aqueous region between the surfactant head groups and offer some interesting possibilities for novel catalysis.[55,58]

Certain sugars are used to preserve freeze-dried biological materials. When the water is removed, the sugar molecules hold the fragile structures apart and protect the system from collapse. The same technology can be applied to liposomes to allow dispersions containing suitable carbohydrates to be freeze-dried. Liposomes containing drugs could then be stored in a dry state without the need for refrigeration, and reconstituted prior to injection simply by adding water.

Adsorption of surfactants on solid surfaces plays important roles in two aspects of detergency. In the removal of oily soils from fibers, a mechanism known as 'roll-up' can occur. Adsorption of the surfactant at the solid/water and the oil/water interfaces causes a dramatic change in the contact angle, which the oil/water interface makes with the solid surface. The change is in direction to reduce the area of oil contact and the oil drop is then more easily removed from the solid by gentle agitation.

The second area is in the removal of particulate soil. Surfactants, especially anionics, can adsorb on the particles and stabilize them as a dispersion of discrete particles in the wash solution, thereby assisting in their removal from fibre surfaces. Redeposition onto the fibres during the wash cycle is hindered by the adsorbed anionic surfactants but is prevented more efficiently by the inclusion of small quantities of anionic co-polymers.

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