ABSTRACT

A surfactant or surface active agent can be defined as the substance which manifests interfacial activity. Generally, surfactants are amphiphilic, i.e., bearing hydrophilic (head) and hydrophobic (tail) moieties. The hydrophobic moiety (usually made of 8-18 carbon atoms) may be straight or branched, or may contain substituted atoms (as in fluorocarbons/siloxanes). The hydrophilic part is generally polar. The polar part of surfactants has strong affinity towards aqueous environment and interacts via dipole-dipole or ion-dipole interactions. Hydrophobic part loves oily environments. In aqueous medium, the amphiphatic nature of surfactants satiates their most appropriate place, interface, where the attractive and repulsive forces are at par. Surfactants are classified on the criterion of physical property or functionality or ionicity. Ionicity distinguishes anionic, cationic, nonionic and zwitterionic surfactants. Most interesting feature in surfactants is to form aggregate structures (micelles) at a particular concentration, called critical micelle concentration (CMC). Micelle formation is significant as it minimizes contact between the hydrophobic parts and water. The main contributing forces to this phenomenon are hydrophobic effect and electrostatic interactions generated by the surfactant head groups. CMC is very important with respect to industrial perspective wherein, in almost all processes, the surfactants are usually used above their CMC values so as to achieve better performance in lowering interfacial tension, foam stability, and other relevant phenomenon.

Surfactants are essential to almost every sphere of life. The relevance of surfactants in industry is quite extensive and has an immense realistic importance. Surfactants may be applied to benefit in the makeup and dispensation of foods, pharmaceuticals, agrochemicals, personal care and laundry products, petroleum (foam drilling fluid, oil flotation process froth, asphalt emulsion, emulsion drilling fluids), mineral ores, fuel additives and lubricants, paints, coatings, adhesives, and in photographic films. They can be found throughout a wide spectrum of biological systems and medical applications. Surfactant replacement therapy may be used in treating the lung diseases, such as meconium aspiration syndrome, neonatal pneumonia and congenital diaphragmatic hernia [1]. Surfactants contribute to key ingredient of edible coatings. Surfactants are also essential in agricultural practices. Many pesticide formulations contain ethoxylated alcohols, alklyphenols, sorbitan and
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Ilkylamines [2]. The spray-application products [2] containing organosilicone surfactants are now available for commercial level.

In the recent years (early 1990s onward) a thrilling maturity in the field of surfactant chemistry is the emergence of dimeric or gemini surfactants. It was Menger [3] who coined term “gemini surfactant” for surfactant molecules bearing two hydrophilic (mostly ionic) groups and two tails per surfactant molecule linked by a spacer group of varying length. In comparison to conventional (single tail-single head) surfactants, gemini surfactants (double head-double tail) own a number of superior properties. These surfactants typically have better surface-active properties and are used as promising surfactants in industrial detergency and have shown efficiency in skin care, antibacterial property, metal-encapped porphyrazine and vesicle formation, construction of high porosity materials, better solubility, better wetting and interesting visco-elastic properties. Among gemini surfactants most of the work is related to m-s-m (m and s, respectively, represent the number of carbon atoms in alkyl tail and spacer of a gemini surfactant) quaternary ammonium gemini surfactants. Despite their exceptional properties they are found to be toxic and non-cleavable [4-6]. This hampers their usage in broader spectrum and raises the need to design eco-friendly surfactants to satiate the environmental pressures and risks associated. In this context, in recent years, cleavable surfactants (i.e., surfactants having a weak bond deliberately included in the molecule) have attracted considerable attention [7]. An illustrative example of such “cleavable surfactants” is the surfactant family of esterquats, cationic surfactants with an ester bond inserted between the hydrocarbon tail or the quaternary ammonium head groups. Esterquats have replaced the conventional stable quats in many large-scale applications, such as fabric softeners, and for their easy degradation they are gaining more attention. Moreover, inducing ethylene oxide (EO) groups in the surfactant moiety reduces the toxicity concerns. Further sophistications for end use to these green moieties are now-a-days added by using them with additives (like salts and proteins), significant for industrial and biomedical purposes.

Salts diminish the electrostatic interaction between the head groups, thus minimize the effective area per head group and thereby endorse the growth. Salts, with increasing concentrations tend to transform the spherical aggregates (the entities formed at or near the CMC) into non-spherical ones (viz., rod, branched or worm-like
micelles—screening of electrostatic repulsion among the polar head groups and movement of the hydrophobic alkyl chains away from the aqueous environment are attributed to micelle growth and birth). This is evidenced by a decrease in CMC and an increase of the micelle aggregation number [8]. Inorganic salts usually generate their effects via electrostatic interactions, change in the water structure, ionic hydratability, etc. Growth and birth of surfactant aggregates in presence of organic salts is the manifestation of both electrostatic as well as hydrophobic effects. Organic salts with hydrophobic moieties (such as sodium salicylate) usually generate cation-π interactions coupled with hydrophobic interactions that lead to transformation of spherical micelles to non spherical ones [9]. Generally, high aggregation numbers are reported with organic salt combinations [10]. Due to the interesting features of such salts, mixed micelles are formed. The formation of micelles from more than one chemical species gives rise to what are known as mixed micelles. The physicochemical properties of mixed micelles are quite different from those of pure micelles of individual components. From the application point of view, mixed micelles are of great importance in biological, technological, pharmaceutical and medicinal formulations, enhanced oil recovery process for the purpose of solabilization, suspension, dispersion, etc. [8]. For interpretation of mixed micelles, generally, according to the conditions, various theoretical models such as Rubingh, Rosen, etc., have been used.

Proteins are awfully vital in living organisms and have ability to bind a wide variety of ligands such as bilirubin, fatty acids, hematin, metal ions, surfactants, and drugs [11-17]. Moreover, in the recent times, protein-surfactant interactions have received considerable attention in the scientific community owing to their technical application in the field of pharmaceuticals, cosmetics, paints, coatings, and biochemical reactions [18]. Protein-surfactant interactions can provide insights into the effect of surfactants on the native structure of proteins in the form of solubilization and denaturing [19] or renaturing the protein [19, 20]. The globular proteins, in particular, are frequently used as functional ingredients in healthcare and pharmaceutical products because of their ability to catalyze biochemical reactions, to be adsorbed on the surface of some substances, and to bind other molecules and form molecular aggregates.
Enzymes are the macro bio-molecules accountable for the variety of metabolic affairs that assist life. They are extremely specific catalysts, substantially enhancing both the rate and selectivity of metabolic reactions. Majority of enzymes are proteins. Generally, enzymes can be denatured, that is, unfolded and inactivated by heating or chemical denaturants (metallic ions, drugs, surfactants, etc.). The chosen globular enzymes are very significant both for industrial as well as in academic realms.

Xanthine oxidase is known to govern the purine metabolism of the living organisms. It converts hypoxanthine to xanthine and that of xanthine to uric acid. The uric acid accumulation in the body parts leads to various clinical disorders like gout and hyperuricemia. In concomitant with uric acid generation there is also sidewise production of peroxides and free radicals which are believed to be provokers of brain and liver abnormalities.

Hen egg white lysozyme (HEWL) is generally used as model system to understand the structure, function, dynamics and folding of proteins [21]. It also possesses various physiological and pharmaceutical (antivirus, antibacterial and antitumor) properties and has a versatile aptitude to interact with metal ions, drugs, dyes and surfactants [22, 23].

Catalase, an anti-oxidant enzyme, found generally in aerobic organisms, is known to catalyze the decomposition of hydrogen peroxide into water and oxygen \(2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}\) and therefore is crucial in regulating oxidative stress [24]. Catalase is alleged to have relevance in apoptosis, aging, mutagenesis and many diseases [25].

Keeping the above significance in view and the fact that surfactant micelles, like many other amphiphilic substances, are potentially important encapsulating and stabilizing agents, we have synthesized novel m-E2-m gemini surfactants (E2 is the diester containing spacer) and studied their interactions with various additives (bile salts, inorganic/organic salts and proteins). The study has been divided into following four chapters.

Chapter-1 is the general introduction, where a detailed account of the behavior of amphiphile systems, micelle formation and critical micelle concentration (CMC), types of micelles, factors affecting the CMC, micellar morphology, effects of additives on structural transitions, micellization, causes of micellization, mixed micelle, theories of mixed micelle, proteins, etc., are outlined.
Chapter-II deals with the experimental details, techniques, synthesis and characterization of m-E2-m (12-E2-12, 14-E2-14, 16-E2-16) novel gemini surfactants. Materials used, their purities, make, etc., are presented in tabular form.

Chapter-III involves the studies on amphiphile-salt systems. It has been divided into two parts:

Chapter-III (A) deals with the effect of salt additives (NaCl, Na₂SO₄, Na₂PO₄, NaTos and NaAn) on the aggregation behavior of the three cleavable biodegradable ester-bonded dicationic gemini surfactants, ethane-1,2-diyl bis(N,N-dimethyl-N-alkyl-ammoniumacetox) dichlorides (m-E2-m; m = 12,14,16). A multi-technique approach employing tensiometry, fluorescence, proton magnetic resonance (¹H NMR), transmission electron microscopy (TEM), absorption spectrophotometry (UV), and Fourier transform infrared (FT-IR) spectroscopy was utilized to probe physicochemical fluctuations. Appreciable changes were observed in various physicochemical parameters viz. critical micelle concentration (CMC), surface excess concentration (Γₘₐₓ), minimum area per head group (Aₘᵢₙ), free energy of micellization (ΔGₘₐₓ), free energy of adsorption (ΔGₐ₅) and aggregation number (Nₕₐ₅). Counter ions were found to affect through electrostatic and hydrophobic influence obeying the overall trend as: NaAn > NaTos > Na₂PO₄ > Na₂SO₄ > NaCl. ¹H NMR, TEM, UV and FT-IR results reveal microstructure evolution and phase transitions. These results thus provide deeper insights in understanding of self-aggregation and microstructure evolution of biocompatible (green) aqueous systems of the gemini surfactants and their implications in the biomedical and pharmaceutical world, which could be helpful to improve their bioavailability and other biochemical aspects like drug delivery and gene transfection.

Chapter-III (B) Due to the potential use of bile salts in drug delivery, mixed micellar, microstructural and mixed monolayer investigations of the biodegradable diester gemini surfactants (m-E2-m) with sodium deoxycholate and sodium cholate in aqueous media have been carried out by tensiometry, dynamic light scattering (DLS) and spectrofluorimetry studies. The micellar and adsorption characteristics like composition, mutual interaction, hydrodynamic radius, aggregation number, activity coefficient, minimum area per molecule, and free energies of micellization and adsorption have been evaluated and compared. A synergistic interaction was observed both in the micelle as well as at interface, as evidenced from interaction parameters.
The results are discussed in terms of the structural characteristics and nature of spacer n the gemini surfactants as well as in terms of the presence of hydroxyl groups in bile salts. Furthermore, the m-E2-m gemini surfactants generate stronger synergistic interactions with sodium cholate as compared to sodium deoxycholate. Dynamic light scattering and spectrofluorimetry results indicate microstructural evolution in the aqueous mixtures of m-E2-m geminis and bile salts which offer potential pharmaceutical applications.

Chapter IV involves studies on amphiphile-enzyme interactions. This chapter has the following three subsections.

Chapter IV A reports interactions of the biodegradable gemini surfactants m-E2-n with bovine milk xanthine oxidase (XO), employing tensiometry, fluorescence spectroscopy, UV spectroscopy, far-UV circular dichroism spectroscopy (CD), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and computational molecular modeling. Surface tension results depict substantial changes in the micellar as well as interfacial parameters (CMC, Π_{CMC}, γ_{CMC}, Γ_{max}, A_{min}, ΔG^{\text{mic}}_n, ΔG^{\text{ads}}) of m-E2-n gemini surfactants upon XO combination, deciphering the interaction of XO with the gemini surfactant. Fluorescence measurements reveal that m-E2-m gemini surfactants cause quenching in the xanthine oxidase (XO) fluorescence spectra via static procedure and the values of various evaluated binding parameters (K_{SV}, K_b, k_q, ΔG^*_b, and n) describe that m-E2-m effectively binds to XO. Three-dimensional fluorescence, 8-anilino-1-naphthalene sulphonic acid (ANS) binding, F1/F3 ratio, UV, CD, FT-IR, SEM and TEM results delineate changes in the secondary structure of xanthine oxidase. Molecular docking results provide complement to the steady-state fluorescence findings and support the view that quenching occurs due to non-polar environment experienced by tyrosine and tryptophan residues of the enzyme. The results of this study can help scientists to tune the conformation of enzyme XO with biocompatible amphiphilic microstructures, which will help to unfold further understanding in the treatment modes of various diseases like gout, hyperuricemia, liver and brain necrosis. Moreover, the m-E2-m+XO interaction assists to unfurl new routes in the designing/selection of green-surfactant-protein mixtures widely used in body processing, cosmetics, and pharmaceutical industries.
Chapter IV (B) provides new insights into binding interaction of m-E2-m gemini surfactants with hen egg white lysozyme (HEWL) through detailed multidimensional techniques viz., fluorescence, UV-visible spectroscopy, circular dichroism (CD), isothermal titration calorimetry (ITC), transmission electron microscopy (TEM) and molecular docking method. Fluorescence and UV measurements indicate m-E2-m-HEWL complex formation via static procedure. Binding isotherms reveal mainly cooperative binding of the m-E2-m surfactants to HEWL. Circular dichroism, ANS fluorescence and pyrene fluorescence depict conformational changes in HEWL upon m-E2-m combination. Synchronous fluorescence shows that addition of m-E2-m has a remarkable effect on the polarity of the microenvironment in HEWL. Far-UV CD spectra demonstrate that the a-helical network of HEWL is disrupted and its content decreases from 30.68% to 20.83% /20.40% /15.86%, respectively, upon 12-E2-12/14-E2-14/16-E2-16 combinations. ITC confirms the endothermicity of m-E2-m-HEWL interactions while slight exothermicity was observed in 14-E2-14-HEWL at higher concentrations of the gemini. TEM micrographs reveal structural change in HEWL upon m-E2-m addition. Molecular docking illustrates that 16-E2-16/14-E2-14 binds principally to predominant fluorophores of lysozyme viz; Trp-108 and Trp-62 while 12-E2-12 binds in proximity of Trp-123. This study provides an important insight, namely the contribution of Trp-123, in the fluorescence besides already known predominant fluorophores Trp-62 and Trp-108. Moreover, this study would be significant to protein-surfactant interactions in terms of special m-E2-m molecular structure, which is essential in determining their future use as excipients in pharmaceutical/drug delivery related compilations.

Chapter IV (C) involves spectroscopic and molecular docking studies on the interaction of bovine liver catalase (BLC) with the ester-functionalized gemini surfactants. We have found that the m-E2-m gemini surfactants interact with BLC through static procedure and binding observed was of moderate type (as revealed by binding parameters). UV, CD and micropolarity assessment indicated conformational change in BLC upon m-E2-m combination. Docking provides support to fluorescence results by presenting the localization of m-E2-m surfactants near to aromatic residues (mainly Tyr, Trp, Phe). Moreover, binding of m-E2-m surfactants near to functionally essential residues (Phe-160, His-74, Tyr-353; these residues are reportedly important for catalytic activity of catalase) infers their probable interactions which may perturb
the functionality of the enzyme. Moreover, since surfactants are generally used as excipients in drug delivery, therefore, this study can be significant in addressing the binding relevance of surfactants to enzymes in general and catalase in particular during such phenomenon. This, in other words, may help the scientific community to cope with the toxic effects of surfactants being used.

References

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