

CHAPTER 8

Biomimetic Amphiphiles: Properties and Potential Use

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Abstract

Surfactants are the amphiphilic molecules that tend to alter the interfacial and surface tension. The fundamental property related to the structure of surfactant molecules is their self-aggregation resulting in the formation of association colloids. Apart from the packing of these molecules into closed structures, the structural network also results in formation of extended bilayers, which are thermodynamically stable and lead to existence of biological membranes and vesicles. From biological point of view the development of new knowledge and techniques in the area of vesicles, bilayers and multiplayer membranes and their polymerizable analogue provide new opportunities for research in the respective area. 'Green Surfactants' or the biologically compatible surfactants are in demand to replace some of the existing surfactants and thereby reduce the environmental impact, in general caused by classic surfactants. In this context, the term 'natural surfactants or biosurfactants' is often used to indicate the natural origin of the surfactant molecules. Most important aspect of biosurfactants is their environmental acceptability, because they are readily biodegradable and have low toxicity than synthetic surfactants. Some of the major applications of biosurfactants in pollution and environmental control are microbial enhanced oil recovery, hydrocarbon degradation, hexa-chloro cyclohexane (HCH) degradation and heavy-metal removal from contaminated soil. In this chapter, we tried to make a hierarchy from vital surfactant molecules toward understanding their behavioral aspects and application potential thereby ending into the higher class of broad spectrum 'biosurfactants'. Pertaining to the budding promise offered by these molecules, the selection of the type and size of each structural moiety enables a delicate balance between surface activity and biological function and this represents the most effective approach of harnessing the power of molecular self-assembly.

Introduction

Surfactants are among the most versatile products of the chemical industry appearing in essential biological systems and industrial processes.¹ Our food, cosmetics, medicines, house-hold items, the drilling mud used in prospecting for petroleum and the floatation agents used in beneficiation of ores, contain a wide range of surfactants. Surfactant is an abbreviation for surface active agent, which literally means: a species, which is active at the interface. In other words, a surfactant is characterized by its tendency to adsorb at the surfaces and interfaces. The term interface denotes a boundary between any two immiscible phases while, the term surface indicates that one of the phases is a gas, generally air.^{2,3} The driving force for surfactant adsorption is the lowering of free energy of the phase boundary.

The interfacial free energy per unit area is what we measure when we determine the interfacial tension between two phases. It is the minimum amount of work required to create unit area of

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the interface or to expand it by unit area. When we measure the surface tension of a liquid, we are measuring the interfacial free energy per unit area of the boundary between the liquid and the air above it. When the interface is expanded, the minimum work required to create the additional amount of that interface is the product of the interfacial tension γ times the increase in the area of the interface: $W_{\min} = \gamma \times \Delta A$. When the boundary is covered by the surfactant molecules, the surface tension (or the amount of work required to expand that interface) is reduced. The denser the surfactant packing at the interface, larger is the reduction in surface tension.^{4,6}

Surfactants may adsorb at all the interfaces listed below:

Solid—Vapor *Surface*

Solid—Liquid

Solid—Solid

Liquid—Vapor *Surface*

Liquid—Liquid

However, the discussion will be restricted to the interface involving a liquid phase. The liquid considered here is usually, but not always water. Examples of different interfaces and products in which these interface are important are given in Table 1. In many formulated products several types of interfaces are present at the same time. Water-based paints and paper coating colors are examples of familiar ones, but from the point of view of a colloidal chemist, the complicated systems contain both solid-liquid (dispersed pigment particles) and liquid-liquid (latex or other binder droplets) interfaces.

In addition, foam formation is a common (though unwanted sometimes) phenomenon at the application stage. All types of interface are well-stabilized by the surfactants.⁷ The total interfacial area of such systems is so immense that oil-water and solid-water interfaces of one liter of paint may cover several football fields. This can be related to an old, saying by Benjamin Franklin in 1974, reported to the British Royal Society:

“At length at Clapman where there is, on the common, a large pond, which I observed to be one day very rough with the wind, I fetched out a cruet of oil and dropped a little of it on the water. I saw it spread itself with surprising swiftness upon the surface. The oil, though not more than a teaspoonful, produced an instant cam over a space several yards square, which spread amazingly and extended itself gradually until it reached the leeside, making all that quarter of the pond, perhaps half an acre, as smooth as a looking glass.”

$$V \sim 2 \text{ ml}, A \sim 2000 \text{ m}^2 \longrightarrow \text{thickness of layer} \sim 1 \text{ nm}$$

It was not until over a hundred years later when Lord Rayleigh suspected that the maximum extension of an oil film on water represented a layer within the thickness of a single molecule.

Coming back to surfactant, these molecules have a strong tendency to accumulate at the interfaces and it can be considered as the fundamental property of these species. In principle stronger is the tendency of accumulation better is the surfactant. The degree of surfactant accumulation at a boundary depends upon the surfactant structure and also upon the nature of the two phases that meet at the interface. Therefore the choice of surfactant depends upon the application potential e.g., some surfactants molecules are soluble only at the oil-water interface.

Table 1. Examples of interfaces involving a liquid phase

Interface	Type of System	Product
Solid-liquid	Suspension	Solvent-borne point
Liquid-liquid	Emulsion	Milk, cream
Liquid-vapour	Foam	Shaving cream

Scope

The purpose of this chapter is to provide an insight into the basics of surfactant molecules, their behavior in solution and most importantly the application of these molecules, in special reference to the biological systems.

Surfactants find applications in almost every chemical industry including detergents, paints, dyestuff, cosmetics, agrochemicals, fibers and pharmaceuticals. Therefore, the fundamental understanding of the physical chemistry of surface-active agents, their usual properties and their phase behavior is essential for most chemical industries. In addition, understanding of the basic phenomenon involved in application of surfactants in the preparation of emulsions, suspensions and micro-emulsions etc. is of vital importance in arriving at right system composition. In pharmaceutical science the usage of surfactants is associated with the control release of trapped drugs along with their in-vivo and in-vitro preparation.

Commercially produced surfactants are not pure chemically and within each chemical type there can be tremendous variation. This is understandable since surfactants are prepared from various feedstocks. It is thus advisable to obtain as much information as possible from the manufacturer about the properties of surfactants e.g., its suitability of the job, variation in the batch, toxicity and impurity, if any.

In the chapter emphasis has been given on the basics of surfactants, their classification, the phenomenon of self-assembly, behavior in solution and microbial surfactants in reference to their properties and commercial potential.

Surfactant Basis

'Surfactants are the amphiphiles': the word being derived from the Greek word "amphi" meaning both and the term is related to the point that all surfactant species consist of at least two parts: a nonpolar hydrophobic portion, usually a straight or branched hydrocarbon chain containing ~8-18 carbon atoms, attached to a polar or ionic portion (hydrophilic). The hydrophilic part is referred to as the head group and hydrophobic part as the tail (Fig. 1). The hydrophobic part of the surfactants may be branched or linear and interacts weakly with water molecules in an aqueous environment. The degree of chain branching, the position of the polar group and the length of the chain are important parameters in deciding the physico-chemical properties of the surfactants.

The polar part of surfactant may be ionic or non-ionic in natural. For non-ionic surfactants the size of the head group can be varied at will but in the case of ionic surfactant this parameter is a fixed one. The polar head group interacts strongly with water molecules (via dipole or ion-dipole interactions) which renders the surfactant soluble in water. The cooperative action of dispersion and hydrogen bonding between the water molecules tends to squeeze the hydrocarbon chain out of water and hence these chain are referred to as hydrophobic. It is the balance between the hydrophobic and hydrophilic parts of the molecule that gives these systems, their special property of accumulation at various interfaces and behavior of self-assembly (i.e., micellization).⁸

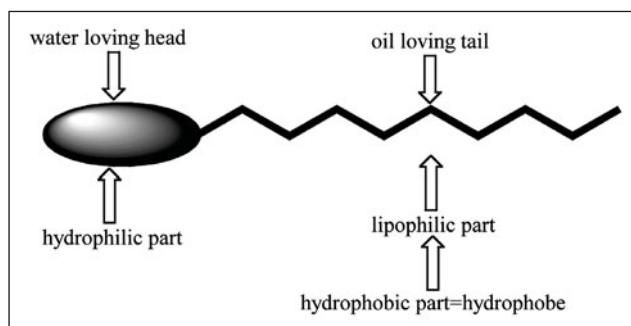
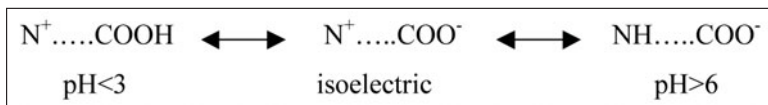


Figure 1. Structure of surfactant molecule.



Scheme 1. pH dependent ionization of surfactant molecules anionics.

Specific Classes of Surfactants

In general surfactants are classified on the basis of the charge of the polar head group and the common practice is to divide the surfactants into anionic, cationic, non-ionic and zwitterions.⁹⁻¹¹ Most ionic surfactants are monovalent but there are also examples of divalent anionic amphiphiles. The most common counterion in anionic surfactants is sodium; however other cations e.g., Li^+ , K^+ , Ca^{+2} and protonated amines are used as counterion for specialty purposes. The counterion of cationic surfactants is usually a halide or sulfate. The hydrophobic group is normally a hydrocarbon (alkyl or arylalkyl) but many vary for polydimethylsiloxane or a fluorocarbon. The common non-ionic surfactants are based on ethylene oxide and are referred as ethoxylated surfactants. The amphoteric or the zwitterionic surfactants contain both the cationic and anionic groups. The characteristic feature of these surfactants is their dependence on the pH of the solution in which they are dissolved. In acidic solutions, the molecules acquire a positive charge and behave like cationic surfactants, whereas in alkaline solutions they become negatively charged and behave like an anionic one. A specific pH can be defined at which both ionic groups show equal ionization, specified as the isoelectric point of the molecule (Scheme 1).

Of the four classes of the surfactants, anionics are the most widely used. Important types of anionic surfactants are carboxylates, sulfonates, sulfates and the phosphates. Figure 2 shows the structure of commonly used anionic surfactants.

Important aspects of anionic surfactants are:

1. Major reason for the popularity of the anionic surfactants is their low cost of manufacture.
2. They possess enhanced foaming and spreading properties.
3. Since sulfonate group is a strong acid, the sulfonate surfactants are soluble and effective in acidic as well as in alkaline media and thus they are useful for textile scouring formulations.
4. Sulfates are more hydrophilic than the sulfonates.
5. Sodium salts are the most common although salts with diethanolamine, thiethanolamine and ammonia are used in cosmetics and shampoos.
6. Phosphate surfactants are excellent emulsifiers under strongly alkaline conditions.

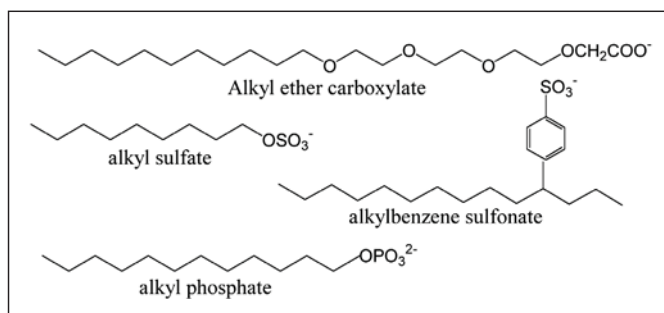


Figure 2. Structures of anionic surfactants.

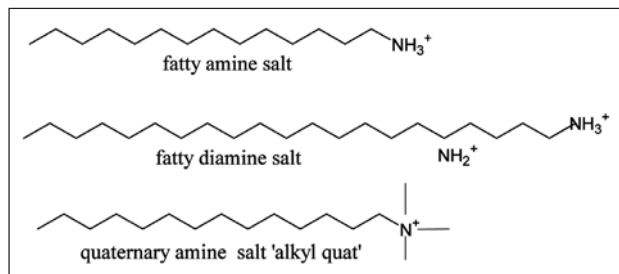


Figure 3. Structure of some cationic surfactants.

Cationics

Most of the uses of cationic surfactants result from their ability to adhere and modify the solid surfaces. Figure 3 represents the structures of some typical cationic surfactants. The common types of cationic surfactants are long chain amines and quaternary amine salts (alkyl 'quat'). Amines function only at the protonated state and thus cannot be used at high pH. The quaternary ammonium compounds are not pH sensitive.

Important facts about cationic surfactants are:

1. They are important as corrosion inhibitors, fuel and lubricating oil additives, germicides and hair conditioners.
2. Other applications include their use as fabric softener, fixatives for anionic dye and drying rate retarder for cationic dye.
3. Cationic surfactants are compatible with non-ionic and zwitterionic surfactants. However their usage is small compared to anionic and non-ionic ones.
4. They adsorb strongly to most surfaces and their main uses are related to in situ surface modification.

Non-Ionics

Non-ionic surfactants have either a polyether or a polyhydroxyl unit as the polar group. In majority of cases, the polar group is a polyether consisting of oxyethylene units, made by the polymerization of ethylene oxide.

The non-ionic surfactants can be specified in terms of the following aspects:

1. These have diverse uses in textiles.
2. The hydrocarbon group is the hydrophobic part of the surfactant while the chains of ethylene oxide group is hydrophilic part. The length of ethylene oxide chain determines the hydrophilicity of the surfactants.
3. Non-ionic surfactants are compatible with all other types of surfactants.
4. Their low foaming tendency can be an advantage to the horticulture industry where they do a good job of breaking water surface tension.
5. They are more effective than the sulfonate surfactants in removing soil from hydrophobic fibres but are inferior to anionic surfactants for soil removal from cotton.
6. The properties of a non-ionic surfactant can be tailored somewhat for a particular use by controlling a relative amount of hydrophilic and hydrophobic characters.

Zwitterionics

Zwitterionic surfactants contain two charged groups of different sign. Whereas the positive charge is almost invariably ammonium, the source of negative charge may vary, although carboxylate is the most common. Common types of zwitterionic surfactants are N-alkyl derivatives of simple amino acids such as glycine ($\text{NH}_2\text{CH}_2\text{COOH}$), betaines ($(\text{CH}_2)_2\text{NCH}_2\text{COOH}$) and amino-propionic acid ($\text{NH}_2\text{CH}_2\text{CH}_2\text{COOH}$). Structures of some of the zwitterionic surfactants are shown in Figure 4.

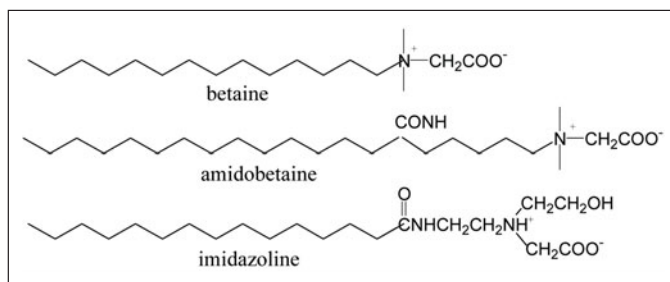


Figure 4. Structure of some zwitterionic surfactants.

Some interesting facts about these surfactants include:

1. These species provide a feel of softness to textile materials.
2. Zwitterionic surfactants are compatible with all other classes of surfactants and are soluble and effective in the presence of high concentrations of electrolytes, acids and alkalis.
3. They exhibit cationic behavior near or below their isoelectric points and anionic behavior at high pH.
4. Their uses in horticulture crop production is very rare.
5. The products from the zwitterionic surfactants are very specifically used to match the properties of specific pesticide formulations and generally are not used in the green house as the stand-alone products.

Surface Active Compounds are Ample in Nature

Nature's own surfactants are the polar lipids and these are abundant in all living organisms. In the biological systems the surface-active agents are used in the similar manner as they are used in the technical systems: to overcome the solubility problems as emulsifiers, as dispersants and to modify the surfaces etc. The examples of polar lipids are given in Figure 5.

A good example of biological surfactants is: bile salts, which are extremely efficient solubilizers of hydrophobic components in the blood. On the other hand the phospholipids packed as ordered bilayer constitute the cell membrane.

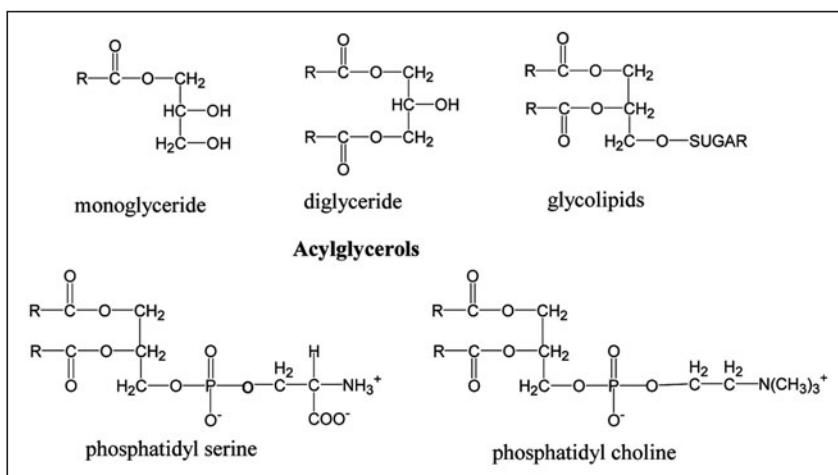


Figure 5. Polar lipids acting as surfactants.

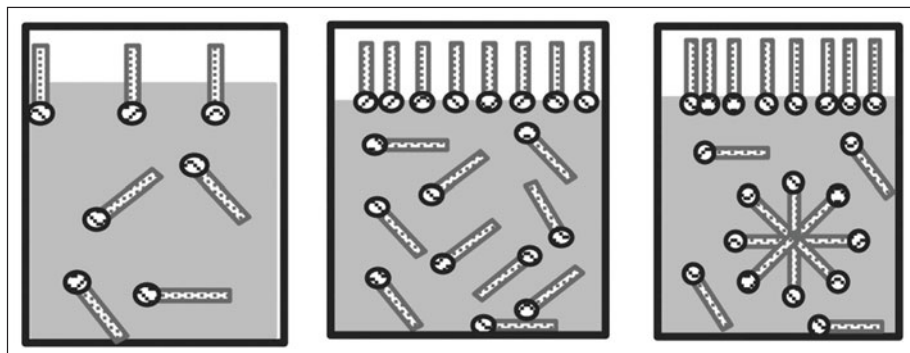


Figure 6. Self-assembly of surfactant monomers.

Self-Assembly Processes

As discussed earlier the characteristic feature of surfactants is their tendency to adsorb at the interface in an oriented fashion.¹²⁻¹⁴ This adsorption has been studied to determine:

- i. The concentration of surfactant at the interface, since this is a measure of how much of the interface has been covered (and thus changed) by the surfactant: the performance of the surfactant in many interfacial processes (e.g., foaming, detergency, emulsification etc.) depends on its concentration at the interface.
- ii. The orientation of the surfactant at the interface, since this determines how the interface will be affected by the adsorption, that is, whether it will become more hydrophilic or more hydrophobic.
- iii. The energy changes i.e., free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) in the system, resulting from the adsorption, since these quantities provide information on the type and mechanism of any interactions involving the surfactant at the interface and the efficiency of its operation as a surface-active material.

The nature of surfactant molecules, having both the lyophilic and the lyophobic groups, is responsible for their tendency to accumulate at the interface and thus reduce the free energy of the system in which they interact. Another fundamental property related to the structure of surfactant molecules is their tendency to form self-associated structures, called the 'micelles'. Micelle formation or the phenomenon of micellization can be viewed as structurally resembling the solid crystals or the crystalline hydrates. Thermodynamically, the formation of micelles favors an increase in solubility of the surfactant molecules. Micelles are generated at very low surfactant concentration in dispersion media (which generally is water).

The concentration at which micelles start to form is called the critical micellization concentration (*cmc*). The *cmc* is an important characteristic of individual surfactant.¹⁵ Figure 6 depicts the self-assembly of surfactant monomers when their concentration exceeds the critical value.

The measurement of bulk properties of solution e.g., surface tension, electric conductivity, light scattering etc. as a function of surfactant concentration at some point reflects the change occurring in the nature of solute species (Fig. 7). This break point corresponds to the *cmc* of a typical surfactant. A *cmc* of 2 mM for any surfactant means that unimer concentration will never exceed this value, regardless of the amount of surfactant added to the solution i.e., after the concentration of 2 mM the surfactant mainly exist in the self-assembled form.

Association Colloids

The various surfactant aggregates in general are categorized as the 'association colloids' where particle size ranges between 10-100 nm. The association colloids formed by the self-aggregation of the surfactant monomers differs from the other colloids in that they are in dynamic equilibrium with the monomers in the solution.

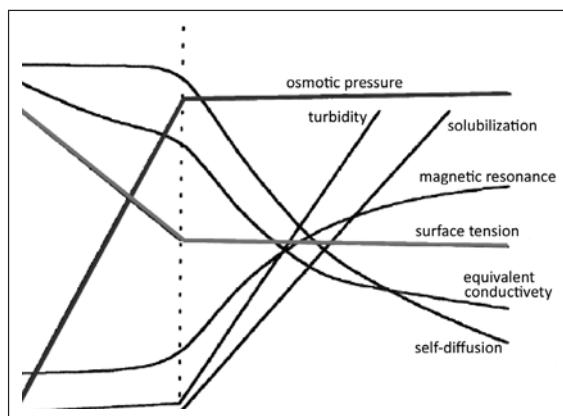


Figure 7. Break point in measured property indicate appearance of *cmc*.

Micelles

McBain¹⁶ proposed the presence of molecular aggregates in soap/surfactant solutions on the basis of the unusual change in the measured bulk property of the system. Micellar colloids represent the dynamic association-dissociation equilibrium. However, ever since the conception of micellization, the structure of micellar aggregates has been a matter of discussion. McBain¹⁶ suggested the formation of two distinct types of micelles: spherical structures composed of ionized salt molecules and lamellar structure comprised of non-ionic aggregates. Subsequently Hartley's¹⁷ model consisted of essentially spherical micelles with diameter equal to approximately twice the length of the hydrocarbon chain. X-ray studies by Harkin's et al¹⁸ suggested the sandwich or the lamellar model. Later Debye and Anacker¹⁹ proposed that micelles are rod shaped rather than spherical or disc like. The cross section of such a rod would be circular, with the polar heads of the detergent lying on the periphery and the hydrocarbon tails filling the interior. The end of the rod would almost certainly have to be rounded and polar. Hartley's (1956) spherical micelle model has been established by Reich²⁰ from the viewpoint of entropy and the spherical form of micelles is now generally accepted as the actual structure. Figure 8 shows various shapes ascribed to the surfactant aggregates.

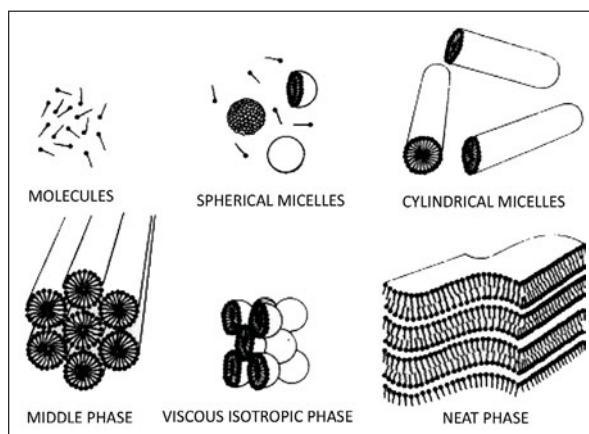


Figure 8. Shapes and the structure of different micelles.

The formation of micelles by ionic-surfactant is ascribed to the balance between the hydrocarbon chain attraction and the ionic repulsion. In general, the net charge of micelles is less than the degree of micellar aggregates indicating the large fraction of counter-ions remains associated with the micelles (These counter-ions form the *stern layer* at the micellar surface). In the case of non-ionic surfactant, the hydrocarbon chain attraction is opposed by the requirement of the hydrophilic group for hydration and space. Therefore, the micellar structure is determined by the equilibrium between repulsive forces among the hydrophilic groups and the short-range attractive forces among hydrophobic groups. In other words, the chemical structure of a given surfactant determines the shape and size of the micelles.

Classical Theories of Micelle Formation

In classical literature two approaches/models for the micellization have been accepted: the mass action model and pseudophase model.²¹ In mass action model it is assumed that an equilibrium exists between the surfactant monomers and the micelles e.g., in the case of non-ionic (or unionized) surfactants, the monomer/micelle equilibrium can be written as:



With a corresponding equilibrium constant, K_m , given by

$$K_m = [\text{M}]/[\text{S}]^n$$

where n is the number of monomers in the micelles, termed as the aggregation number. In such systems the activity of the surfactants may increase with the total concentration above *cmc*, although the size of that increase may be negligibly small.

In the phase separation or the pseudophase model the micelle is assumed to be a separate but soluble phase, which appears when the surfactant concentration reaches the *cmc*. The *cmc* therefore may be viewed as a solubility limit or the saturation concentration for the monomeric species. According to such a concept the concentration of individual surfactant molecules should not increase beyond that attained at the point of micelle formation. The assumption for the pseudophase model that the activity of the monomeric species remains constant above the *cmc* is related to the observation that the surface tension of a surfactant solution normally remains constant above that concentration.²

Micelles and Ahead

The associations of surfactants into simpler structures like spheres, rods and discs allow a direct analysis of the fundamental aspects of their behavior.

However, the amphiphiles which can not pack themselves into closed structures result in the assembly of extended bilayers. Such molecules have relatively small head groups or bulky hydrocarbon network. Although extended bilayers are thermodynamically favorable, there are the conditions under which it is more desirable to form the closer bilayers systems, leading to existence of biological membranes and the vesicles. Considering the impact of surfactants and the membranes on the biological systems, it has been a goal in many allied fields to develop a well-characterized synthetic model of biological membranes and enzymes. From biological point of view the development of new knowledge and techniques in the area of vesicles, bilayers and multiplayer membranes and their polymerizable analogue provide new opportunities for research in the respective area.

Emulsification

Emulsification—the formation of emulsions from two immiscible liquid phases is probably the most versatile property of surface-active agents for practical applications.²² Paints, polishes, pesticides, mettle cutting oils, margarine, ice-cream, cosmetics, metal cleaners and textile processing oils are all examples of emulsions in one form or the other.

An emulsion is a significantly stable suspension of particles of one liquid (say water) of certain particle size within the second, immiscible liquid (say oil). For this suspension, surfactant due to their polarity acts as a good stabilizing agent. The solution eventually remains transparent and aggregation of surfactants encircling the oil becomes sufficiently large that the oil in the center has the properties similar to that of bulk oil. This oil could be considered to be emulsified by the surfactant and not the solubilized. So, as long as the mixture forms spontaneously, is not turbid and does not separate, it would merit the name microemulsion. Basically the size of dispersed particles count for the three types of formulations: (i) macroemulsions, the opaque emulsions with particle size >400 nm that are easily visible under the microscope, (ii) microemulsions, transparent dispersion with particle size <100 nm and (iii) miniemulsions, a recently suggested type that is blue with particle size between 100-400 nm.

Recently explored are the multiemulsions where the dispersed particles themselves are the emulsions of the all types. Microemulsions has remained the area of significant research because of their wide spread application potential which vary from miniature house hold products to large scale enhanced oil recovery. In the case of microemulsions the dual character of surfactants provides a way to the stable mixing of two entirely different phases (say: water and oil). These two phases in turn provide the microenvironment to the solubilization of external entity, which occupy the space in microemulsion media according to their physico-chemical aspects. The avenue of such formulations has largely been explored in biological applications e.g., in pharmaceuticals where microemulsions act as drug carrier molecules in vivo, in agricultural and house hold products, as dry cleaning fluids, in beverages and as dyeing agents.

Biosurfactants and Their Potential Uses

The particulate properties of surfactants confer excellent detergency, emulsifying, foaming and dispersing traits, which makes them one of the versatile chemical products.²³ More than 4500 tons of environmentally harmful surfactants (classified as emulsifiers, floatation aids and surfactants) were used in Sweden during 1999. A good surfactant should begin with the user and end up friendly to the environment.²⁴ 'Green Surfactants' or the biologically compatible surfactants are in demand to replace some of the existing surfactants and thereby reduce the environmental impact. In this context, the term 'natural surfactants or biosurfactants' is often used to indicate the natural origin of the surfactant molecules. Biosurfactants are the amphiphilic compounds produced on living surfaces, mostly on the microbial cell surfaces and contain the hydrophobic and hydrophilic moieties that have the ability to accumulate between the liquid interface, thus reducing the surface and interfacial tension.²⁵ Originally, biosurfactants attracted attention in the late 1960s and their applications have been greatly extended in the past five decades as an improved alternative to the chemical surfactants (carboxylates, sulphates and esters), preferably in food, pharmaceuticals and the oil industry.^{26,27} The reason for their popularity as high-value microbial products is primarily because of their specific action, low toxicity, higher biodegradability, effectiveness at extreme temperatures, wide spread applicability and their structure which provide different properties than that of the classical surfactants.

Activity of Biosurfactants

The activities of biosurfactants can be determined by measuring the change in physico-chemical property, stabilization or destabilization of emulsion and the hydrophilic-lipophilic balance (HLB). When surfactant is added to air/water or oil/water system at increasing concentration, a change in measured property is observed up to a critical level, above which supramolecular structures corresponding to surfactant assemblies are formed. This critical value is called the *cmc* and is the parameter to measure the efficiency of any surfactant.

Biosurfactants may stabilize (emulsifiers) or destabilize (de-emulsifiers) the emulsion. The emulsification activity is determined by the ability of surfactant to generate turbidity due to suspended hydrocarbons such as a hexadecane-2-methylnepthalene in an aqueous assay system.²⁸ The de-emulsification activity is derived by determining the effect of surfactant on a standard emulsion by using a synthetic surfactant.²⁹

The HLB value indicates whether a surfactant will promote water-in-oil or the oil-in-water emulsion. Emulsifiers with HLB value less than 6 favor stabilization of water-in-oil emulsification whereas the HLB value in the range 10-18 gives the opposite effect and favors the oil-in-water emulsification.

Classification of Biosurfactants

Unlike chemically synthesized surfactants, which are classified on the basis of polar head-groups, biosurfactants are classified by their chemical composition and microbial origin. Rosenberg and Ron³⁰ have suggested that biosurfactants can be divided into low-molecular-mass molecules, which efficiently lower the surface and interfacial tension and the high-molecular-mass polymers, which are more effective as emulsion-stabilizing agents. In general, the structure of biosurfactants includes the hydrophilic moiety consisting of amino acids or peptide anions or cations; mono-, di-, polysaccharides; and a hydrophobic moiety consisting of unsaturated or saturated fatty-acids.³¹ The major types of biosurfactants and the microbial species of origin are listed in Table 2.

Glycolipids

Most of the biosurfactants are the glycolipids, which are the carbohydrates in combination with long chain aliphatic acids or hydroxyaliphatic acids. The linkage is by means of either ether or an ester group.

Rhamnolipids: In this class one or two molecules of rhamnose are linked to one or two molecules of β -hydroxydecanoic acid and are the best studied glycolipids. While the $-OH$ group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the $-OH$ group of the second acid is involved in ester formation. Production of rhamnose containing glycolipids was first described in *Pseudomonas aeruginosa* by Jarvis and Johnson.³² L-Rhamnopyranosyl-L-rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate (Fig. 9) and L-Rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate, referred to as rhamnolipids 1 and 2, respectively, are the principal glycolipids produced by *P. aeruginosa*.

Trehalolipids: Various types of microbial trehalolipid biosurfactants have been reported.³³ Disaccharide trehalose linked at C-6 and C-6' to mycolic acids is associated with most species of *Mycobacterium*, *Nocardia* and *Corynebacterium*. Mycolic acids are long-chain, α -branched- β -hydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms and the degree of unsaturation. Trehalose dimycolate produced by *R. erythropolis* (Fig. 9) has been extensively studied. Trehalose lipids from *R. erythropolis* lowered the surface and interfacial tension in the culture broth to 25-40 and 1-5 mN m^{-1} , respectively.

Sophorolipids: Sophorolipids, which are produced mainly by yeasts such as *T. bombicola*, *T. apicola* and *T. Petrophilum* consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid (Fig. 9). These biosurfactants are a mixture of at least six to nine different

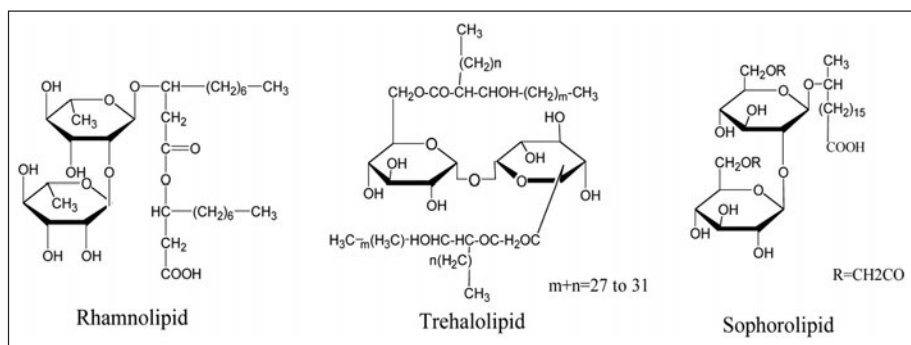


Figure 9. Structures of some common glycolipids.

Table 2. Microbial sources and major types of microbial surfactants

Biosurfactant	Organism	Surface Tension (mN m ⁻¹)
Glycolipids		
Rhamnolipids	<i>P. aeruginosa</i>	29
	<i>Pseudomonas sp.</i>	25-30
Trehalolipids	<i>R. erythropolis</i>	32-36
	<i>N. erythropolis</i>	30
	<i>Mycobacterium sp.</i>	38
Sophorolipids	<i>T. bombicola</i>	33
	<i>T. apicola</i>	30
	<i>T. petrophilum</i>	
Cellobiolipids	<i>U. zeae</i>	
	<i>U. maydis</i>	
Lipopeptides and lipoproteins		
Peptide-lipid	<i>B. licheniformis</i>	27
Serrawettin	<i>S. marcescens</i>	28-33
Viscosin	<i>P. fluorescens</i>	26.5
Surfactin	<i>B. subtilis</i>	27-32
Subtilisin	<i>B. subtilis</i>	
Gramicidins	<i>B. brevis</i>	
Polymyxins	<i>B. polymyxa</i>	
Fatty acids, neutral lipids, phospholipids		
Fatty acids	<i>C. lepus</i>	30
Neutral lipids	<i>N. erythropolis</i>	32
Phospholipids	<i>T. thiooxidans</i>	
Polymeric surfactants		
Emulsan	<i>A. calcoaceticus</i>	
Biodispersan	<i>A. calcoaceticus</i>	
Mannan-lipid-protein	<i>C. tropicalis</i>	
Liposan	<i>C. lipolytica</i>	
Carbohydrate-protein-lipid	<i>P. fluorescens</i>	27
	<i>D. polymorphis</i>	
Protein PA	<i>P. aeruginosa</i>	
Particulate biosurfactants		
Vesicles and fimbriae	<i>A. calcoaceticus</i>	
Whole cells	Variety of bacteria	

hydrophobic sophorosides. Although sophorolipids can lower the surface and interfacial tension, they are not effective emulsifying agents.

Lipopeptides and Lipoproteins

A large number of cyclic lipopeptides including decapeptide antibiotics and lipopeptide antibiotics, produced by *Bacillus brevis* and *Bacillus polymyxa*, respectively, possess remarkable surface-active properties. An aminolipid biosurfactant called serratamolide has been isolated from *Serratia marcescens* NS.38. Studies on serratamolide-negative mutants showed that the biosurfactants increased cell hydrophilicity by blocking the hydrophobic sites on the cell surface.

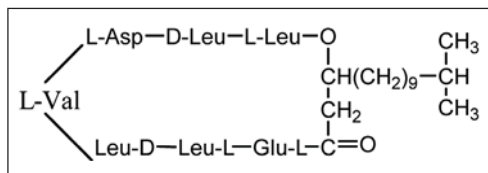


Figure 10. Structure of surfactin obtained from *Bacillus subtilis*.

The cyclic lipopeptide surfactin (Fig. 10) produced by *B. subtilis* ATCC21332, is one of the most powerful biosurfactant. It lowers the surface tension from 72 to 27.9 mN m⁻¹ at concentration as low as 0.005%.

Recently, Yakimov et al³⁴ have showed the production of a new lipopeptide surfactant, lichenysin A, by *B. licheniformis* BAS-50 containing the long-chain β -hydroxy fatty acids.

Fatty Acids, Neutral Lipids, Phospholipids

Several bacteria and yeasts produce large quantities of fatty acids and phospholipid surfactants during growth on n-alkanes. The HLB is directly related to the length of the hydrocarbon chain in their structure. In *Acinetobacter sp.* strain HO1-N phosphatidylethanolamine (Fig. 11) rich vesicles are produced, which form optically clear microemulsion of alkanes in water.

Phosphatidylethanolamine produced by *R. erythropolis* grown on n-alkane caused a lowering of interfacial tension between water and hexadecane to less than 1 mN m⁻¹ and a *cmc* of 30 mg l⁻¹.

Polymeric Surfactants

The best-studied polymeric biosurfactants are emulsan, liposan, mannoprotein and other polysaccharide-protein complexes. *Acinetobacter calcoaceticus* RAG-1 produces a potent polyanionic amphipathic heteropolysaccharide bioemulsifier called emulsan (Fig. 12). Emulsan is a very effective emulsifying agent for hydrocarbon in water even at a concentration as low as 0.001 to 0.01%. It is one of the most powerful emulsion stabilizers known today and resists inversion even at a water-to-oil ratio of 1:4. Biodispersan is an extracellular, nondialyzable dispersing agent produced by *A. calcoaceticus* A2. It is an anionic heteropolysaccharide, with an average molecular weight of 51,400 and contains four reducing sugars.

Liposan is an extracellular water soluble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate and 17% protein. The carbohydrate portion is a heteropolysaccharide consisting of glucose, galactose, galactosamine and galacturonic acid.

Particulate Biosurfactants

Extracellular membrane vesicles partition hydrocarbons, to form a microemulsion which plays an important role in alkane uptake by microbial cells. Vesicles of *Acinetobacter sp.* strain HO1-N with a diameter of 20 to 50 nm and a buoyant density of 1.158 g cm⁻³ are composed of protein,

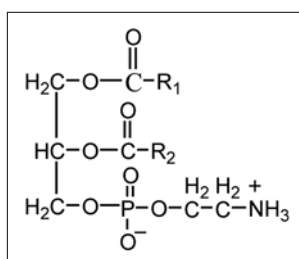


Figure 11. Structure of phosphatidylethanolamine, a microbial surfactant produced by *Acinetobacter sp.*

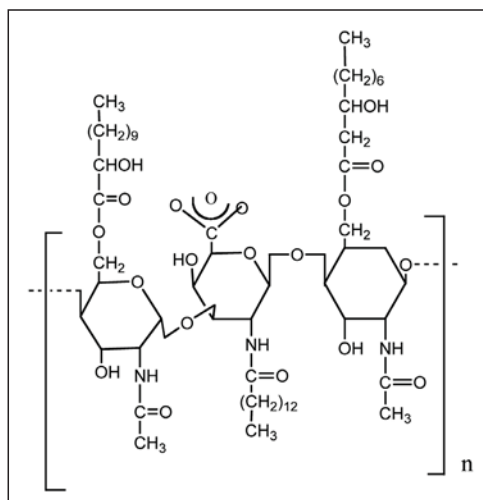


Figure 12. Structure of emulsin, produced by *Acinetobacter calcoaceticus*.

phospholipids and lipopolysaccharide.³⁵ Surfactant activity in most hydrocarbon-degrading and pathogenic bacteria is attributed to the cell surface components.

Properties of Biosurfactants

Biosurfactants are of increasing interest for commercial use because of the continually growing spectrum of available substances. The main distinctive features of biosurfactants and a brief description of their properties are as given below:

Surface and Interfacial Activity

A good surfactant can lower the surface tension of water from 72 to 35 mN m^{-1} and the interfacial tension of water/hexadecane from 40 to 1 mN m^{-1} . Surfactin from *B. subtilis* can reduce the surface tension of water to 25 mN m^{-1} and interfacial tension of water/hexadecane to <1 mN m^{-1} .³⁶ Rhamnolipids from *P. aeruginosa* decreases the surface tension of water to 26 mN m^{-1} and the interfacial tension of water/hexadecane to <1 mN m^{-1} .³⁷

Temperature, pH and Ionic Strength Tolerance

Many biosurfactants and their surface activities are not affected by environmental conditions such as temperature and pH. McInerney et al³⁸ reported that lichenysin from *B. licheniformis* JF-2 was not affected by temperature up to 50 °C, pH 4.5-9.0 and by NaCl and Ca^{+2} concentrations up to 50 and 25 g l^{-1} , respectively.

Biodegradability

Unlike synthetic surfactants, microbial-produced compounds are easily degraded and particularly suited for environmental applications such as bioremediation and dispersion of oil spills.

Emulsion Forming and Emulsion Breaking

Stable emulsion can be produced with a life span of months and year. Higher molecular-mass biosurfactants are in general better emulsifier than the low-molecular-mass biosurfactants. Sophorolipids from *T. bombicola* have been shown to reduce surface tension, but are not good emulsifiers. By contrast, liposan does not reduce the surface tension, but has been used successfully to emulsify edible oils. Polymeric surfactants offer additional advantages because they coat droplets of oil, thereby forming the stable emulsions. This property is especially useful for making oil/water emulsion for cosmetics and food.

Chemical Diversity

The chemical diversity of naturally produced biosurfactants offer a wide selection of surface-active agents with properties closely related to specific applications.

Low Toxicity

Microbial surfactants are generally considered as the low or nontoxic products and therefore are appropriate for pharmaceutical, cosmetic and food industries. A report suggested that a synthetic anionic surfactant (corexit) displayed an LC₅₀ (concentration lethal to 50% of test species) against *Photobacterium phosphoreum* ten times lower than rhamnolipids, demonstrating the higher toxicity of chemical-based surfactants. It was also reported that biosurfactants showed higher EC₅₀ (effective concentration to decrease 50% of test population) value than synthetic surfactants.³⁹

Potential Applications of Biosurfactants

Most important aspect of biosurfactants is their environmental acceptability, because they are readily biodegradable and have low toxicity than synthetic surfactants. These unique properties of biosurfactants allow their use and possible replacement of chemically synthesized surfactants in a great number of industrial applications. Some of the major applications of biosurfactants in pollution and environmental control are microbial enhanced oil recovery, hydrocarbon degradation, hexa-chloro cyclohexane (HCH) degradation and heavy-metal removal from contaminated soil⁴⁰:

Microbial Enhanced Oil Recovery (MEOR)

An area of considerable potential for biosurfactant application is microbial enhanced oil recovery. In MEOR, microorganisms in reservoir are stimulated to produce polymers and surfactants, which aid MEOR by lowering interfacial tension at the oil—rock interface. To produce microbial surfactants in situ, microorganisms in the reservoir are usually provided with low-cost substrates, such as molasses and inorganic nutrients. However, to be useful for MEOR in situ, bacteria must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity and low oxygen level. Several aerobic and anaerobic thermophiles tolerant of pressure and moderate salinity have been isolated which are able to mobilize crude oil in the laboratory.⁴¹

Hydrocarbon Degradation

Hydrocarbon-utilizing microorganisms excrete a variety of biosurfactants. An important group of such surfactants is mycolic acids which are the α -alkyl, β -hydroxy very long-chain fatty acids contributing to some characteristic properties of a cell such as acid fastness, hydrophobicity, adhesion and pathogenicity. This product has many applications in agrochemistry, mineral flotation and bitumen production and processing. Further, the product may be used as an emulsifying and dispersing agent while formulating herbicides, pesticides and growth regulator preparations. The constituent fatty acids of biolipid extract also have antiphytoviral and antifungal activities and therefore, can be applied in controlling plant diseases.⁴²

Hydrocarbon Degradation in the Soil Environment

Degradation is dependent on presence in soil of hydrocarbon-degrading species of microorganisms, hydrocarbon composition, oxygen availability, water, temperature, pH and inorganic nutrients. Addition of synthetic surfactants or microbial surfactants results in increased mobility and solubility of hydrocarbon, which is essential for effective microbial degradation.

Lindley and Heydeman⁴³ have reported that the fungus *Cladosporium resinae*, grown on alkane mixtures, produces extracellular fatty acids and phospholipids, mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhances the alkane degradation rate by 30%. Foght et al⁴⁴ has reported that the emulsifier, Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used.

Hydrocarbon Degradation in Aquatic Environment

When oil is spilled in aquatic environment, the lighter hydrocarbon components volatilize while the polar hydrocarbon components dissolve in water. However, because of low solubility (<1 ppm) of oil, most of the oil components will remain on the water surface. The primary means of hydrocarbon removal are photooxidation, evaporation and microbial degradation. Since hydrocarbon-degrading organisms are present in seawater, biodegradation may be one of the most efficient methods of removing pollutants.⁴⁵

Emulsan, a high MW lipopolysaccharide produced by *A. calcaoceticus* RAG-1, has been proposed for a number of applications in the petroleum industry such as to clean oil and sludge from barges and tanks, reduce viscosity of heavy oils, enhance oil recovery and stabilize water-in-oil emulsions in fuels.

Biosurfactant and HCH Degradation

Hexa-chlorocyclohexane (HCH) is still the highest ranking pesticide used in India and many other countries. Of the eight known isomers of HCH, the alpha-form constitutes more than 70% of the technical product, which is not only known insecticidal but also a suspected carcinogen. The poor solubility is one of the limiting factors in the microbial degradation of alpha-HCH. Presence of six chlorines in the molecule is another factor that renders HCH lipophilic and persistent in the biosphere.

It has been reported that addition of biosurfactant from *Pseudomonas* Ptm⁺ strain facilitated 250-fold increase in dispersion of HCH in water. Addition of either this organism or biosurfactant dislodged surface-borne HCH residues from many types of fruits, seeds and vegetables as well.⁴⁶ Laboratory-scale studies have revealed that microbial surfactants are very efficient in cleaning the containers where HCH residues were sticking to the wall.

Some more applications of biosurfactants include:

- i. Binding of heavy metals. A rhamnolipid biosurfactant has been shown to be capable of removing Cd, Pb and Zn from soil. The mechanism by which rhamnolipid reduces metal toxicity may involve a combination of rhamnolipid complexation of Cd and rhamnolipid interaction with the cell surface to alter Cd uptake.
- ii. Food industry. Lecithin and its derivatives, fatty acid esters containing glycerol, sorbitan or ethylene glycol and ethoxylated derivatives of monoglycerides including a recently synthesized oligopeptide are currently in use as emulsifier in the food industry.
- iii. Cosmetic industry. A large number of compounds for cosmetic applications are prepared by enzymatic conversion of hydrophobic molecules by various lipases and whole cells.⁴⁷ The cosmetic industry demands surfactants with a minimum shelf life of 3 years. Therefore, saturated acyl groups are preferred over the unsaturated compounds. Monoglycerides, one of the widely used surfactants in the cosmetic industry, has been reported to be produced from glycerol-tallow (1.5:2) with a 90% yield by using *P. fluorescens* lipase treatment.
- iv. Medicinal uses. A deficiency of pulmonary surfactant, a phospholipid-protein complex, is responsible for the failure of respiration in prematurely born infants. The isolation of genes for protein molecules of this surfactant and cloning in bacteria has made possible its fermentative production for medical application. 1% emulsion of rhamnolipids is successfully used for the treatment of *Nicotiana glutinosa* infected with tobacco mosaic virus and for the control of potato virus-x disease.

Association Properties of Biosurfactants

The association properties or self-assembly of biologically based amphiphilic molecules into potentially useful structures has been the area of interest. Owing to their dualistic structure these molecules self-assemble to form wide variety of morphologies including micelles, vesicles, tubes and coacervates. The micellar aggregation of biosurfactants is originated at the critical micellar concentration (*cmc*) and interestingly, they have about 10- to 40-fold lower *cmc* compared to chemical surfactants. This fact narrows the gap between the cost and efficiency of biosurfactants. However, bulkier structure of biosurfactants makes them more prone to the result in the formation

of bilayered aggregates prior to the formation of routine microaggregates. It has been found that the single component of glycolipid biosurfactants, mannosyl-erythritol lipid-A (MEL-A) forms the sponge phase (L_3) together with the usual vesicle formation.⁴⁸ Later, it has been observed that the addition of phospholipids to glycolipid sponge phase (L_3) induces the formation of thermodynamically stable vesicle ($L_{\alpha 1}$).⁴⁹ The formation of micellar aggregates followed by higher order aggregates for dirhamnolipids (diRL) extracellular biosurfactant has been observed by Sanchez et al.⁵⁰ As determined by surface tension measurements, at pH 7.4, the *cmc* of dirhamnolipid is 0.110 mM whereas at pH 4.0 the value falls to 0.010 mM, indicating that the negatively charged diRL has a much higher *cmc* than its neutral species. In comparison to other relevant biosurfactants like surfactin (*cmc* = 0.0075 mM), the *cmc* of diRL is one order higher in magnitude, suggesting that dirhamnolipids behave as weak detergents. At higher concentration the diRL results in the formation of mainly multilamellar vesicles of heterogeneous size.

The chemical character of respective hydrophobic portion and the hydrophilic part allows a wide range of variation in the physical and biological properties. Selection of the type and size of each moiety enables a delicate balance between surface activity and biological function and this represents the most effective approach of harnessing the power of molecular self-assembly.

Toxicological and Ecological Aspects of Surfactants

The environmental impact of surfactant volume merging directly to the surrounding has become an important area of concern. The rate of biodegradation of surfactant in combination with the degree of toxicity produced, majorly determines the ecological impact.⁵¹

Dermatological Aspects

A number of dermatological problems of day life can be related to exposure of skin to surfactant solutions. Many of the formulations contain significant amount of surfactants e.g., cutting fluids, rolling oil emulsion, house-hold cleaning formulations and personal care products. The physiological aspects of surfactant on the skin has been investigated by various dermatological laboratories, starting with the surface of skin and progressing via the horny layer and its barrier function to the deeper layer of the vessel cells. Surfactant classes that are generally known to be mild to the skin include the polyol surfactants (alkyl polyglucosides), zwitterionic surfactant (betaines, amidobetaines and isothionates) and many polymeric surfactants. Alcohol ethoxylates are relatively mild but not as mild as the polyol-based non-ionics (the alkyl polyglucoside). In addition, alcohol ethoxylates may undergo oxidation to give by-products (hydroperoxide and aldehydes) that are skin irritants.

Anionic surfactants are generally greater skin irritant than non-ionics. For examples, sodium dodecyl sulphate, which is commonly used in toothpaste, has relatively high skin toxicity. In contrast, ether sulphates are milder and are recommended for use in hand dishwashing formulations. However, some amphoteric surfactants such as betaines can also reduce the skin irritation of anionics.

Aquatic Toxicity

Aquatic toxicity may be measured on fish, daphnia or algae. Toxicity is given as LC_{50} (for fish) or EC_{50} (for daphnia or algae), where LC and EC stand for lethal and effective concentration, respectively. Values below 1 mg l⁻¹ after 96 h testing on fish and algae and 48 h on daphnia are considered toxic.

Bioaccumulation

Bioaccumulation can be measured directly on fish in experimental way but is more often calculated from a model experiment. Partitioning of the surfactant/compound between two phases, organic and water is measured and logarithm of the values, log P, is used. The value of log P usually tells us about the hydrophobicity of the surfactant. A surfactant is considered to be bioaccumulated if:

$$\text{Log } P_{\text{organic/water}} > 3$$

Most of the surfactant have log P values below 3. Bioaccumulation therefore is not considered to be a critical issue.

Biodegradability

This is the biological process carried by bacteria in nature. Through a series of enzymatic reactions, a surfactant molecule is finally converted into CO₂, H₂O and oxides of other elements. However, stable and persistent compound does not undergo natural biodegradation. For surfactants the rate of biodegradation varies from 1-2 h for fatty acids, 1-2 days for linear alkylbenzene sulfonates and months for branched alkylbenzene sulfonates. The rate of biodegradation depends upon the factors such as concentration, pH and temperature. The temperature effect is the most important factor. The rate at which chemicals are broken down in sewage plants may vary by as much as a factor of five between summer and winter in Northern Europe.

Conclusion

The chapter provides an insight into the basics of surfactant molecules, their behavior in solution and most importantly the application of these molecules. The dualistic structure of surfactant molecules results in the subtle balance between the hydrophobic and hydrophilic interactions. This results in their special property of accumulation at various interfaces and behavior of self-assembly (i.e., micellization).

The properties of surfactant molecules make them the most versatile of process chemicals appearing in wide range of product starting from house-hold usage, to medicinal chemistry and then to industries. The last decade has seen the extension of surfactant applications to high-technology areas such as electronic printing, magnetic recording, microelectronic, biotechnology and diversified medicinal research. In surge of green chemistry, the biologically compatible surfactants are in demand to replace some of the existing chemical surfactants. The reason for the popularity of biosurfactants as high-value microbial products is primarily because of their specific action, low toxicity, higher biodegradability, effectiveness at extreme temperatures, wide spread applicability and their structure which provide different properties than that of the classical surfactants.

Biological surfactants are highly sought after biomolecules as fine specialty chemicals, biological control agents and new generation molecules for pharmaceutical, cosmetic and health care industries.

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