Synopsis

In recent years, there has been extensive fundamental and applied research about the properties of surfactants and polymers in aqueous solutions. This is because of their uses in a wide range of chemical and technological areas such as organic and physical chemistry, biochemistry, polymer chemistry, pharmaceuticals, petroleum recovery, detergents, cosmetics, paints and coatings, photographic films, minerals processing, and food science. A vast amount of experimental and theoretical work devoted to the understanding of the aggregation of surface-active molecules (surfactants). Synthetic surfactants undergo concentration and temperature dependent aggregation to form various types of microstructures in aqueous solutions. Depending upon the shape and size of these structures they are called spherical, rod, disk and worm like micelles, planar and flexible bilayer, bilayer tubules, ribbons and vesicles. Among these structures vesicles have recently become a fascinating subject in surfactant science because of their importance in chemistry, biology as well as in the industry. There is a considerable body of literature that addresses vesicles and liposomes used in various scientific fields. Since vesicles can entrap large quantities of reagents either in the lipophilic membrane or in the aqueous cavity, they have been used as encapsulants of cosmetic substances and pharmaceutical drugs. They are also of great interest in organic reaction chemistry, sensor technology and wastewater treatment. Vesicles are widely used as micro reactors for artificial photosynthesis, and as substrates for a variety of enzymes and proteins. Researchers have also used liposomes for separating biomolecules. Recently, the separations of other types of molecules have been also suggested in the literature. Indeed, the use of giant vesicles as pseudostationary phase in the enantiomeric separation of (±) binaphthol and (±) binaphthol phosphate by use of micellar electrokinetic capillary chromatographic technique has been reported.

The stability of vesicles is an important issue as far as applications are concerned. Vesicles investigated for applications are almost exclusively kinetically stabilized. The properties of these types of vesicles depend on their method of preparation (e.g., the length of sonication or the rate of extrusion) and they gradually revert back to their native lamellar phase state over time. The surfactants used are relatively expensive and are often of biological origin. Therefore, the major drawback of both kinetically stabilized and

equilibrium vesicles are their inherent mechanical instability in response to environmental changes. To overcome this problem, many researchers have attempted to polymerize vesicles to produce a more robust polymer membrane that would resist degradation. The most widely used process is the polymerization of synthetic surfactants that contain a polymerizable group. Recognizing the need for stable vesicles, many authors have synthesized both cationic and anionic polymerized surfactant vesicles. The subject has been reviewed recently. The vesicles formed by polysoaps are reported to be more stable and less permeable than respective monomeric ones. Therefore, polymerized vesicles are expected to act as better encapsulants of cosmetic substances and pharmaceutical drugs. Polymerized vesicles in the bulk phase have been shown as biomimetic systems that are capable of carrying out cell-like functions.

Vesicles are normally formed by double chain surfactants. However, recently many researchers have demonstrated formation of vesicles by single hydrocarbon chain synthetic surfactants. Among them N-acylamino acid (NAA) surfactants have attracted tremendous attention in the recent past because the salts of long-chain amino acids are currently used in detergents, foaming agents and shampoos because they are mild, nonirritating to human skin and easily biodegradable. They have been shown to be useful in stereoselective synthesis. More recently, it has been reported that optically active NAAs self-organize in dilute aqueous dispersions to form aggregates with various morphologies: spherical micelles and vesicles, fibers, chiral bilayer ribbons, tubules and helical structures. It has been demonstrated that chirality of amphiphilic molecules imparts stability to the hydrophobic aggregates. One of the most important properties of these supramolecular assemblies is chiral recognition. The chiral recognition properties of the monolayers of optically active NAAs have recently been reported. Several monomeric as well as polymerized chiral NAAs have been synthesized and used as stationary and pseudo-stationary phases in liquid chromatography for enantiomeric separation of pharmaceutical drugs, pesticides and insecticides. It has been found that in most of the cases the chiral polymerized surfactant acts as a better chiral selector than its monomeric counterpart. It has been reported that the nature of vesicular structures used for controlled drug delivery depends on the stereochemistry of the chiral amphiphile. Further biopolymers such as polynucleotides, polypeptides and polysaccharides can take the helical structure, which is very important in the occurrence of biological functions. The formation of

similar helical structures has recently been reported for assemblies of some native and artificial amphiphiles.

The focus of this work is to synthesize and characterize chiral surfactants that either by itself or upon polymerization form stable vesicles in aqueous solutions. In this work, a series of novel polymerizable chiral surfactants sodium N-(11-acrylamidoundecanoyl)-glycinate (SAUG), L-alaninate (SAUA), L-phenylalaninate (SAUPA), L-serinate (SAUS), L-valinate (SAUV), L-threoninate (SAUT), L-asparaginate (SAUAS), and L-glutaminate (SAUGL) have been synthesized from 11-acrylamidoundecanoic acid (AU) and corresponding amino acid. The surfactants SAUG, SAUA, SAUV and SAUS including sodium salt of AU were polymerized to obtain the polysoaps PSAUG, PSAUA, PSAUV, PSAUS and PSAU, respectively. The self-asemblies formed by these surfactants and polysoaps were characterized by a number of methods including surface tension, fluorescence probe, circular dichroism, light scattering, and microscopy.

The surface tension (γ) measurements were performed to determine different physicochemical properties such as critical micelles concentration (*cmc*), surface pressure (π_{cmc}) at the *cmc*, maximum surface excess concentration (Γ_2), minimum surface area per surfactant headgroup (a_o), standard free energy change of adsorption ($\Delta_{ad}G^o$) and standard free energy change of micelle formation, Δ_mG^o. The γ-log (concentration) plot exhibit two break points in the case of SAUA in contrast to one break for SAU. That is SAUA has two *cmc*s corresponding to formation of primary and secondary aggregates and SAU has one *cmc*. Two *cmc*s were also observed with the other surfactants. The first *cmc* of SAUAS (0.08 mM) and SAUGL (0.03 mM) was found to be much lower than those of the other amphiphiles (~0.4 mM). The corresponding Δ_mG^o values were also less as compared to other surfactants. This has been attributed to the hydrogen bonding capability of the amino acid side chains of the surfactants. The surface area per surfactant molecule at the interface, a_o, was calculated using Gibb's adsorption equation. The packing parameter was calculated using a_o value. The packing parameters of all the surfactants except SAUPA (0.35) fall in the range 0.5-1.0 indicating the presence of bilayer aggregates.

To further examine the existence of two break points in the surface tension plots steady-state fluorescence spectra of pyrene as probe molecule were measured in the presence of different concentrations of the amphiphiles. The plot of intensity ratio I_1/I_3 of the first (372

nm) and the third (384 nm) vibronic peaks of the pyrene fluorescence spectrum as a function of surfactant concentration showed two-step process confirming two break points in the surface tension plots. However, in consistence with the surface tension plot, the I_1/I_3 plot indicated only one-step process in the case of SAU. The plots show a fall of I_1/I_3 ratio, which indicates that pyrene probe moves from high polarity region to low polarity. The plots clearly indicate that the polarity of the microenvironment of pyrene probe solubilized within the primary aggregates is higher than that when it is solubilized into the secondary aggregates.

In order to understand the nature of the aggregates formed by the amphiphiles, steady-state fluorescence anisotropy (*r*) measurements using 1,6-diphenyl-1,3,5-hexatrine (DPH) as probe were performed. The r-values at surfactant concentrations above and below second *cmc* of the surfactants were found to be in the range 0.14-0.22 and were equal. The relatively high value of *r* suggests an ordered environment around the DPH probe in the self-assemblies. That is bilayer aggregates are formed by the amphiphiles above their first *cmc*. The low micropolarity (I_1/I_3) and high anisotropy of DPH probe in the presence of surfactants having concentrations above the second *cmc* indicated the presence of closed spherical vesicles. The steady-state fluorescence anisotropy value and fluorescence lifetime of the DPH probe in the presence of the amphiphiles were used to estimate the microviscosity of the probe environment. The microviscosity values in all the cases are very high (60-138 mPa s) and are comparable to liposomes. On the other hand, relatively high I_1/I_3 ratio and r-values below the second *cmc* suggests formation of flat lamellar structures.

To obtain the size of the aggregates dynamic light scattering (DLS) measurements were performed. The angular dependence of translational diffusion constant suggested presence of polydisperse aggregates. The apparent diffusion coefficients of the amphiphiles are about 10^{-12} m^2 s^{-1}, which is much smaller than that of normal spherical micelles ($\sim 10^{-10}$ m^2 s^{-1}). Assuming spherical aggregates, the hydrodynamic radius, R_h was calculated from the measured D value by use of Stokes-Einstein relationship. The R_h values of the aggregates were found to be in the range 40-75 nm. Thus the results of DLS studies indicate the existence of large aggregates in solution of surfactants. The mean aggregation number (N_{agg}) of the self-assemblies for all the surfactants was estimated to be of the order of 10^4-10^5. Based on the results of surface tension, fluorescence probe and DLS studies it has been

concluded that the large aggregates formed by the amphiphiles above their second *cmc* are closed vesicles and those formed below second *cmc* are flat lamellar type aggregates.

The formation of bilayer self-assemblies by the monomeric surfactants has been explained in terms of the intermolecular amide hydrogen bonding between surfactant molecules. The molecular structures of the amphiphiles suggest that there can be two stable intermolecular amide hydrogen bonds. The first one is between –NH- and –CO- in the amide group near the chiral center that induces a stable linear state. The second one is between the terminal amide groups of the hydrophobic chains. The influence of the amide linkage near the hydrophilic headgroup on the Langmuir monolayer formation by the NAA surfactant has already been established in the literature. In this work it has been demonstrated intermolecular hydrogen bonding between the terminal amide linkages of hydrocarbon chain. The multiple hydrogen bonds between N-H and C=O groups assists the surfactant molecules to self-assemble to form a parallel arrangement of the corresponding hydrophobic tails such that the surfactant molecules can self-organize into bilayer structures in water. The low *cmc* of the amphiphiles and large size of the aggregates compared to that of corresponding fatty acid salts suggest that the intermolecular hydrogen-bonding interaction between amide groups enhances the hydrophobic interaction that leads to tight packing of the hydrocarbon chains. This is manifested by the low micropolaity and high microviscosity of the vesicles. However, the bilayer sheets with strong surface binding interactions may also tend to form spherical vesicles, ribbons, mono or multilayer tubules, and rodlike micelles. The twisting of the bilayer sheet results in formation of twisted ribbons or helical strands.

The aqueous solutions of all the chiral surfactants were also examined for chiral organization by circular dichroism (CD) spectroscopy. The CD spectra of SAUA in water above first *cmc* show a negative band at 212 nm. However, at concentrations below *cmc*, the peak at 212 nm disappears and a new peak at 200 nm appears. This means that the drastic change in the CD spectrum accompanies aggregate formation. The disappearance of the CD band at 212 nm below *cmc* indicates formation of chiral structures through aggregation. Similar CD spectra were observed with all other chiral amphiphiles. Thus all the surfactants were found to form chiral aggregates in aqueous solution.

To investigate the microstructure of the self-assemblies transmission electron microscopic (TEM) pictures were taken in aqueous solution of the amphiphiles. The TEM

pictures showed the presence of polydisperse spherical vesicular structures. The sizes obtained are consistent with those obtained from DLS measurements. The TEM images however, did not reveal any helical aggregate. This might be due to the inherent artifact of the technique that involves drying of the sample. However, twisted ribbons in the case of SAUV, and closed tubules in the case of SAUS were observed. Optical microscopic images also showed the formation of two-strand helical structures in the case of SAUV.

The phase transitions of the bilayer membranes formed by the amphiphiles were followed by fluorescence anisotropy measurements of the DPH probe. The plot of r as a function of temperature of the solution revealed the phase transition temperature, T_m. The T_m values were recorded from the inflection point of the respective plots. The vesicles formed by the surfactants have relatively high T_m values. The surfactants with headgroup having amino acid side chain capable of forming hydrogen bonds were found to exhibit to transitions, one due to change in hydrocarbon chain mobility and the other due to transition from gel to liquid-crystal state. The hydrocarbon chains were not found to melt even at 60 °C. This means that the vesicles formed by the amphiphiles are quite stable.

Since the surfactants contain a carboxylate group, there should be an effect of pH on the microstructure formation. Therefore, the stability of the bilayer structures was also tested against change of pH of the solution. The fluorescence anisotropy of DPH probe in the presence of the surfactants was measured at different pH. The r-value was found to increase with the decrease in pH of the solution. This means an increase of ordering at the interface of the bilayer aggregates. The sigmoid change of anisotropy value with the change in pH suggested a two-state process. The inflection point of the plot was taken as the pK_a of the amphiphile. The pK_a value thus obtained is higher than the pK_a value of the corresponding fatty acids. The pH adjustment provides a way for the bilayer surface charge density to be varied precisely through changing the degree of protonation of the amino acid head group. As a result, the concomitant electrostatic and hydrogen-bonding interactions may force the bilayers to adopt spherical shape to form closed vesicles. This study suggested that the vesicles are more stable below neutral pH.

Effects of organic additives such as methanol, acetonitrile and urea on the stability of the vesicles of a representative surfactant, SAUV were also studied. Surface tension measurement indicated decrease of *cmc* with the addition of additives in the low

concentration range. Aggregation number of the self-assemblies in the presence of additives was observed to increase in consistence with the decrease in *cmc*. Therefore, the bilayer formation is facilitated in the presence of low concentration of the additives. Fluorescence anisotropy of DPH was used to monitor the changes in the local environment of the self-assemblies in the presence of urea. Relatively high concentration of urea is required to break the intermolecular amide-amide hydrogen bonding in the bilayer assemblies of SAUV. However, higher concentration of the additives results in disruption of the organized assemblies as indicated by the decrease in N_{agg} and fluorescence anisotropy values. It is evident from the plot that in the presence of small amount of urea the anisotropy increases indicating tighter packing in the bilayer aggregates. However, at higher concentrations the anisotropy decreases showing denaturation of the vesicles. For complete disruption of the bilayer assemblies it requires a very high concentration of urea. Circular dichroism spectra showed the formation of chiral aggregates in aqueous solutions in the presence of the additives.

Similar studies as described above for the surfactant monomers were also performed with the polysoaps (PSAU, PSAUG, PSAUA, PSAUV and PSAUS). Average molecular weight of the polymers was determined using static light scattering technique. The molecular weight of the polymers was around 100 kD except for PSAU the molecular weight of which was found to be 740 kD. Like the monomeric counterparts the polysoaps are also found to be surface-active. As indicated by surface tension and fluorescence probe studies, the polysoaps formed both intra-chain and inter-chain aggregates in aqueous solutions. The high anisotropy and low micropolarity of the self-assemblies indicated formation of bilayer aggregates. Intra-chain aggregates are unilamellar and inter-chain aggregates are multilamellar vesicles. The DLS measurements showed the existence of large aggregates. The stability of the bilayer aggregates was examined against temperature, pH, urea and Triton X-100 surfactant. CD spectra of the chiral polysoaps showed the existence of secondary structures. The TEM images of all the polysoaps showed the existence of polydisperse spherical vesicular structures.