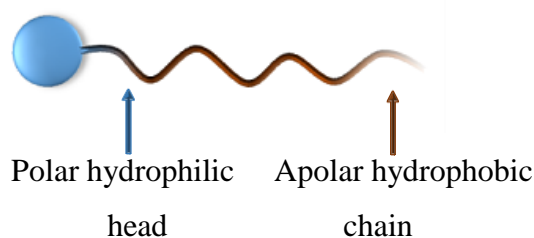


# Chapter I

## Introduction and review of previous works

### 1.1. Self-assembly of amphiphilic molecules : An overview

Self-assembly refers to spontaneous organization of molecules in solution driven by noncovalent interactions into stable aggregates. Self-assembly is highly recognized in biological systems, e.g., lipid bilayers, the DNA duplex, and tertiary and quaternary structure of proteins. The process of spontaneous aggregation of single molecules in solution into larger structures with a certain order is also an important phenomenon in every-day-life as well as in science. The best-known example of aggregation in every-day-life is the formation of micelles by detergent molecules. The most important type of aggregation, which is essential to life, is the formation of the lipid bilayer membrane by phospholipids. It has inspired chemists and physicists to study and mimic this and other types of aggregates.<sup>1</sup> Aggregation of molecules often occurs at the borderline of solubility. An important molecular property in this respect is polarity, for which solubility follows the rule 'like dissolves like'. Polar (hydrophilic) compounds are well soluble in polar solvents, e.g. salt in water, and the same goes for apolar (hydrophobic) compounds and solvents, e.g. vitamin E in oil. Furthermore, polar compounds are insoluble in apolar solvents and vice versa. Interestingly, there is a major class of compound which are amphiphilic in nature, i.e. these contain a polar as well as an apolar part. The polar part is called “head” and the apolar part, usually a long chain hydrocarbon, is called “tail” (Figure 1).



**Figure 1. Representation of a surfactant molecule**

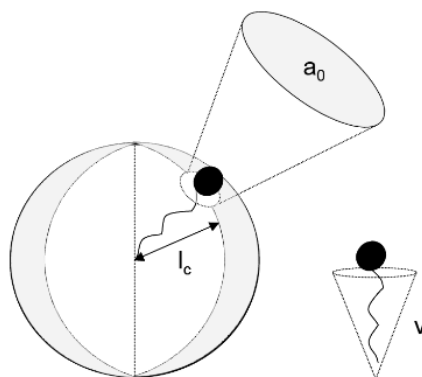
These compounds are most comfortable in a situation when each part is located in an appropriate environment, which is only possible at the interface between two media. Therefore, amphiphilic compounds are also called surface-active agents, or in short,

surfactants.<sup>2</sup> The hydrophobe is usually the equivalent of 8 to 18 hydrocarbon, and can be aliphatic, aromatic, or a mixture of both. The source of hydrophobes are normally natural fats and oils, petroleum fractions, relatively short synthetic polymers, or relatively high molecular weight synthetic alcohols. The hydrophilic group gives the primary classification to surfactants, and are anionic, cationic and nonionic in nature. The anionic hydrophiles are the carboxylates (soaps), sulphates, sulphonates and phosphates. The cationic hydrophiles are usually quaternary ammonium, pyridinium, imidazolium cations etc. The nonionic hydrophiles are dominated by long chain ethoxylates. These hydrophiles associate with water at the ether oxygens of a polyethelene glycol chain. There are some surface active amphiphilic molecules that contain both anionic and cationic centres at the head group. These are called zwitterionic surfactants. Surfactants can also have two hydrocarbon chains attached to a polar head and are called double chained surfactants. On the other hand, surfactants containing two hydrophobic and two hydrophilic groups are called “gemini” surfactants. Amphiphilic molecules can also have two head groups (both anionic, both cationic or one anionic and the other cationic) joined by hydrophobic spacer.<sup>1</sup> These type of molecules are termed “bola-amphiphiles” commonly known as “bolaforms”. Surface activity of these molecules depend on both the hydrocarbon chain length and the nature of the head group(s).

## **1.2. Classifications of diverse self-organized assemblies on the basis of packing parameter**

The concept of molecular packing parameter has been widely cited in chemistry, physics, and biology literature because it allows a simple and intuitive insight into the self-assembly phenomenon.<sup>3</sup> The packing parameter approach permits indeed to relate the shape of the surfactant monomer to the aggregate morphology.<sup>4-6</sup> The molecular packing parameter  $P$  is defined as the ratio  $v/(a_0 l_c)$ , where  $v$  and  $l_c$  are the volume and the extended length of the surfactant tail, respectively and  $a_0$  is the *equilibrium* area per molecule at the aggregate interface (or mean cross-sectional (effective) head-group surface area), as illustrated in Figure 2. If we consider a spherical micelle with a core radius  $R$ , made up of  $N_{agg}$  molecules, the volume of the core is  $V = N_{agg} \times v = 4\pi R^3/3$ , the surface area of the core  $A = N_{agg} \times a_0 = 4\pi R^2$ . Hence, it can be deduced that  $R = 3v / a_0$ , from simple geometrical relations. If the micelle core is packed with surfactant tails without any empty space, then the radius  $R$  cannot exceed the extended length  $l_c$  of the

tail. Introducing this constraint in the expression for  $R$ , one obtains  $0 \leq v / a_0 \cdot l_c \leq 1/3$ , for spherical micelles. These geometrical relations, together with the constraint that at least one dimension of the aggregate (the radius of the sphere or the cylinder, or the half-bilayer thickness, all denoted by  $R$ ) cannot exceed  $l_c$ , lead to the following well-known connection between the molecular packing parameter and the aggregate shape<sup>4</sup>:  $0 \leq P \leq 1/3$  for sphere,  $1/3 \leq P \leq 1/2$  for cylinder, and  $1/2 \leq P \leq 1$  for bilayer. Inverted structures are formed when  $P > 1$ .

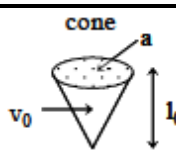
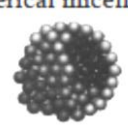



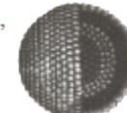
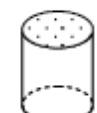





**Figure 2. The critical packing parameter  $P$  (or surfactant number) relates the head group area, the extended length and the volume of the hydrophobic part of a surfactant molecule into a dimensionless number  $P=v/(a_0 l_c)$**

Therefore, if the molecular packing parameter is known, the shape and size of the equilibrium aggregate can be readily identified as shown below (Table 1). It is noteworthy that  $a_0$  is often referred to as the “headgroup area” in the literature. However, for all practical purposes this has led to the erroneous identification of  $a_0$  as a simple geometrical area based on the chemical structure of the headgroup in many papers, although  $a_0$  is actually an equilibrium parameter derived from thermodynamic considerations.<sup>3</sup> Needless to say, that for the same surfactant molecule, the area  $a_0$  can assume widely different values depending on the solution conditions such as temperature, salt concentration, additives present, etc.; hence, it is meaningless to associate one specific area with a given head group. For example, sodium dodecylbenzene sulfonate forms micelles in aqueous solution whereas bilayer structures are formed when alkali metal chlorides are added.<sup>7</sup> Moreover, the role of the surfactant tail has been virtually neglected. This is in part because the ratio  $v / l_c$  appearing in the molecular packing parameter is independent of the chain length for common surfactants ( $0.21 \text{ nm}^2$  for single tail surfactants) and the area  $a_0$  depends only on the head group interaction parameter. Nagarajan showed that the tail length

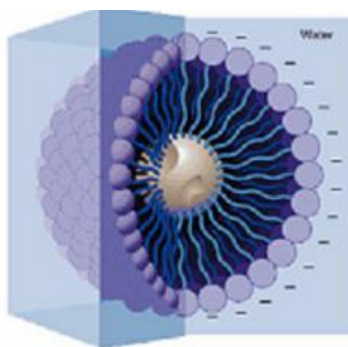
influences the head group area (consideration of tail packing constraints) and thereby the micellar shape.<sup>8</sup>

**Table 1. Schematic representation of surfactant structures and shapes derived from various packing parameters**

Possible surfactant type	$P(=v/a_0l_c)$	Shape	Structures formed
Single-tail surfactants with large headgroups	$< 1/3$	 cone	 spherical micelles
Single-chain surfactants with small headgroups	$1/3 < P < 1/2$	 truncated cone	 cylindrical micelles
Double-chain surfactants with large headgroups and flexible chains	$1/2 < P < 1$	 truncated cone	 flexible bilayers, vesicles
Double-chain surfactants with small headgroups or rigid, immobile chains	$P \sim 1$	 cylinder	 planar bilayers
double-chain surfactants with small headgroups, and bulky chains	$P > 1$	 inverted truncated cone or wedge	 inverted micelles

### 1.2.1 Micelles

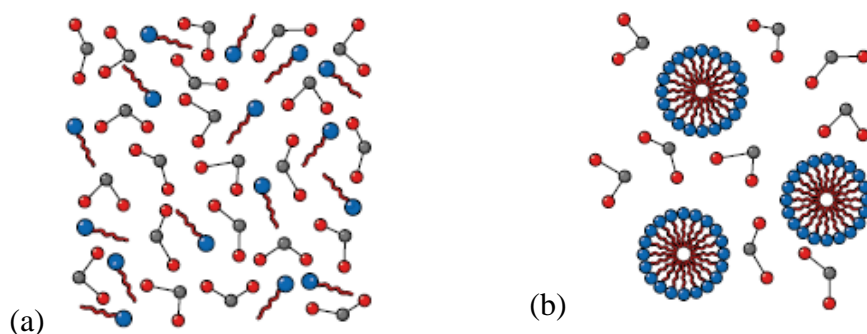
The most intensely studied and debated molecular self-assembly and perhaps the simplest in terms of the structure of the aggregate is the micelle. Micelles formed by ionic amphiphilic molecules in aqueous solution are dynamic associations of surfactant molecules that achieve segregation of their hydrophobic portions from the solvent via self-assembly. They are loose, mostly spherical aggregates above their critical micellization concentration (cmc) in water or organic solvents.<sup>1</sup> Also, micellar aggregates are short-lived dynamic species, which rapidly disassemble and reassemble.<sup>9</sup> Hence, only average shape and aggregation numbers of micelles can be determined. Micellization of surfactants is an example of the hydrophobic effect. In micellization there are two opposing forces at work. The first is the hydrophobicity of the hydrocarbon tail, favoring the formation of micelles and the second is the repulsion between the surfactant head groups. The mere fact that micelles are also formed from ionic surfactants is an indication of the fact that the hydrophobic driving force is large enough to overcome the electrostatic repulsion arising from the surfactant head groups. Figure 3 represents a spherical micelle formed in aqueous solution, where the hydrophobic chains are directed towards the interior of the aggregate and the polar head-groups point towards water, hence allowing the solubility / stability of the aggregate (no phase separation).



**Figure 3. Schematic representation of a spherical micelle in aqueous solution.**

Micelles are also known to be disorganized assemblies whose interiors consist of mobile, non-stretched hydrophobic chains.<sup>10</sup> Water molecules can penetrate partially into the micelle core to interact with surfactant hydrophobic tails.<sup>11</sup> There are a huge number of publications related to the micelles, micelle structures, and the thermodynamics of micelle formation. A huge amount of experimental and theoretical work devoted to the understanding of the aggregation of surface-active molecules has

been carried out.<sup>12-14</sup> Micelles are formed by cationic, anionic, zwitterionic as well as surfactants having short alkyl chains. The environment of a micelle varies in a regular manner as a function of distance from the center of the micelle, going from a relatively dense aliphatic medium near the center to a relatively diffuse region known as either Stern layer in ionic micelles, or as palisade layer in neutral micelles<sup>15-17</sup> where the headgroups, bound counterions, and solvent molecules coexist. The remaining counterions are contained in the Gouy-Chapman portion of the double layer that extends further into the aqueous phase. Fluorescence probe studies have indicated that micellar core is nonpolar, but less fluid than hydrocarbon solvents of equivalent chain length.<sup>18</sup> On the other hand, the Stern layer has polarity equal to that of alcohols.<sup>19</sup> When a nonpolar group is introduced into an aqueous solution, the hydrogen bonding network formed by the existing water molecules is disrupted and the water molecules order themselves around the nonpolar entity to satisfy hydrogen bonds (Figure 4 (a)). This results in an unfavorable decrease in entropy in the bulk water phase. As additional nonpolar groups are added to the solution, they self-associate thus reducing the total water-accessible surface of the complex relative to the monodisperse state. (Figure 4 (b))

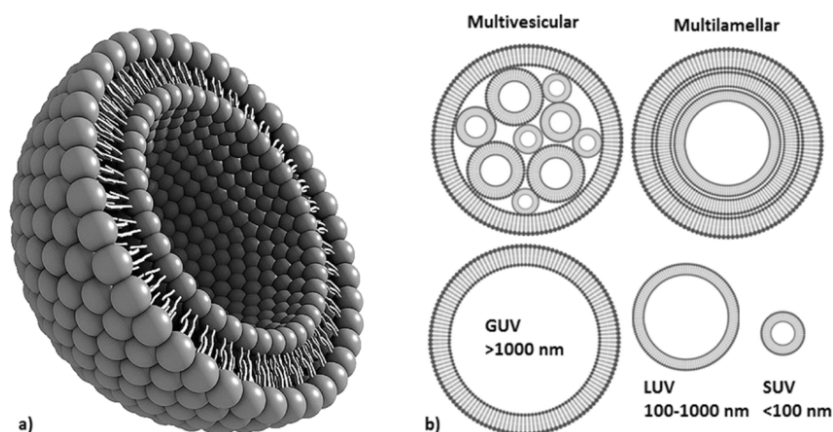


**Figure 4. (a) Water molecules ordered around surfactant monomers, (b) Loss of total water-accessible surface as a result of micellization.**

Now, fewer water molecules are required to rearrange around the collection of nonpolar groups. Therefore, the entropy associated with the complex is less unfavorable than for the monodisperse detergents. In short, hydrophobic association and the formation of micelles is driven by the favorable thermodynamic effect on the bulk water phase. An extensive compilation of the cmc's of surfactants in aqueous media has been published.<sup>20</sup>

### 1.2.2 Vesicles

Vesicles are closed bilayered or hollow spherical structures similar to those of the lamellar phase characterized by two distinct water compartments, one forming the core and other the external medium. Like micelles, the formation of vesicles is a result of energetically favorable hydrophobic association of the hydrocarbon tail(s) of an amphiphilic molecule. However, unlike micelles, two distinct domains are present in vesicles: a lipophilic membrane and the aqueous interior cavity. Precisely, the surface of micelles is a lipid monolayer, while that of liposomes is a lipid bilayer. Whereas, the inner core of micelles is composed of hydrocarbon chains, and that of vesicles is an aqueous phase.<sup>21,22</sup> Vesicles are dynamic supramolecular structures generally composed of amphiphilic molecules that form bilayers enclosing a small aqueous compartment. Besides the common phospholipids, synthetic amphiphiles can also form vesicles and bilayers that encapsulate an aqueous compartment. Due to the close analogy of synthetic vesicles with biological membranes, research on vesicles have been undertaken extensively giving rise to a blossoming field of biomimetic membrane chemistry.<sup>4,23</sup> Specific molecules can bind to the bilayer membrane and mediate interactions between the vesicles or, in selected cases, can be transported across the membrane. For this reason, synthetic vesicles are versatile model systems for protein and carbohydrate mediated recognition, fusion, adhesion and transport phenomena that are known from ion and water channels, endocytosis, viral infection, cell adhesion and the development of tissue from individual cells.<sup>24</sup> Vesicles are differentiated on basis of size and structure as follows: small unilamellar vesicles (SUVs, <100 nm), large unilamellar vesicles (LUVs, 100 -1000 nm), giant unilamellar vesicles (GUVs, >1  $\mu\text{m}$ ), multilamellar vesicles (MLVs, >1  $\mu\text{m}$ ) and multivesicular liposomes (MVLs, >1  $\mu\text{m}$ ). The term “liposome” is generally used for vesicles composed of natural phospholipids, while the term “vesicle” includes vesicles composed of synthetic amphiphiles, phospholipids, and other components. A schematic representation is shown in Figure 5.



**Figure 5. Schematic representation of (a) liposome with assembly of phospholipids in a bilayer showing both inner aqueous and outer lipid compartments within the structure and (b) various forms and sizes of vesicles; small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), giant unilamellar vesicles (GUVs), multilamella and multivesicular liposomes.<sup>25</sup>**

Vesicles can also be formed by mixing a single-tailed cationic surfactant with an aromatic acid. This is similar to the mixtures of surfactants because the surfactant and the aromatic acid tend to bind very strongly. The difference is that only one of the two components in the mixture is a surfactant. Mixtures of CTAB and 5-methyl salicylic acid (5mS) have been previously studied by Davis et al.<sup>26</sup> Surprisingly, at a molar ratio around 1.1, it was found that their solution contained unilamellar vesicles. Cryogenic transmission electron microscopy (cryo-TEM) was employed, which showed that vesicles were around 100 nm in diameter. Zheng, Zakin and co-workers<sup>27</sup> also used cryo-TEM to infer the presence of vesicles in mixtures of CTAB and the sodium salt of 3-methyl salicylic acid (3mS). These vesicles transformed into cylindrical micelles upon shearing. In the system studied by Manohar et al., a surfactant was obtained by mixing of CTAB with sodium 3-hydroxynaphthalene-2-carboxylate (SHNC) in equimolar amounts, followed by removal of excess counterions.<sup>28</sup> In aqueous solutions, at room temperature, the surfactant molecules assembled into MLVs with diameter around 1 - 10  $\mu\text{m}$ .<sup>29</sup> Due to the formation of these large MLVs, highly turbid and viscous solutions were obtained (viscosities ca. 100 times that of water). On increasing the temperature, the samples transformed into clear solutions containing wormlike micelles (discussed later), thereby leading to an increase in viscosity by about an order of magnitude. Davies and co-workers, using turbidimetry, rheological techniques, and small-angle neutron scattering (SANS) measurements, have demonstrated that the aromatic derivative, 5-methyl salicylic acid (5mS), can induce the cationic surfactant,

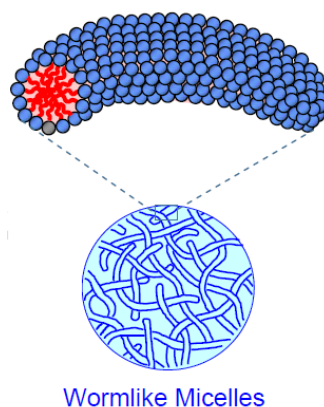


CTAB, to form either wormlike micelles or unilamellar vesicles depending on the solution composition.<sup>30</sup> Eisenberg and Zhang reported the formation of vesicles from self-assembly of polystyrene-poly (acrylic acid) (PS-PAA) block copolymer.<sup>31</sup> Due to the wide array of intramolecular and intermolecular interactions within block copolymer assemblies, the block copolymer vesicles (or polymerosomes) has superior mechanical and physical properties, compared to the lipid based vesicles (or liposomes).<sup>32</sup> Following this, hundreds of papers and reviews have been published reporting the formation of vesicles from single, di-block and tri-block copolymers.<sup>33-37</sup>

Biocompatible vesicles show high promise in applications for the in vivo delivery of anti-cancer drugs for tumour treatment<sup>38</sup> and even in the treatment of degenerative brain conditions.<sup>39</sup> Vesicles with responses to pH stimulus also exhibit applications for the rapid and non-cytotoxic cellular delivery of DNA sequences, which opens the possibilities for efficient gene-delivery for the treatment of specific genetic diseases.<sup>40,41</sup> Polymer vesicles has also been used as synthetic nano-reactors<sup>42</sup> for several biological connotations.<sup>43</sup> Furthermore, the potential applications of polymer vesicles as synthetic intra-cellular organelles have been the basis for several striking reports, with possibilities for the treatment of genetic diseases caused by either enzyme inactivity or deficiency.<sup>44</sup>

### 1.2.3. Worm like micelles

Under specific conditions like salinity, pH, counter ion variation etc., micelles can grow into ‘polymer-like’ elongated and flexible aggregates, referred to as ‘wormlike’ (or ‘threadlike’) micelles (Figure 6).



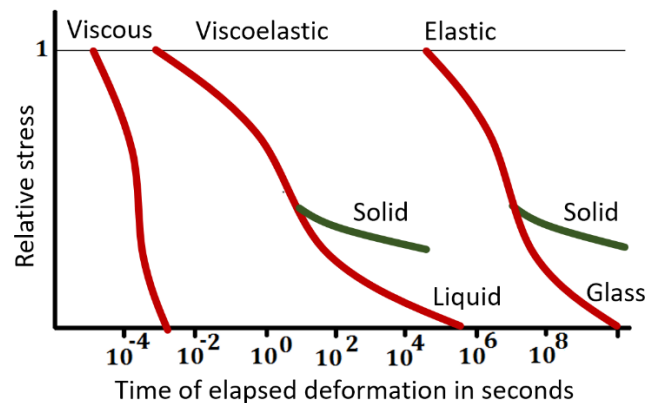
**Figure 6. Microstructure of a typical wormlike micelle.**

The formation of worm like micelles is a consequence of the system minimizing the excess free energy by reducing the number of end caps as the spontaneous curvature of the end caps is higher than the curvature along the cylindrical body. The dynamics of these systems are particularly interesting, because subtle changes in the surfactant, counterion and added electrolyte alter the dimensions, flexibility and interactions of the micelles which lead to marked effects on the macroscopic rheological behavior.<sup>45</sup> The rheological behavior of these surfactant solutions is known to follow “reaction-reptation model” which is an extension of the reptation model of polymer relaxation to cylindrical micelles of surfactant molecules undergoing reversible scission and recombination processes.<sup>46</sup> Wormlike micelles have drawn considerable interest over the past three decades, both from a theoretical viewpoint as well as for industrial and technological applications, the range of which keeps expanding. From a fundamental perspective, wormlike micelles are of specific interest, as they are a model of ‘equilibrium polymers’ (or ‘living’ polymers)<sup>47-49</sup>: extended linear objects that constantly break and reform. Due to their transient nature, they exhibit novel static and dynamic properties on time-scales both long and short compared to their finite lifetime.<sup>49-51</sup> The unique viscoelastic properties of worm like micelles have been exploited to tune the rheology in various applications without the use of polymers or additives.<sup>52</sup> Industrial significance include their use as heat-transfer fluids, hard-surface cleaners, drag reduction agents,<sup>53-56</sup> fracturing fluids in oil fields,<sup>57</sup> solids transports and personal care products,<sup>58</sup> for which both their high viscosity and elastic properties are exploited. Several theoretical approaches and simulations<sup>59-61</sup> have been proposed to predict structure-property relationships and understand how the structural features of the aggregates can be tuned by specific control parameters, and how these dictate the bulk properties. The bulk properties and dynamics have been measured by rheological measurements, recently by microrheology and neutron spin-echo while detailed structural information is typically obtained by small-angle neutron scattering (SANS), static and dynamic light scattering (LS), and cryogenic transmission electron microscopy (cryo-TEM).<sup>62</sup>

### **1.3. Rheology of viscoelastic fluids**

Rheology is the science of deformation and flow. The term originates from Greek ‘rheos’ meaning ‘to flow’. One common factor between solids, liquids, and all materials

whose behavior is intermediate between solids and liquid is that if we apply a stress or load on any of them they will deform or strain. The response of materials towards the externally applied strain or deformation describes its rheological behavior. The experimental characterization of a material's rheological behavior is known as rheometry. Typically, materials are either elastic or viscous. Elasticity is the tendency of solid materials to return to their original shape after forces are applied on them. An ideal elastic material stores all imposed deformation energy and recovers totally on release of stress. Viscosity is a measure of a fluid's resistance to flow. An ideal viscous material is unable to store any deformation energy. It is irreversibly deformed when subjected to stress i.e., it flows and the deformation energy is dissipated as heat resulting in rise in temperature. Materials whose properties are intermediate between viscous and elastic are known as viscoelastic i.e., they store some of the deformation energy in their structure while some is lost by flow. The relationship between stress and strain of viscoelastic materials depends on time or, in the frequency domain, on frequency. One of the way of characterization of materials is by its relaxation time- i.e. the time required to reduce the stress in the material by its flow. Some of the typical relaxation time for materials are- gas  $<10^{-6}$  sec, liquid  $10^{-6} - 2$  sec, solid  $> 10^2$  sec. Figure 7 represent the difference in viscous, elastic and viscoelastic fluids in terms of relaxation time.



**Figure 7. Curves showing the differences between viscous, viscoelastic and elastic materials when subjected to deformation.<sup>63</sup>**

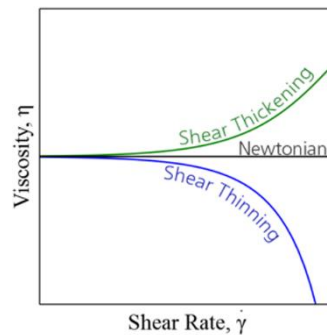
Shearing of a substance is the key to the knowledge of its flow behavior and structure. A sheared flow is achieved through: (a) flow between two parallel plates, (b) rotational flow between two coaxial cylinders, of which one is stationary and other is rotating, (c) telescopic flow through capillaries and pipes (d) torsional flow between parallel plates. Depending on their viscosity behavior as a function of shear rate, stress, deformation,

fluids are characterized as Newtonian or non-Newtonian. Newtonian fluids are named after Sir Issac Newton (1642 - 1726) who described the flow behavior of fluids with a simple linear relation between shear stress [mPa] and shear rate [1/s]. This relationship is now known as Newton's Law of Viscosity, where the proportionality constant  $\eta$  is the viscosity [mPa.s] of the fluid:

$$\sigma = \eta * \dot{\gamma} \quad (1)$$

Where,  $\sigma$  is the shear stress,  $\dot{\gamma}$  is the shear rate and  $\eta$  is the viscosity.

Newtonian fluids are characterized by a single co-efficient of viscosity and thus they have constant viscosity dependent on temperature but independent of shear rates. Typical examples are water, mineral and vegetable oils and pure sucrose solutions. In reality most fluids are non-Newtonian, i.e., their viscosity is dependent on shear rate (Shear Thinning or Thickening) or the deformation history (Thixotropic fluids). In contrast to Newtonian fluids, non-Newtonian fluids display either a non-linear relation between shear stress and shear rate (Figure 8), have a yield stress, or viscosity that is dependent on time or deformation history or a combination of all the above.



**Figure 8. Viscosity of Newtonian, shear thinning or shear thickening fluids as function of shear rate.**

A fluid is shear thickening if the viscosity of the fluid increases as the shear rate increases (Figure 8). A common example of shear thickening fluids is a mixture of cornstarch and water. Fluids are shear thinning if the viscosity decreases as the shear rate increases. Shear thinning fluids, also known as pseudo-plastics, are ubiquitous in industrial and biological processes. Common examples include ketchup, paints and blood. A fluid while flowing under constant stress may show some elastic properties as well. When such bodies are subjected to a sinusoidally oscillating stress, the strain is neither exactly in phase with the stress (as it would be for a perfectly elastic solid) nor 90 degrees out of phase (as it would be for a perfectly viscous liquid) but rather exhibits a strain that lags the stress at a value between zero and 90 degrees:

The sinusoidal deformation (strain) can be expressed as

$$\gamma(t) = \gamma_0 \exp(i\omega t) \quad (2)$$

Where,  $\gamma_0$  is the amplitude of the strain,  $\omega$  is the angular frequency and  $t$  is the time.

The shear stress developed will also be sinusoidal with a phase angle  $\delta$ , thus

$$\sigma(t) = \sigma_0 \exp(i[\omega t + \delta]) \quad (3)$$

For an oscillatory shear, we can define a complex shear modulus  $G^*$  as:

$$G^* = \frac{\sigma(t)}{\gamma(t)} = \frac{\sigma_0 (\cos\delta + i\sin\delta)}{\gamma_0 \exp(i\omega t)}$$

$$\text{or, } G^* = G' + iG'' \quad (4)$$

$G'$  and  $G''$  are referred as the storage or elastic modulus and loss or viscous modulus respectively and are measure of the elastic or viscous property of the system.

Oscillatory shear measurements are a convenient way to measure viscoelasticity of entangled wormlike micelles formed in various surfactant solutions. Wormlike micelles have two principal mechanisms of stress relaxation: first, reptation occurs when the system is sufficiently concentrated to be entangled, as for classic polymers.<sup>64</sup> Reptation describes how a polymer chain escapes from the tube formed by the surrounding entangled chains. The characteristic time of stress relaxation by reptation,  $\tau_{rep}$ , is directly proportional to  $\frac{L^2}{D_c}$  with  $L$  the micellar contour length and  $D_c$  the curvilinear diffusion constant of the micelle. Second, micelle breaking (scission) and recombination is expressed by the characteristic time  $\tau_{br}$ , which is inversely proportional to  $L$ .<sup>47</sup> When the system is sufficiently entangled, reptation is much slower than breaking. In this fast breaking limit ( $\tau_{br} \ll \tau_{rep}$ ) several scission and recombination events take place within the reptation time scale. In this limit, the stress relaxation is close to that of a Maxwell fluid, with a single exponential stress decay,<sup>47,48</sup> characterized by one relaxation time,  $\tau_R$ :

$$\tau_R = (\tau_R \cdot \tau_{rep})^{1/2} \quad (5)$$

The viscoelastic behaviour of giant micelles is often close to that of a Maxwell fluid, for which  $G'$  and  $G''$  are related as:

$$G'(\omega) = \frac{G_0 \omega^2 \tau_R^2}{(1 + \omega^2 \tau_R^2)} \quad (6)$$

$$G''(\omega) = \frac{G_0 \omega \tau_R}{(1 + \omega^2 \tau_R^2)} \quad (7)$$

where  $G_0$  is the elastic modulus extrapolated to infinite frequency, also referred to as the plateau

modulus.  $G_0$  is proportional to the number density of the entanglement points, therefore it characterizes the network structure whereas  $\tau_R$  is a measure of the time required for the original state to be reestablished after the application of a stress and therefore characterizes the dynamics of the system. The zero shear viscosity,  $\eta_0$ , for Maxwell fluids is given by:

$$G_0 = \eta_0 \cdot \tau_R \quad (8)$$

The network mesh size of the entangled wormlike micelles is characterized by  $\xi$ , the hydrodynamic correlation length. This length scale can be obtained directly from the plateau modulus  $G_0$ .<sup>65</sup>

$$\xi = \left( \frac{k_B T}{G_0} \right)^{1/3} \quad (9)$$

$\xi$  is related to the persistence length,  $l_p$ , of the micelles and the entanglement length,  $l_e$  the average distance along the micelle between two entanglement points in the micellar network as:

$$l_e = \frac{\xi^{1/3}}{l_p^{2/3}} \quad (10)$$

The entanglement length can be determined from measurements of viscoelasticity using<sup>66</sup>

$$\frac{G_0}{G''_{min}} = \frac{L_{avg}}{l_e} \quad (11)$$

where  $L_{avg}$ , is the average micelle length and  $G''_{min}$  is the local minimum of the  $G''$  curve at frequencies above  $1/\tau_R$ . The ratio  $L_{avg}/l_e$  defines the average number of entanglements per micelle.

Quantitative rheological measurements show that this viscoelasticity is characterized by a single relaxation time, a property which is rather unusual for fluids with complex

microstructures.<sup>67</sup> This rule is indeed so general that it is now admitted that a single relaxation time in the linear mechanical response is a strong indication of the wormlike character of self-assembled structures. The kinetics of the micelle formation and breakdown above the cmc is based on multiple equilibria, in which the micelles grow or shrink by stepwise incorporations or dissociations of monomers.<sup>19,68,69</sup> For surfactant showing preferentially the cylindrical aggregation, the end-cap energy,  $E$ , denotes the excess in packing energy (between a spherical and a cylindrical environment) for the molecules located in the two hemispherical end-caps. The growth laws for neutral and polyelectrolyte micelles are presented as follows.

### Neutral micelles

The end-cap energy  $E$  is here equivalent to the scission energy necessary to create two new chain ends. For a dispersion of micelles of length  $L$  and molecular weight distribution  $c(L)$ , the minimization of a free energy that takes into account the end-cap energy and the translational entropy yields for the average micellar length,  $L_{avg}$ ,<sup>70-72</sup>

$$L_{avg} = \frac{2}{n_0} c^{1/2} \exp\left(\frac{E}{2k_B T}\right) \quad (12)$$

where  $n_0$  is the number of surfactant per unit length of the linear aggregate.  $n_0$  (in  $\text{\AA}^{-1}$ ) is of the order of unity. The distribution in length  $c(L)$  is broad and given by:

$$c(L) = \frac{c}{L_{avg}^2} \exp\left(-\frac{L}{L_{avg}}\right) \quad (13)$$

where  $c(L)dL$  denotes the number density of chains of length comprised between  $L$  and  $L + dL$ .

### Polyelectrolyte Micelles

MacKintosh and coworkers have proposed a model to demonstrate that the electrostatic interactions reduce the scission energy and favor the breaking of micelles.<sup>73,74</sup> For polyelectrolyte

micelles, the end-cap energy is not equivalent to the scission energy. The electrostatic contribution to the free energy results in a broad dilute regime. There, the micelles are rather monodisperse and their length increases very slowly with concentration. The overlap concentration,  $c^*$  between the dilute and semidilute regimes depends on the effective linear charge density,  $\nu$ , through the relationship:

$$c' = \left(\frac{k_B T l_b R_c v^2}{E}\right)^2 \quad (14)$$

where  $l_b$  is the Bjerrum length ( $l_b = 7.15 \text{ \AA}$  in water) and  $R_c$  the radius of the cylinder. Above the overlap threshold, which also corresponds to the screening of the electrostatic interactions by the counterions, the micelles grow more rapidly according to:

$$L_{avg} = \frac{2}{n_0} c^{1/2} \exp\left(\frac{E}{2k_B T} [1 - (c'/c)^{1/2}]\right) \quad (15)$$

The predictions for the dynamics of growth of neutral and polyelectrolyte micelles agree qualitatively with experiments. For instance, it is known that the addition of salt to a solution of polyelectrolyte micelles can result in a strong increase of viscosity, the solution passing from a viscous fluid to a viscoelastic gel.<sup>75</sup>

#### 1.4. Formation and Physical Chemistry of Wormlike Micelles

Wormlike micelles can form spontaneously at ambient temperature using cationic surfactants, e.g., with 16 carbon atoms in the aliphatic chain. This is the case for cetyltrimethylammonium bromide (CTAB)<sup>76,77</sup> and cetylpyridinium bromide (CPBr).<sup>78</sup> The growth of the aggregates can be promoted however if cosurfactants or other low molecular weight additives are incorporated to the solutions. These additives are short alcohol chains, strongly binding counterions, oppositely charged surfactants etc. the different classes of surfactants and cosurfactants/additives which form such structure. These are discussed elaborately as follows.

**(a) Surfactant and simple salt.** The addition of simple salts such as sodium chloride (NaCl) or potassium bromide (KBr) to ionic surfactant solutions results in the screening of the electrostatic interactions between the charges, and thus in the growth of the aggregates. The archetype system of class A is CTAB with KBr.<sup>49,79-83</sup> Other well-known examples are sodium dodecyl sulfate (SDS) with monovalent<sup>84-90</sup> or multivalent counterions.<sup>91,92</sup>

**(b) Surfactant and cosurfactant,** where the cosurfactant is a short alcohol chain. Classical examples are the ternary systems sodium alkylsulfate-decanol (Dec)-water, Sodiumdecyl sulfate (SdS)-Dec<sup>93-96</sup> and Sodiumdodecyl sulfate (SDS)-Dec<sup>97-99</sup> and cetylpyridinium chloride (CPC)-hexanol-Hexanol (Hex)-brine.<sup>100-104</sup> In these systems,



the ratio between the alcohol and surfactant concentrations controls the polymorphism of the self-assembly.

**(c) Surfactant and strongly binding counterion.** Strongly binding counterions are small molecules of opposite charge with respect to that of the surfactant. They are sometimes called hydrotopes. Well-known examples of hydrotopes are salicylate, tosylate and chlorobenzoate counterions, which all contain an aromatic phenyl group. CTAB and CPC with sodium salicylate (NaSal) have been probably the most studied micellar systems during the last two decades.<sup>105-120</sup> Contrary to simple salts (class A), a large proportion of these counterions (~ 80 %) is assumed to be incorporated into the micelles. It was found that in CPC-NaSal, long wormlike micelles are immediately formed at the cmc (0.04 wt. %), without passing through any intermediate spherical morphology.<sup>105,106,121</sup>

**(d) Amphoteric surfactant.** Amphoteric surfactants are surface active molecules that contain positive and negative charges in the head group. Betaine-type molecules with quaternary ammonium and carboxylate groups are the representatives of this class. They associate at low concentrations and aqueous solutions exhibit strong gel-like properties. These properties are attributed to the existence of an entangled network of micelles.<sup>122,123</sup>

**(e) Gemini surfactants and surfactant oligomers.** The covalent linking of amphiphilic moieties at the level of the head group yields to Gemini surfactants and surfactant oligomers.<sup>124</sup> In aqueous solutions, these molecules present a broad polymorphism of aggregation.<sup>125-129</sup> Gemini surfactants are one of the rare examples for which cylindrical micelles close on themselves spontaneously, forming loops or rings. This property has been attributed to large end-cap energies.<sup>130</sup>

**(f) Cationic and anionic surfactant mixtures.** Oppositely charged surfactants have shown synergistic enhancements of rheological properties, and notably through the formation of mixed wormlike micelles. The growth of the micelles is assumed to arise from the charge neutralization of the surface potential and from the related increase of the ionic strength (as in type (a)). Recent examples studied are the mixtures of sodium dodecylsulfate (SDS) and dodecyltrimethylammonium bromide (DTAB)<sup>131,132</sup> or the mixtures made from cetyltrimethylammonium tosylate and sodium dodecyl benzenesulfonate.<sup>133, 134</sup>

**(g) Block copolymers.** Cylindrical self-assembly have also been reported in aqueous solutions of low molecular weight block copolymers.<sup>135-137</sup> The system investigated is poly(ethylene oxide)-poly(butadiene) with a weight fraction around 50 % for the first block.<sup>135,136</sup> “Giant” micelles have been found by cryo-transmission electron microscopy and the cylindrical morphology of the poly(butadiene) core was confirmed by neutron scattering. Beside the ionic surfactants, non-ionic surfactants such as polyoxyethylene ethers such as C<sub>12</sub>E<sub>5</sub>,<sup>138</sup> C<sub>10</sub>E<sub>51</sub>,<sup>139</sup> and C<sub>16</sub>E<sub>6</sub><sup>140</sup> are also reported to form worm like micelles in presence of co-surfactants. Lecithin (or phosphatidylcholine),<sup>141-144</sup> is one of the most widely studied lipid-based surfactant forming wormlike micelles and is used in pharmaceutical formulations as it is well-tolerated and non-toxic. Since the past decade, an increasing number of studies have been focusing on wormlike micelles made from biocompatible or biodegradable surfactants, which could find potential applications as drug-delivery systems. Examples include block-copolymers based on methoxypoly(ethylene glycol) and poly(caprolactone-b-L-lactide)<sup>145</sup> and biomimetic glycopolymer–polypeptide triblock copolymers for site-specific drug delivery.<sup>146</sup>

### 1.5. Morphology transition in presence of aromatic hydrotropes/counterions

Organic  $\pi$ -conjugated molecules are effective tuners in the formation of various nanostructured materials, and the entailed route is potentially facile and efficient for the development of functional materials of technological and biological importance.<sup>147-150</sup> Microstructural transitions of micellar aggregates, especially the nature of transition from ordinary micelles to long worm-like giant micelles and the vesicles, mediated by organic  $\pi$ -electron systems are of fundamental scientific interest and have been investigated. Interactions of aromatic hydroxy compound, phenol (PhOH) with micellar aggregates of CTAB in aqueous solutions have been investigated by means of titration calorimetry, solution conductimetry, and <sup>1</sup>H NMR spectroscopy.<sup>151, 152</sup> Results indicate that PhOH molecules can interact directly with CTAB micelles formed either in H<sub>2</sub>O or D<sub>2</sub>O and reside both at palisade layer<sup>152</sup> as well as at the hydrophobic core of the micelles.<sup>151</sup> The strongly negative enthalpy change of micellization, The  $\Delta H_{mic}^0$ , values measured for the PhOH/CTAB/H<sub>2</sub>O system suggest that the main driving force of phenol solubilization is the increased PhOH - CTAB interaction upon transfer from the unmicellized aqueous phase. Influence of electrolyte on the interaction between the micelles of CPC and phenol was studied by Xu et. al.<sup>153</sup> Compared with sodium chloride

(NaCl) and sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), the presence of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) promoted the solubilization of phenol in CPC micelle significantly due to the enhancement in degree of dissociation of phenol. The addition of  $\text{Na}_2\text{CO}_3$  produced more (phenolate ion)  $\text{C}_6\text{H}_5\text{O}^-$  at the same phenol concentration. The ion interaction between  $\text{C}_6\text{H}_5\text{O}^-$  and (pyridinium ion)  $\text{C}_5\text{H}_5\text{N}^+$  played more important role in the solubilization of phenol in CPC micelle than the polar interaction between phenol and CPC micelle in the presence of  $\text{Na}_2\text{CO}_3$ . A thermo-responsive phase transition from a viscoelastic wormlike micelle solution to an elastic hydrogel in a mixture of an imidazole-type surfactant, 1-hexadecyl-3-methylimidazolium bromide ( $\text{C}_{16}\text{mimBr}$ ), and sodium salicylate (NaSal) was reported by Lin. et al.<sup>154</sup> They speculated that  $\pi$ - $\pi$  interactions between aromatic groups may play a key role in the observed thermo-responsive phase transition. You et. al.<sup>155</sup> reported the structural transitions of wormlike micelles triggered by pH in system comprised of ionic liquid (IL) *N*-hexadecyl-*N*-methylpyrrolidinium bromide ( $\text{C}_{16}\text{MPBr}$ ) and anthranilic acid (AA). For 80mM  $\text{C}_{16}\text{MPBr}$ /80 mM AA composition, highly entangled wormlike micelle were observed at pH 5.01 which disappeared at pH 2.03 as well as at pH 7.03. The  $\text{pK}_a$  values of the AA are 2.14 and 4.92 and its isoelectric point is 3.2. When pH value increases from  $\sim 2$  to  $\sim 5$ , an increased binding of AA on the headgroups of  $\text{C}_{16}\text{MPBr}$  occurred due to reduced electrostatic repulsion, which led to the morphology of aggregates transit from spherical micelles to WLMs. The structural transitions occurring with change in pH for aqueous mixtures of CTAB and NaSal were further investigated by Umeasiegbu et. al. at various temperatures using dynamic light scattering and small-angle neutron scattering study.<sup>156</sup> Beside structural transition from rigid cylindrical micelles at neutral pH to spherical micelles at  $\sim$  pH 2 upon protonation of salicylate molecules; an unanticipated reversion to flexible cylindrical micelles with further decrease in pH was observed. Their results suggested that, in addition to the well-described electrostatic and hydrophobic interactions in cationic surfactant-hydrotrope mixtures, the pH-induced microstructural changes are potentially governed by complementary cation- $\pi$  and hydrogen bonding interactions. The effect of extended aromatic  $\pi$ -conjugated compounds 1 and 2-Naphthols on the shape transition of CTAB and cetylpyridinium bromide (CPB) micelles were studied by Saha et. al.<sup>157</sup> They revealed that in the absence of charge screening of surfactant head groups, H bonding among micelle embedded naphthol molecules probably played the key role in micellar shape transition. While fluorescence quenching via H-bond strengthening was not observed in the

micellar phase, UV absorption spectra demonstrated the presence of inter molecular H bond in micelle-embedded naphthols in their ground electronic states, which was confirmed by FTIR. In a subsequent paper,<sup>158</sup> they established that the success of naphthols in effecting microstructural transition of micelles lie in their unique ability to form H-bonds with interfacial water molecules, which have shown unusual H-bond donating properties compared to bulk water. They reported that OH groups of micelle-embedded naphthols remain protruded toward the Stern layer through  $\sim 1 \text{ \AA}$  and the dielectric constant of OH sites has been measured as  $45 \pm 2$  by observing the pKa shift of acid-base equilibrium of naphthols at the interface relative to that in bulk water. This observation was significant in relation to orientation of aromatic moieties in biological membrane. In studying the the location, orientation, and dynamics of small hydrophobes 1 naphthol and 1-methylnaphthalene in lipid dimyristoylphosphatidylcholine (DMPC) membrane, via <sup>1</sup>H- nuclear overhauser effect spectroscopy (NOESY) and molecular dynamics simulation, Shintani et. al. have shown that 1-naphthol exhibits a slight preference for pointing its OH group toward the hydrophilic domain of the membrane while no definite preference was concluded for the orientation of 1-methylnaphthalene.<sup>159</sup> Stronger NOE was observed for 1-naphthol due to the restricted motion of the OH group along with the slowdown of the 1-naphthol motion within the lipid membrane. Study on 1-hexadecyl-3-methylimidazolium cation, [C<sub>16</sub>mim]<sup>+</sup> with aromatic anions, 4-hydroxybenzenesulfonate, [HBS]<sup>-</sup>, benzenesulfonate, [BS]<sup>-</sup>, and p-toluenesulfonate [PTS]<sup>-</sup> have shown that the ILs exhibited 2-3 fold lower cmc values as compared to that of conventional surfactants or ILs having inorganic anions as counterions.<sup>160</sup> The high promise and applicability of aromatic  $\pi$ -conjugated systems in promoting structural transition and their industrial and biological relevance have been explored in this dissertation.

## **1.6. Need for innovation in novel surfactant development**

Besides the constant challenge of finding ways to minimize the manufacturing cost for existing surfactants, the market pull for ‘greener’ products is the overriding driving force for surfactants development. Some recent methodology that are being followed are - to synthesize the surfactant from natural building blocks; to insert one or more weak bonds into the structure in order to speed up the degradation, such surfactants are sometimes referred to as ‘cleavable surfactants’; to use starting materials – natural or synthetic – that give proper rate of biodegradation and to make more efficient

surfactants, i.e., surfactants that can be used in lower amounts; gemini surfactants are examples of that approach. With the rising challenges of environmentally benign chemical processing, ionic liquids (ILs), consisting of a large organic cation and a corresponding small anion, have emerged in recent years as discussed earlier. ILs have drawn increasing interest for their promising role as alternative media in a variety of catalytical, separation, and electrochemical processes as a result of their unique chemical and physical properties.<sup>161-163</sup> According to the generally accepted definition, these salts are liquid below 100°C, and have a negligible vapor pressure.<sup>164</sup> They have been shown to be excellent candidates in the fields of catalysis, nanostructure materials, organic synthesis, electrochemistry, and liquid/liquid extraction.<sup>165-169</sup> More importantly, their chemical and physical properties can be effectively and easily tailored by changing the cation, anion, and substituent components.<sup>170</sup> ILs bearing long alkyl chain can be regarded as a novel category of amphiphiles. ILs are considered environment friendly compounds, mainly because they are non-flammable and non-volatile. Additionally, enhanced reaction rates obtained in ILs allows the reduction of solvent volumes in the given technological process, thus reducing costs, risks and possible waste. These properties prompt the interest to replace common volatile organic solvent with ILs. The opportunity of cation modification and anion selection as well as enormous number of their possible combinations let to improve their physical and chemical properties. This, in turn, fulfills technological demands and provides for contemporary technology and especially chemical industry, novel so called task-specific media.<sup>171</sup> The high interest is also due to the capability of ILs to form a wider range of intermolecular interactions than typical volatile organic solvents. This includes interactions of the type: strong and weak ionic, hydrogen bonding, van der Waals, dispersive,  $n-\pi$  and  $\pi-\pi$  interactions.<sup>172,173</sup> A number of ILs also exhibit surface activity at air/water interface and due to this inherent nature, they are called surface-active ionic liquids (SAILs), and they can form aggregates with specific structures, shapes, and properties. The aggregation behavior of SAILs has therefore been extensively investigated in the field of colloid and interface chemistry.<sup>174,175</sup> Bowers et al. investigated the aggregation behavior of three ionic liquids based on the 1-alkyl-3-methyl-imidazolium cation, 1-butyl-3-methylimidazolium tetrafluoro-borate ([BMIM][BF<sub>4</sub>]), 1-methyl-3-octylimidazolium chloride and iodide ([OMIM][Cl] and [OMIM][I], respectively) in aqueous solutions by means of surface tension, conductivity, and small angle neutron scattering (SANS) measurements.<sup>176</sup> Their

results revealed that SAILs behaved like short chain surfactants in aqueous solutions and formed micelles at concentrations higher than their respective cmc. The conductivity data for the [OMIM][Cl] and [OMIM][I] also indicated the possible presence of aggregates at concentrations below the cmc. Fluorescence quenching technique was used by Vanyur et. al to determine the aggregation number of long chain imidazolium based ionic liquids viz., 1-dodecyl-3-methyl-imidazolium bromide ([DDMIM][Br]), 1-methyl-3-tetradecyl-imidazolium bromide ([TDMIM][Br]) and 1-hexadecyl-3-methyl-imidazolium bromide ([HDMIM][Br]).<sup>177</sup> They observed that the aggregation number of the micelles were smaller than values characteristic for parallel alkyltrimethylammonium bromide (C<sub>n</sub>TAB) surfactants containing the same chain length. The fluorescent behavior of pyrene probe indicated higher polarity in [C<sub>n</sub>MIM][Br] micelles than in the corresponding C<sub>n</sub>TAB micelles. A novel class of zwitterionic SAIL N-alkyl-N-carboxymethylimidazolium inner salts ([N-C<sub>n</sub>, N'-CO<sub>2</sub>-Im], n = 10, 12, 14) was synthesized and reported by Wang. et. al.<sup>178</sup> They showed that these class of SAIL exhibited higher surface active property and lower cmc than conventional imidazolium based SAILs. Detailed experimental and theoretical investigations on another newly synthesized halogen-free and low-cost alkylcarboxylate-based anionic SAIL namely, 1-butyl-3-methylimidazolium alkylcarboxylates ([C<sub>4</sub>mim][C<sub>n</sub>H<sub>2n-1</sub>O<sub>2</sub>], n = 8, 10, 12) was reported by Cheng et. al.<sup>179</sup> An interesting study on C<sub>16</sub>mimBr, with an azobenzene derivative, sodium azobenzene 4-carboxylate (AzoCOONa) in aqueous solution was done by Yu et.al. Initially, viscoelastic wormlike micelles with a viscosity of 0.65 Pa·s were constructed in the C<sub>16</sub>mimBr/AzoCOONa system. Upon irradiation by UV light (365 nm), the wormlike micelles become much longer and more entangled, exhibiting a high viscosity of 6.9 Pa.s.<sup>180</sup> This can be attributed to photoisomerization of the AzoCOONa molecule from trans to cis form. It is the first time that, with exposure to UV or visible light, the aggregate type of the photoresponsive system has been found to remain unchanged, with only a change of internal property parameters. The cation- $\pi$  interaction prevailing over the hydrophobic interaction and electrostatic interaction between C<sub>16</sub>mimBr and AzoCOONa molecules was supposed to be responsible for this peculiar phase behavior. Apart from these, a large number of reports on aggregation of different imidazolium based surface active ionic liquids are available in literature.<sup>181</sup>

The diverse application of the self-assembled aggregates of molecules has also led to the research in increasing the extent of adaptiveness of these aggregates towards different chemical and physical environment. Modification in adaptive nature of self-assembly of molecules towards parameters like temperature, pH, pressure, moisture etc. have also been a major field of interest. The environmental adaptiveness, including smart self-assemblies adaptive to pH, temperature, pressure, and moisture; special chemical adaptiveness, including nanostructures adaptive to important chemicals, such as enzymes, CO<sub>2</sub>, metal ions, redox agents, explosives, biomolecules; field adaptiveness, including selfassembled materials that are capable of adapting to external fields such as magnetic field, electric field have been extensively researched in the past and ongoing decade.<sup>182</sup>

Amino acids, on the other hand, are natural products and the linkage between the amino acid residue and the hydrophobic tail is often an ester or an amide bond. Such bonds are easily degraded by the action of hydrolytic enzymes such as lipases and peptidases. This means that the majority of amino acid-based surfactants are readily biodegradable and non-toxic, especially to marine organisms. In addition, the most common of the amino acid-based surfactants have proven to be mild to the skin and non-sensitizing. Long chain N-alkyl derivatives of amino acids are amphoteric surfactants. There has been an interest in N-alkyl cysteine-based surfactants because of the possibilities that the thiol group in the polar head can provide.<sup>183,184</sup> Task specific surfactants based on l-glycine, l-alanine, l-valine, l-glutamic acid, and l-proline having superior surface activity compared to conventional surfactants, were prepared by Trivedi et al.<sup>185</sup> Amino acid-based surfactants have many of the characteristics that are needed for surfactants in consumer products. The transmembrane domains of integral membrane proteins show an astounding accumulation of the aromatic amino acids tyrosine and tryptophan residues, especially in the region of the highest lipid density. It has been found that these residues perform vital antioxidant functions inside lipid bilayers and protect cells from oxidative destruction.<sup>186</sup> Long-chain acylated tyrosine and tryptophan, but not phenylalanine or short-chain acylated derivatives, are potent inhibitors of lipid peroxidation and oxidative cell death. Statistical analyses of amino-acid distribution patterns in integral membrane proteins reveal a pronounced enrichment of tyrosine and tryptophan in the transmembrane domains of all major classes of membrane proteins: Single-span as well as multi-span membrane proteins, proteins of the  $\alpha$ -helix bundle as

well as of the  $\beta$ -barrel type.<sup>187-189</sup> Tyrosine and Tryptophan are especially enriched in the region contacting the membrane zone of the highest lipid density, which comprises the inner portion of the lipid headgroups and the beginning of the hydrocarbon tails. The antioxidant functions of tyrosine and tryptophan provide a specific explanation for (a) their unique transmembrane distribution pattern and (b) the high vulnerability of low-protein neuronal membranes to oxidative stress, as seen in neurodegenerative disorders. With the growing concern about the biological effects of chemicals it is likely that the class of amino acid surfactants will become even more important in the future.

References are provided in BIBLIOGRAPHY under “References for Chapter I” (Page 155-168)