Chapter 1
General Introduction

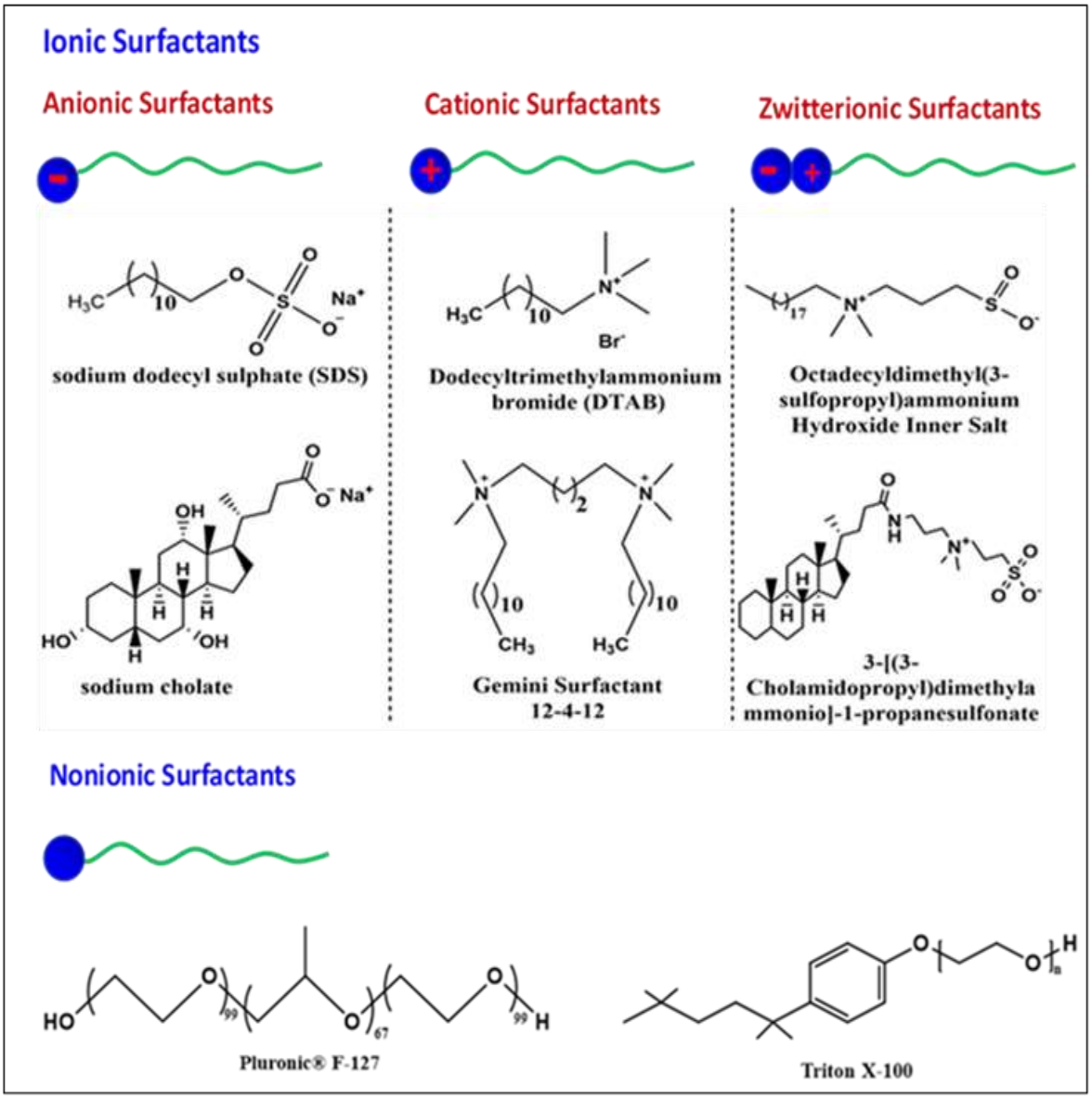
1. Introduction

Organized morphologies are one of the hot research areas due to their diverse applications, from micellar catalysis to transport of pharmaceutical material to desired site [1–8]. However, the possibility to control the physico-biochemical behaviour of the above self-organized assemblies may enlarge the window of potential applications [9–11]. The conventional strategy to control the responsiveness of such assemblies (micelles, vesicles, mesophases, etc.) directed towards variation in the chemical structure of the single component involved (surfactant/bio-surfactant, ionic liquids, bio-polymer, copolymer, block co-polymer) [12–15]. Though, this approach was quite successful but is tedious and time consuming as the synthesis of desired material to meet the requirement of stipulated applications. During last two decades, an alternative approach of physical blending of above-mentioned material has been pick the momentum to meet the applicability [16–20]. In this direction, the mixing of two surfactants (having different charges), two co-polymers (of different lengths and architecture) or the combination of individuals from both categories has been adopted (by varying composition) to obtain various morphologies with desired utility [21–28]. In the present thesis, an attempt has been made to use a blending approach to obtain the above-mentioned morphologies and their solubilization and drug release applications. In the process, various characterization techniques are used to establish different morphologies in the blended solution.

1.1. Surface Active Agents or Surfactants

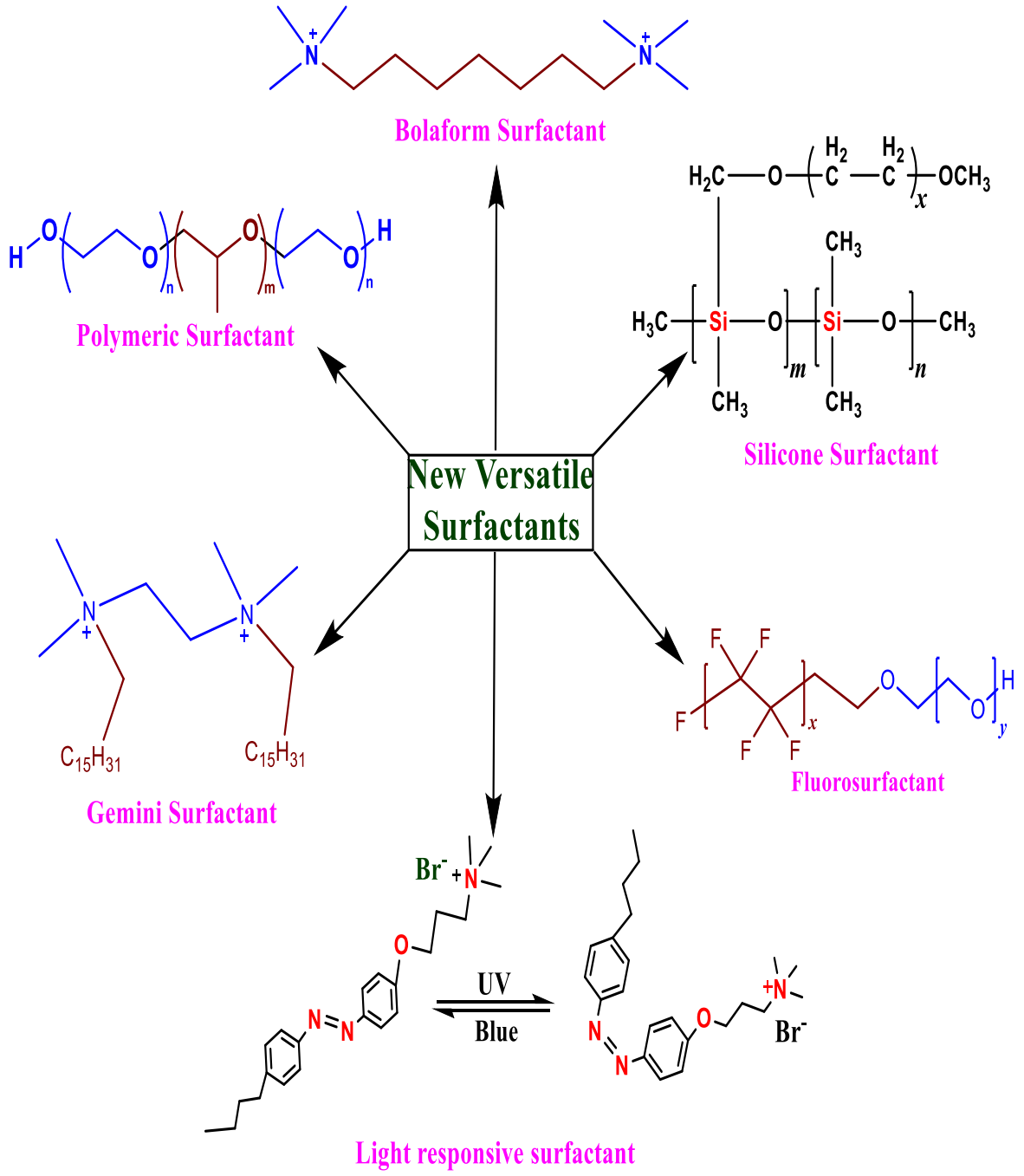
Material with distinct polarities (polar and non-polar groups in the same molecule) in the molecular structure imparts a distinct solution behaviour when dissolved in an aqueous solution. Such materials prefer to accumulate at the junction of two phases (*e.g.*, air-water,

liquid-liquid, solid-liquid, etc.). This accumulation results in changes such as a decrease in surface tension, a change in interfacial energy, mixing of two mutually insoluble materials, etc. Above properties are shown by *surface active agents* or **Surfactants** [29–32]. Due to the presence of polar and non-polar sections in the same molecule, surfactants are also known as amphiphiles, polar-nonpolar or amphipathic compounds. Conventionally, polar and non-polar parts of surfactants are known as 'head' and 'tail' groups, respectively. Solution properties of surfactants have been found to be dependent on molecular architecture (one head-one tail, one head-two tail, two head-two tail, etc.), electrostatics together with external stimuli, or nature of the medium [33]. Electrostatics of head group (charged or neutral) also dictates the category/classification of surfactants. Surfactant can be ionic or non-ionic depending upon the nature of charge (present and absent), respectively [34,35]. Surfactants with a charged head group comes under the category of ionic surfactants, while those with a no charge comes under *nonionic surfactants*. Similarly, surfactants with a head group having a positive charge are known as *cationic surfactants,* while those with a negative charge are categorized as anionic *surfactants*. Moreover, if both types of charges (cationic and anionic) are present in a surfactant molecule than it comes under the category of amphoteric or zwitterionic surfactants. All the above categories of surfactants together with their examples are shown in Scheme 1.



Scheme 1. Molecular structures typical of the four classes of surfactants: (a) cationic surfactant, (b) anionic surfactant, (c) nonionic surfactant, and (d) zwitterionic surfactant.

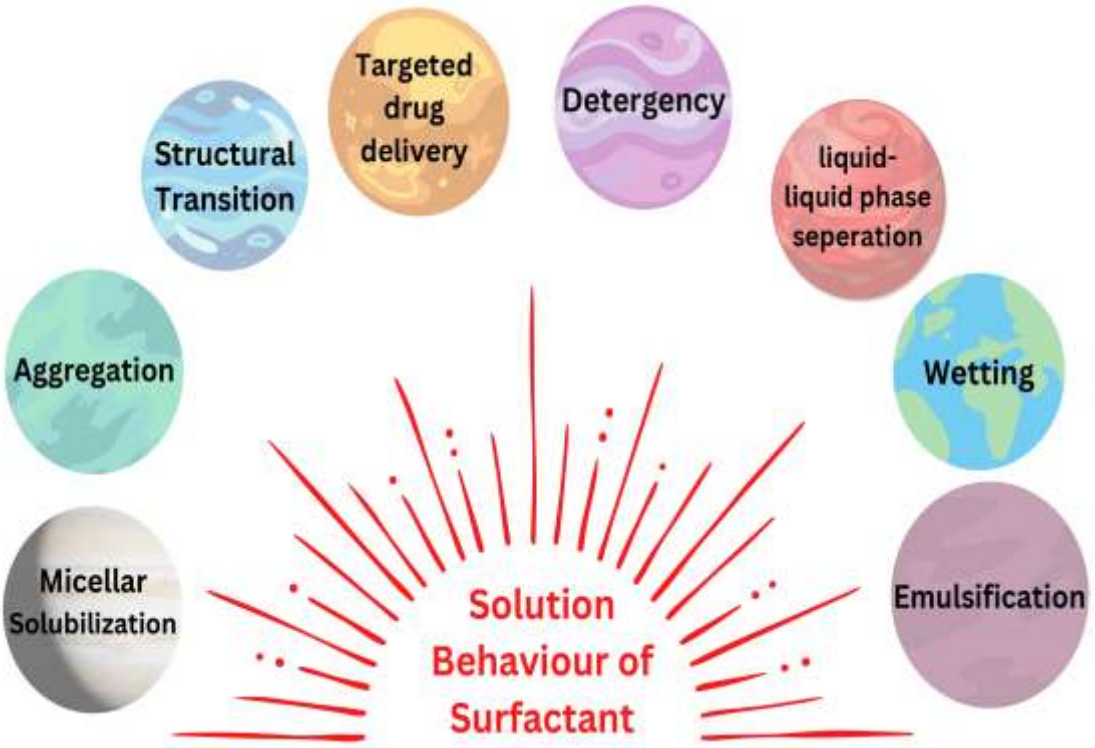
Apart from surfactant structures given in Scheme 1, a variety of novel types of amphiphiles have been reported in last two-to-three decades, e.g., dimeric or gemini surfactants, bola-form type surfactants, polymeric surfactants, bio-surfactants, fluorocarbon surfactants, etc. [31,36–40] (Scheme 2).



Scheme 2. Different type of new versatile surfactants with their molecular structure.

1.2. Solution Behaviour of Surfactants

Surfactants are known to show different characteristics in solutions depending upon their nature, structure, and medium. Scheme 3 shows some of the phenomena generally shown by surfactants when they are present in the solution phase. The present thesis is based on studies related to the solution behaviour of dimeric or gemini or twin heads-twin tails surfactants.



Scheme 3. Phenomena shown by surfactants in solution

A representative structure of a typical gemini surfactant has been shown in Figure 1. This material was first made and patented in the 4th decade of the last century [41]. But in open literature, such material was found in early seventies of the last century [42]. However, Magner and Littau coined the name ‘Gemini’ in 1991 [40]. Availability of geminis in open literature caused a drastic increase in research and applications in diverse areas [43–52].

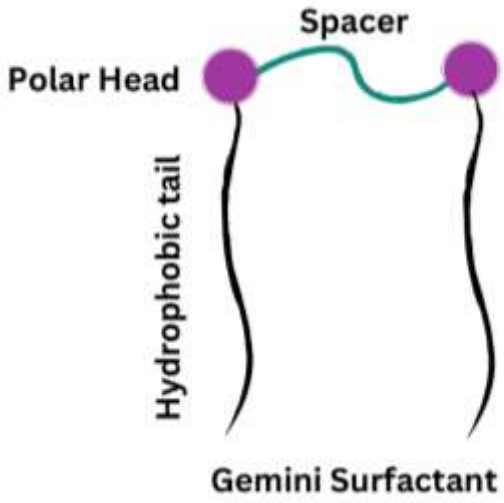


Figure 1. Schematic representation of gemini surfactant

The phenomenon shown in Scheme 3 are general but present thesis exclusively related to aggregation, structural transitions, solubilization, and solubilized drug release. Literature related to other phenomena (not covered in this thesis) can be found in published works or monographs [53–60].

1.3. Aggregation Behaviour of Surfactants

Owing to the amphipathic nature of surfactants, they prefer to accumulate both at the surface and in bulk solvent in the form of monolayer or aggregate, respectively. 'Micelle' is the most fundamental aggregate (first formed in the solution) formed by the surfactant in an aqueous solution when [Surfactant] increases [61]. Micelles are not present at all concentrations as some surfactant molecules are used to saturate the air-solution interface. Above saturation of interface is followed by aggregation in solution under critical solution conditions such as critical micelle concentration (CMC) and critical micellar temperature (CMT). Therefore, CMC is the concentration at/above which the presence of micelles can be

detected in the solution (Figure 2) [62,63]. Similarly, CMT is the temperature at/above micelle formation takes place [64–66]. The phenomenon of conversion of surfactant monomers into micelle is known as *'micellization'*. At CMC, micelles are roughly *spherical (or ellipsoidal)* which can take other structural shapes depending upon various factors such as concentration, temperature, internal/external stimuli together with foreign material [67–70].

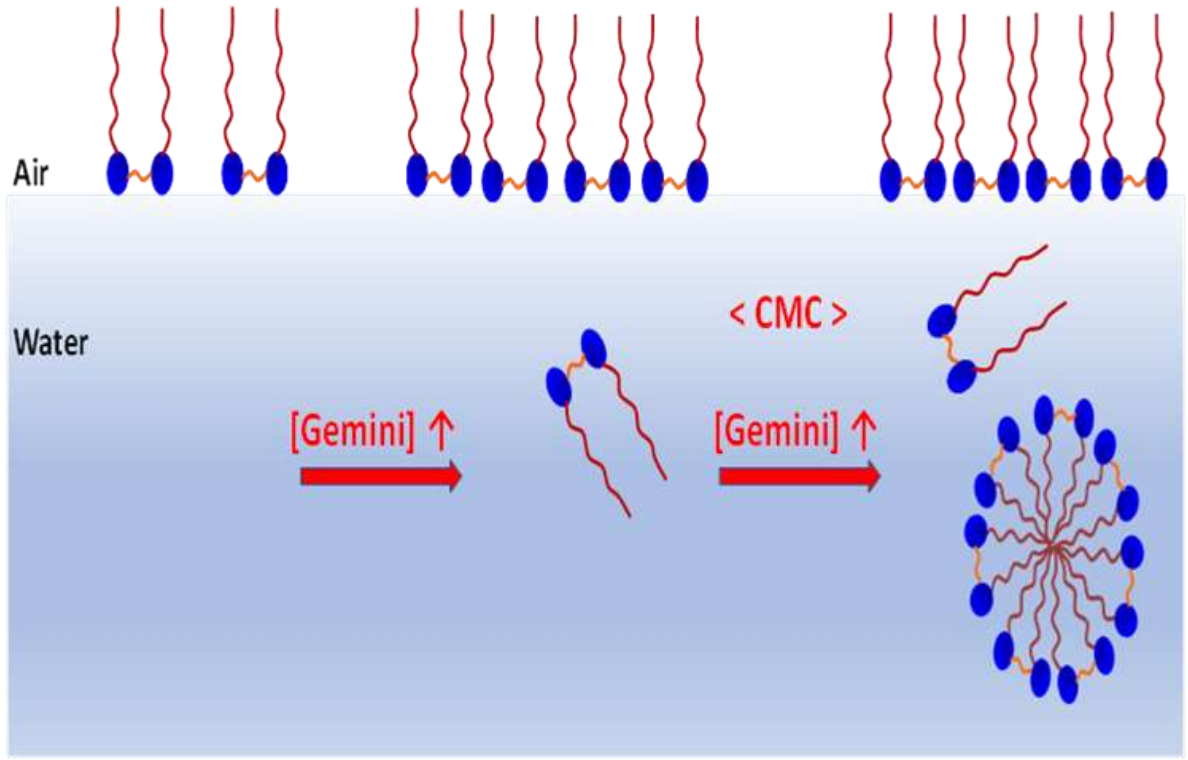


Figure 2. Schematic representation of gemini monomers and micelles in aqueous solution below and above CMC.

To detect the micelle, various physico-chemical methods are available depending upon the ionic nature/structure and experimental conditions. Table 1 shows a list of various methodologies used to obtain/compute micellar parameters, micellar structure, micellar polarity/environment, etc. [71].

Table 1. Different techniques to determine critical micelle concentration (CMC)

Sr No.	Technique	Nature of Surfactant	Micellar parameters obtain from	Ref.
1.	Surface tension	All type	CMC, C_{20}, area of the head group, purity, surface activity	[72,73]
2.	Conductometry	Ionic	CMC, degree of micellar ionization, structural transition	[74,75]
3.	Fluorescence	All type	CMC, aggregation number, micellar polarity, microenvironment	[76–78]
4.	Small angle neutron scattering (SANS)	All type	Micellar structure, aggregation number, structural transition, charge on micelle	[79,80]
5.	Small angle X-ray scattering (SAXS)	All type	Head group area, magnitude of charge, micellar size	[81,82]
6.	Dynamic light scattering (DLS)	All type	Micellar size, charge on micelle, isotropy/anisotropy	[83,84]

7.	Nuclear magnetic resonance (NMR)	All type	CMC, micellar structure, micellar environment, micellar interactions	[85,86]
8.	TEM / Cryo-TEM	All type	Micellar structure/ morphology, size	[87]
9.	UV-Visible spectroscopy	All type	CMC, micellar solubilization, surfactant-dye/drug interaction	[88]
10.	Atomic force microscopy (AFM)	All type	Surface structure of micelle, surface interaction with foreign material	[89]
11.	Refractometric	All type	CMC, refractive index, density	[90]
12.	Freezing point depression	All type	CMC, aggregation number	[91]
13.	Viscometry	All type	CMC, viscosity	[92]
14.	Isothermal titration calorimetry	All type	CMC, surfactant purity, saturation determination, drug-excipient interactions	[93]
15.	Capillary electrophoresis	Ionic	CMC	[94]

1.4. Mixed Micellization

As mentioned in the opening paragraph of this section, the presence of more than one surfactant in an aqueous solution can change the solution behaviour of individual components. Mixing or blending may results micelles constituted by both the mixing components. Such an aggregation process after blending is known as mixed micellization and the aggregates so formed are known as *'mixed micelle'*. Blending of surfactants is important in pharmacy, environmental remediation, and the development of various industrial fields (with polymers) [95–100]. To produce mixed micelles in the solution, various combinations of surfactants such as ionic-ionic, ionic-nonionic, and nonionic-nonionic are investigated in light of changes in solution properties and possible applications [101–103]. Mixing of surfactants may result tuning of interfacial characteristics at the meeting point of two discreate phases (*e.g.,* oil and water or air and water). The presence of a nonionic surfactant as one of the components (or both the components) may find application in biological systems and can be treated as a safe alternative (compared to other combinations) for environmental point of view [104–107]. Depending upon the composition and nature of the two components of the mixture, one can observe micellar structural changes in the solution phase or the formation of a biphasic system with condensed lamellar structures in coexistent of dilute medium [28,108–110]. In former case, various types of micellar structures are formed depending upon mixing composition, chain length, type etc. [27,111–113]. In latter case, surfactant association/phase separation has been found to be dependent on composition, pH, temperature, etc. [26,114,115]. Spontaneous separation and formation of lamellar structures can find applications in pre-concentrating biomolecules, carbon tubes, metal ions, and lower hydrocarbon chain molecules [116–119]. The structure of a typical mixed micelle formed by the combination of a conventional surfactant and a gemini surfactant has been shown in Figure 3.

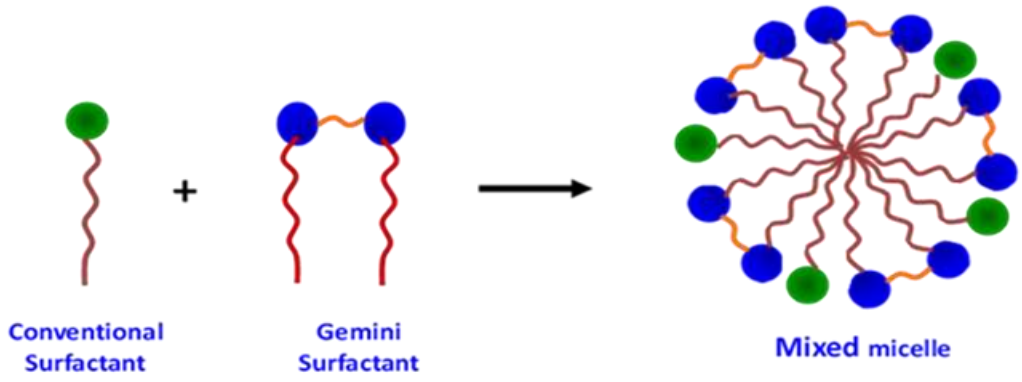


Figure 3. Representative structure of mixed micelle in aqueous solution

1.5. Micellar Morphologies in Aqueous Solution

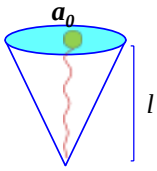
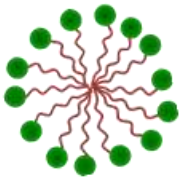
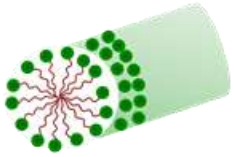
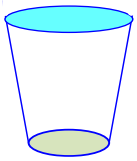
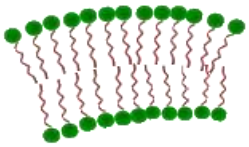
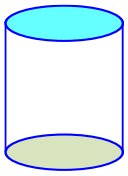
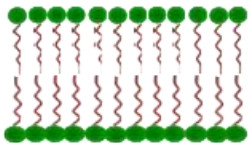
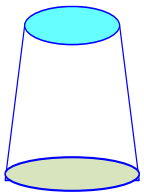
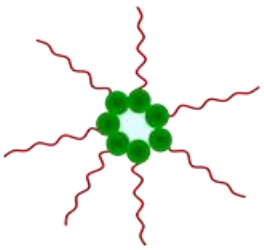
Surfactant molecules can assemble into a variety of structures depending upon solution/experimental conditions. Micellar shape can be governed by various factors namely, *i)* steric repulsion forces among similar charged heads, *ii)* cumulative hydrophobic interactions among hydrocarbon chains of surfactant(s) with or without additives; and *iii)* changes in surface regions (area per head group) by external/internal stimuli. A generalised packing parameter (*P*), named the Mitchell-Ninham parameter, has been proposed to theoretically predict possible morphology based on surfactant structure (volume of hydrocarbon tail(*V*), average head group area (a_0) and hydrocarbon tail length (*l*)). This surfactant structural information and *P* are correlated by a mathematical correlation given below.

$$P = V / a_0 l \qquad \text{eq. (1)}$$

The values of *V* and *l* can be computed by the method of C. Tandford [35] while a_0 can be obtained by experiment [120]. The values of *V*, *l*, and a_0 can be tuned by structural variation in surfactant and/or by the addition of different counter ions [121–125]. It may be mentioned here that *V* can be increase without change in *l* in case of gemini surfactant. Probably this is the

reason of getting higher order aggregates with geminis in comparison to their conventional counter parts [126,127]. Dependence of morphology on *P* has been depicted in Table 2.

Table 2. Dependence of shape on critical packing parameter ($P=V/a_0l$)

Effective shape of surfactant molecule in	Packing Parameter	Aggregate morphology (Geometry of Micelle)
 Cone	< 1/3 (0.33)	Spherical micelle 
 Truncated cone	1/3 – 1/2 (0.33 – 0.5)	Cylindrical micelle 
 Truncated cone	1/2 – 1.0 (0.5 – 1.0)	Flexible bilayer, vesicle 
 Cylinder	~ 1	Planar bilayer 
 Inverted cone	>1	Reverse Micelle 

1.6. Micellar Solubilization

An increase aqueous solubility of otherwise sparingly soluble material due to the presence of micelles is commonly called micellar solubilization. Solubilization has applications both in industries as well as biology due to incorporation of hydrophobic material. Sometimes, solubilization process can be considered as partitioning of insoluble material between micellar interior and background solution. Depending upon nature and structure of solubilizate and surfactant, the process is governed by locus of solubilization. This information can be well gathered by NMR technique by obtaining self-diffusion co-efficient of the desired material [128,129]. The micellar morphology holds the key to the deciding solubilization potential of a typical micelle forming material. The micellar morphology may or may not change by the incorporation of solubilizate. The behaviour is well exploited in environmentally benign solubilization of hydrophobic material e.g., drug or dyes [130–133]. It may be mentioned here that micelles provide a number of sites of varying polarity (Figure 4), where additives of matching polarity are stationed at a typical site [134–139].

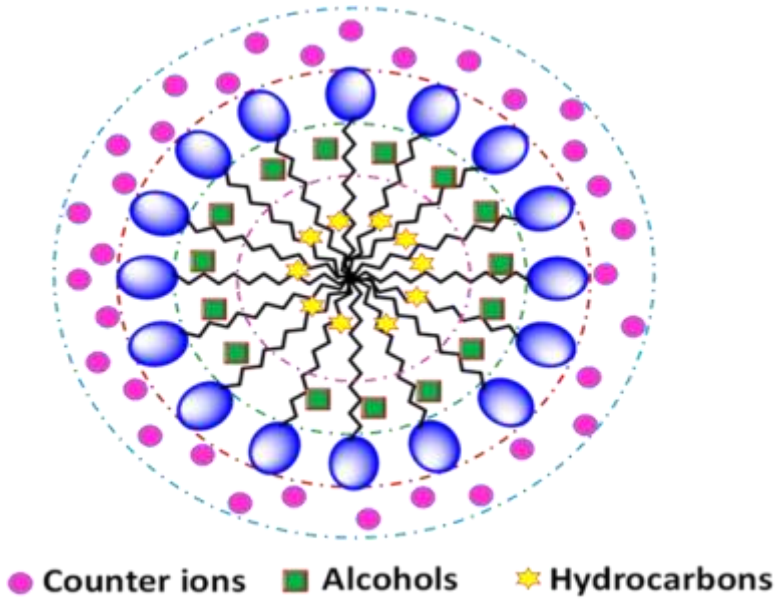


Figure 4. Schematic representation of solubilization process in a typical micelle

As mentioned in the previous section, mixed micelles are formed by more than one surfactant. This mixing may result in various structural transitions together with an augmentation in solubilization efficacy [28,111,112,140–142]. This approach can be extended to increase the solubility of other hydrophobic materials (pesticides, pharmaceuticals, cosmetic ingredients, etc.) which are industrially important ingredients. Therefore, the mixing approach is an ideal methodology which can be optimized depending upon case-to-case [143–150].

1.7. Micellar Solution as Drug Delivery Vehicle

Processes such as surfactant-drug interactions, micellar drug solubilization, and surfactant-based drug delivery systems are of ongoing importance in the pharmaceutical field [151]. The size of the micelle and stability are critical factors in optimizing surfactant-based delivery vehicles. Surfactant micelles have been studied from last few decades from the point of view of drug-to-gene carriers [152]. Mixed micellar structures have been reported to be better systems than individual micelles due to differences in the micellar interior environment [153,154]. The micellar interior can serve as a drug carbo space for incorporating various nearly insoluble therapeutic materials and can act in controlling release kinetics. The size information of micellar systems can help in the bio-distribution and accumulation of drug at the desired delivery site [155–158]. The release profile is an essential part of the overall drug transportation process. The prolongation of release time of a drug is of paramount importance to provide opportunities to bind with receptor at the site (cell membrane). It has been reported that drugs can form micellar aggregates in water and can form mixed micelles with an oppositely charged surfactant [159–161]. Liposomes are the first lipid-based vehicle to deliver drugs in dermis or epidermis regions but unable to deliver required drug concentration with gradual circulation [162]. This problem has been solved by taking phospholipid and surfactant to make optimised system [163]. This approach has been found successful with a wide variety

of drugs [164]. The development of drug specific optimised delivery systems is the topic of present time in which application-based carriers are search to achieve the desired goal of release kinetics [165–170].

1.8. Relevance of Research Work

Preceding topics of the present sections show the importance of surfactant association process and its modification for required surfactant applications. Blending of surfactants is one of the simplest methods to achieve desired solution conditions for targeted applications. Morphological changes can affect the desired process by providing an alternative better environment for solubilization or drug carrier/release ability. In these directions, studies were performed with various conventional surfactants, with or without lipids or phospholipids. As we know, gemini has better solution properties (low CMC, high aggregation number, anisotropic morphologies, etc.) than its conventional counterparts, people have started using geminis with conventional surfactants for blending and achieving desired properties [171–176]. The last decade witnessed the solution studies on mixtures obtained from exclusive blending of gemini surfactants [87,177,178]. However, their potential has not been exploited for solubilization efficacy, drug solubilization, or pollutant solubilization. The initial researches suggest that such systems show better solubilization efficacy and potential of forming higher order aggregates by small changes in chemical structure or in composition of the blend [27,28,179,180].

The present thesis embodies several such instances of the blending of oppositely charged gemini surfactants and the resulting influence on solution properties, association patterns, and performance towards solubilization efficacy among others. In these directions, various ionic gemini surfactants are synthesised and characterised. Such surfactants are having

various alkyl chain lengths and spacers and are blended in various fixed concentration to get different morphologies of varied electrostatics and shape. The resulted oppositely charged gemini mixtures were characterized in terms of their interactions, solubilizing efficacies, and nature of the microenvironment. For the purpose, conductance and fluorescence measurements are employed to obtain micellar association parameters for further treatment. Various regular solution theories are applied and thermodynamic data of interaction together with interaction parameter are computed.

The resulted systems were further studied in context of the presence of various morphologies and their charged modulation by blending. For the purpose, DLS, TEM, and zeta potential data are collected and combined for the establishment of the presence of various charged higher-order aggregates. The size and shape information have been used to formulate mixed micellar systems for solubilization experiments. A series of solubilization experiments have been performed by taking various polyaromatic hydrocarbons and both extracted natural and synthetic drugs. UV-visible spectrophotometry has been employed to generate solubilization data and judge the efficacy of the studied systems.

Based upon above solubilization experiments, few optimized systems are selected for the invitro release studies with various drugs. Various release kinetic models were applied to observe the release process under physiological conditions. The study provides a hint regarding controlled and sustainable release with the selected systems in comparison to conventional releasing medium. Antioxidant activity, in the case of an extracted natural drug, has been computed using a mathematical formula. A well-known procedure has been used to determine the functional stability of the extracted natural drug.

The cytotoxicity of the optimised systems against MCF-7 (breast cancer cell) has been

obtained by a tetrazolium salt MTT assay. IC_{50} data are acquired by the well-known MTT procedure (details are given in Chapters 5 and 6). It has been found that blended systems are better for cell proliferation.

1.9. Constitution of the Thesis

The thesis entitles "**Solution Behaviour of Aqueous Mixed Surfactant Systems with and without Additives"** consists of eight chapters including : ***i)*** General Introduction; ***ii)*** Materials and Methodologies; ***iii)*** Association Behaviour and Interaction of Oppositely Charged Gemini Surfactants in Aqueous Solution; ***iv)*** Composition Triggered Morphologies of Mixed Oppositely Charged Geminis having Different Chain Length and Spacers; ***v)*** Counter Charged Geminis Mixture for Solubilization/Release of Raloxifene Hydrochloride; ***vi)*** Amplification of Curcumin Entrapment/Release in Aqueous Counter Charged Gemini Mixtures; ***vii)*** Solubilization of Polycyclic Aromatic Hydrocarbons (PAHs) in Individual and Mixed Geminis: Implications of Blending and ***viii)*** Overall Conclusion.

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