Introduction

1. Self-assembled systems and their classifications

1.1. Organized assemblies

An organized molecular assembly is a group of atoms or molecules built in a solid matrix due to chemical or intermolecular interactions and possessing, in total, the ability to produce a definite response to the given perturbation.

Fig. 1.1. Pictorial representation of organized assemblies (liposome, micelle, bilayer sheet).

Self-assembly is a fabricating ordered molecular structures with spatial precision. In other words, a phenomenon in which a number of independent molecules suspended in an isotropic (disordered) solution spontaneously come together to form an ordered aggregate which is of molecular size in one dimension at least. The most impressive examples of self-assembly in biology: the ribosome-a complicated machine for building up a polypeptide chain according to the set of instructions encoded in a chain of messenger RNA. The organization of monomolecular assemblies at the solid surfaces provides a rational approach for fabricating interfaces with an advance. Such assemblies could provide a means to control the chemical and physical properties of the interfaces for a variety of heterogeneous phenomena including catalysis, corrosion, lubrication, and adhesion [1, 2]. Surfactants act as a barrier between the lipid and aqueous phase in a small domain in order to produce different kinds of aggregates such as
monolayers, micelles, reverse micelles, microemulsion, bilayers (vesicles, liposomes etc.) (vide. Fig. 1.1). These assemblies are known to mimic biological aggregate owing to their ability to compartmentalize the substrate.

1.1.1. **Micelles**
Surfactants are organic substances, which significantly decrease the surface tension of water at relatively low concentrations, and possess at least partial solubility in water. As surfactants are absorbed mainly on the surface of the solution, creating a thin monolayer, they are called surface active substances (SAS). At a certain concentration of the surfactant, its molecules or ions begin to associate and organize themselves into more complex units, also called micelles. The characteristic concentration value, where the association process begins, is called the *critical micelle concentration* and abbreviated as CMC. CMC is one of the most useful physicochemical characteristics of many biologically active substances and drugs. Beyond CMC in water, surfactants aggregate to form micelles, where the polar head groups of the surfactant are in contact with the bulk aqueous medium, forming a Stern layer, while the hydrophobic chains are directed towards the centre to from a non-polar core. The central core has properties similar to those of the liquid hydrocarbon. The surface charge density and the hydrophobic tail size are the main controlling factors for the micellar size [*vide. pictorial representation Fig. 1.2(a)*]

1.1.2. **Microemulsions**
Microemulsions can be thought to be oil swollen micelles (o/w microemulsion) or water swollen reverse micelles (w/o microemulsion). The physical characteristics of water/oil microdroplet differ from those of the bulk water or oil. Being a ternary or quaternary system, it has the advantage of offering better compartmentalization than the other organized assemblies. In addition, microemulsions have experienced continuous scientific and industrial development since their inception. The knowledge gained on the fundamental aspects of these systems (both microemulsions and reverse micelles) has made it possible to improve some established applications and to develop new ones. In view of these growing research activities in this area, this review is devoted to the detailed discussion on the various aspects, more precisely, the basic aspects viz. formation characteristics, properties and structural characterization of microemulsions and reverse micelles stabilized by single and mixed surfactants in nonpolar, polar and biocompatible oils. However, numerous books and review articles appeared concerning various aspects of microemulsions/reverse micelles, which will be discussed in
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subsequent sections. Hence it is obvious that everything known about microemulsions cannot be mentioned within a few pages. An attempt has been made to present the most important facts and concepts that are closely related with the topic of the thesis, in the following sections. The basic intention of this thesis is an attempt on the studies emphasizing on the formation and characterization of microemulsions/reverse micelles formed from both single and mixed surfactants of different charge types and mixing ratio, in linear hydrocarbons and alkyl ester oils, as mixtures of surfactants can provide better performance than pure surfactants for a wide variety of applications. Further, major aspect of my dissertation aims at physicochemical studies of mixed surfactant microemulsions. As the thesis contains one chapter on ionic liquid based microemulsions, one section (Sec. 6.0) has been devoted to various aspects of ionic liquids (ILs) and ionic liquid based microemulsions including biocompatible ILs, applications etc.

1.1.3. Reverse micelles

Surfactants associate to form inverted or reversed micelles in non-polar solvents containing a little water. The polar head groups of the surfactant monomers are directed to the centre containing water with the hydrophobic tails extending outwards into the bulk organic solvent phase. Reverse micelles can solubilize an appreciable amount of water to form a spherical pool in the centre [vide. pictorial representation Fig. 1.2(b)]
1.1.4. Vesicles

Vesicles are colloidal structures representing a special class of amphiphile bilayer architecture that enclose a volume with a thin membrane (vide. Fig. 1.1). Vesicles of amphiphilic molecules can be formed in aqueous medium either by external stimuli or can be obtained spontaneously by judicious mixing of cationic and anionic surfactants. Synthetic vesicles have attracted considerable attention because of their diverse applications in the fields, viz., colloids, materials, drug delivery and mimicking biological phospholipid membranes.

1.2. What is microemulsion?

Microemulsion is a system of water, oil and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution, according to Danielsson and Lindman [3]. The word “amphiphile” [amphi means (both sides) and philos means (liking)] was coined by Winsor [4] to describe substances with an affinity towards both non-polar and polar phases. Herein, surfactants are the most important amphiphiles. Their amphiphilic character is strong enough to be driven to the interface where the polar part is located in the polar phase and vice versa. The term “oil” refers to an organic phase which is immiscible or at least partially miscible with the polar phase. Therefore, nonpolar substances such as hydrocarbons, partially or totally chlorinated or fluorinated hydrocarbons, single-chain alkanes, cyclic or aromatic hydrocarbons, but also triglyceride natural oils or polar lipophilic oils can be used [5, 6]. Straight chain hydrocarbons or n-alkanes are the most frequently used non-polar phases in microemulsions.

Microemulsions were not really recognized until the work of Hoar and Schulman in 1943, who reported a spontaneous formation of emulsion of water and oil upon addition of a strong surface-active agent [7]. The term “microemulsion” was first used even later by Schulman et al. [8] in 1959 to describe a multiphase system consisting of water, oil, surfactant and alcohol, which forms a transparent solution. There has been much debate about the word “microemulsion” to describe such systems [9]. Although not systematically used today, some authors prefer the names “micellar emulsion” [10] or “swollen micelles” [11].

1.3. Classification of microemulsions

Three types of microemulsions are most likely to be formed depending on the composition: oil-in-water (o/w), water-in-oil (w/o) and bicontinuous. A schematic representation is also depicted in Fig. 1.3.

Oil-in-water microemulsions are droplets of oil surrounded by a surfactant (and possibly cosurfactant) film that forms the internal phase distributed in water, which is the continuous phase. The monolayer of surfactant forms the interfacial film, which is oriented in a “positive” curve, where the polar head groups face the continuous water phase and the hydrophobic tails
face into the oil droplets. The o/w systems are interesting because they enable hydrophobic drugs to be more soluble in an aqueous based system, by solubilizing it in the internal oil droplets [12]. Water-in-oil (w/o) microemulsions are made up of droplets of water surrounded by an oil continuous phase. These are generally known as “reverse micelles” (RMs), where the polar head groups of surfactant are facing into the droplets of water, with the hydrophobic tails facing into the oil phase. In the RMs, the amount of water present is low and is limited to the maximum capacity of hydration of the hydrophilic head group of the surfactants; hence, the water pool is rigid. In a w/o microemulsion, when the amount of water exceeds the hydration requirement of the surfactant head groups, both bound and free water prevail in the water pool. A term \( \omega \) defined as [water]/[surfactant] has been taken as a criterion as to whether a RMs or a w/o microemulsion has been formed [13]. It was suggested that when \( \omega < 10 \), it is a RMs and when \( \omega > 10 \), it is a w/o microemulsion [14]. However, some evidence exists that the cut-off point may be \( \omega = 15 \) [15]. The hydrophilic peptides can be easily incorporated into the water internal phase and are more protected from enzymatic proteolysis by the continuous oil phase than other oral dosage forms [12]. Reverse micellar aggregates have the ability to host hydrophilic compounds like water, forming water-in-oil microemulsions and generally enhancing the solubility of immiscible solvents [16]. When the amount of water and oil present are at equivalence, a bicontinuous microemulsion system may results. In this case, both water and oil exist as a continuous phase. Transition from o/w to w/o microemulsion may passes through this bicontinuous state. Bicontinuous microemulsions are useful for topical delivery of drugs or for intravenous administration, where upon dilution with aqueous biological fluids, form an o/w microemulsion [12]. Irregular channels of oil and water are intertwined, resulting in what looks like a “sponge phase”.

Fig. 1.3. Schematic representation of oil-in-water (o/w) (a), bicontinuous (b) and water-in-oil (w/o) (c) microemulsions.
1.4. Winsor representation

Winsor representation [4] is a cut of the phase prism/tetrahedron where only one variable is varied and measures the relative volume of the phases obtained on varying the single variable. Each data point represents one sample and is a direct picture of the observed phases in the test tube. There are four commonly observed phase behaviors as described by Winsor (Fig. 1.4),

1. Winsor I (2), a water rich microemulsion in equilibrium with an excess oil phase.
2. Winsor II (\(\overline{2}\)), an oil-rich microemulsion in equilibrium with an excess water phase.
3. Winsor III (3), a microemulsion phase in equilibrium with an excess oil phase and an excess water phase.

Fig. 1.4. Winsor classifications of microemulsion systems.

2.0. Basic aspects of microemulsions

2.1. Role of surfactants in microemulsions

Surface active agents (usually referred to as surfactants) are amphiphilic molecules that consist of a non-polar hydrophobic portion, usually a straight or branched hydrocarbon or fluorocarbon chain containing 8-18 carbon atoms, which is attached to a polar or ionic portion (hydrophilic). The hydrophilic portion can, therefore, be nonionic, ionic or zwitterionic, and accompanied by counter ions in the last two cases. The hydrocarbon chain interacts weakly with the water molecules in an aqueous environment, whereas the polar or ionic head group interacts strongly
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with water molecules via dipole or ion-dipole interactions. Because of this strong interaction with the water molecules that renders the surfactant soluble in water. However, both the cooperative action of dispersion and hydrogen bonding between the water molecules tend to squeeze the hydrocarbon chains out of the water and hence, these chains are referred to as hydrophobic. The balance between hydrophobic and hydrophilic parts of the molecule gives these systems their special properties, e.g. accumulation at various interfaces and association in solution (to form micelles) (vide. Fig. 1.5).

Fig. 1.5. Schematic diagram of aggregated structure of surfactant.

Surfactants find applications in almost every chemical industry, including detergents, paints, dyestuffs, cosmetics, pharmaceuticals, agrochemicals, fibers, plastics [17]. Moreover, surfactants play a major role in the oil industry, for example in enhanced and tertiary oil recovery [18]. They are also occasionally used for environmental protection, e.g. in oil slick dispersants [17]. Therefore, a fundamental understanding of the physical chemistry of the surfactants including their unusual properties and phase behaviors are essential for most of the applications. In addition, an understanding of the basic phenomena involved in the application of surfactants, such as in the preparation of emulsions and suspensions and their subsequent stabilization, in microemulsions, in wetting, spreading and adhesion, etc., is of vital importance in arriving at the right composition and control of the system involved [19]. A simple classification of surfactants based on the nature of the hydrophilic group is commonly used. Two main classes may be distinguished, namely ionic and nonionic [20]. Ionic surfactants can be anionic, cationic or zwitterionic.
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Anionic surfactants are the most widely used class of surfactants in industrial applications [21] due to their relatively low cost of manufacture and are used in practically every type of detergent. For optimum detergency, the hydrophobic chain should be a linear alkyl group with a chain length in the region of 12–16 carbon atoms. Linear chains are preferred since they are more effective and also degradable than branched ones. A general formula may be ascribed to anionic surfactants as follows:

- Carboxylates: $C_nH_{2n+1}COO^-X$
- Sulphates: $C_nH_{2n+1}OSO_3^-X$
- Sulphonates: $C_nH_{2n+1}SO_3^-X$
- Phosphates: $C_nH_{2n+1}OPO(OH)O^-X$

Where, $n = 8-16$ atoms and the counter ion $X$ is usually $Na^+$. Alkali alkanoates, also known as soaps, are the most common anionic surfactant. The anionic charge in this surfactant comes from the ionized carboxyl group. Dioctyl sodium sulfosuccinate (DOSS) is the most widely studied anionic surfactant. It has twin tails and is a particularly good stabilizer of w/o microemulsions. Other important classes of anionic surfactants include, alkyl sulphates, alkyl ether sulphates, methylester sulphonates, aryl sulphonates.

Cationic surfactants generally fall into the class of quaternary ammonium alkyl salts. Alkylammonium halides are excellent hydrogen bond donors and interact strongly with water. The most well known examples from the cationic surfactant class are hexadecyltrimethylammonium bromide (CTAB) and didodecylammonium bromide (DDAB). Although less numerous, phosphorous can be quaternarized with alkyl groups to create alkyl phosphonium cationic surfactants as well. Cationic surfactants are generally water soluble when there is only one long alkyl group. They are generally compatible with most inorganic ions and hard water, but they are incompatible with metasilicates and highly condensed phosphates. They are also incompatible with protein-like materials. Cationic surfactants are generally stable towards pH changes, both acid and alkaline. They are incompatible with most anionic surfactants, but they are compatible with nonionic surfactants. These cationic surfactants are insoluble in hydrocarbon oils. In contrast, cationic surfactants with two or more long alkyl chains are soluble in hydrocarbon solvents, but they become only dispersible in water (sometimes forming bilayer vesicle type structures). In most cases, they are chemically stable and can tolerate electrolytes. The CMC of cationic surfactants is close to that of anionic surfactants with the same alkyl chain length. The prime use of cationic surfactants is their tendency to adsorb at negatively charged surfaces, e.g. anticorrosive agents for steel, flotation collectors for mineral
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ores, dispersants for inorganic pigments, antistatic agents for plastics, other antistatic agents and fabric softeners, hair conditioners, anticaking agent for fertilizers and as bactericides [22, 23].

Amphoteric (Zwitterionic) Surfactants are surfactants containing both cationic and anionic groups [24, 25]. The most common amphoteric surfactants are the N-alkyl betaines, which are the derivatives of trimethyl glycine (CH$_3$)$_3$NCH$_2$COOH (described as betaine). An example of betaine surfactant is lauryl amido propyl dimethyl betaine, C$_{12}$H$_{25}$CON(CH$_3$)$_2$CH$_2$COOH. These alkyl betaines are sometimes described as alkyl dimethyl glycinites. Phospholipids, such as lecithin are common zwitterionic surfactants. Unlike other ionic surfactants, which are somewhat toxic, they show excellent biocompatibility. This is most likely due to the fact that lecithin is obtained naturally from soyabean or egg, which contains diacylphosphatidylcholine as the major constituent. The main characteristic of amphoteric surfactants is their dependence on the pH of the solution in which they are dissolved. In acid pH solutions, the molecule acquires a positive charge and behaves like a cationic surfactant, whereas in alkaline pH 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 (the isoelectric point of the molecule). The change in charge with pH of amphoteric surfactants affects their properties, such as wetting, detergency, foaming, etc. At the isoelectric point (i.e.p.), the properties of amphoteric surfactants resemble those of nonionic surfactants very closely. Below and above the i.e.p, the properties shift towards those of cationic and anionic surfactants, respectively. Zwitterionic surfactants have excellent dermatological properties. They also exhibit low eye irritation and are frequently used in shampoos and other personal care products (cosmetics) [24].

Fig. 1.6. Pictorial representation of various types of surfactants.
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Most of the nonionic surfactants are structurally similar to the ionic surfactants, except the head groups are uncharged or neutral. The interaction between these nonionic head groups are governed by steric forces, as no electrostatic charges emerges from their head groups. Ethoxylated alcohols are the most common nonionic surfactants. These alcohols contain a wide-ranging degree of ethoxylation, where ethylene oxide is added to fatty acids to make them more water soluble. They are considered to be amphiphiles, with oil loving hydrocarbon tail and a water loving ethoxylated alcohol group. Examples of nonionic surfactants include polyoxyethylene surfactants, such as Brij-35, Brij-58, or sugar esters, such as sorbitan monooleate (Span-80). Ethoxylated derivatives of Spans are known as Tweens. Polyoxyethylene sorbitan monooleate (TWEEN-80) and polyoxyethylene sorbitan monolaurate (TWEEN-20) are safe and acceptable for oral and parenteral use [26, 27]. Polysorbates are partially fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 5 or 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides. Sorbitans are partial esters of sorbitol and mono and di-anhydrides with fatty acids. These are considered lipophilic nonionic surfactants. Alkanol amides and polyamines are the primary nitrogen-based nonionic surfactant types [28, 29]. Fig. 1.6 depicts different type of surfactants.

Sugar-based surfactants such as alkyl glucosides can be made from renewable material and are biodegradable. They can be used in many applications as substitutes for other surfactants that are not environmentally sound. In addition, sugar-based surfactants exhibit some unusual properties. As nonionic surfactants, their solubility comes from the hydroxyl groups and not the ether oxygens (as in alkyl polyethyleneglycol ethers) (C_i E_j). They do not show the inverse solubility versus temperature, unlike nonionic alkyl polyethyleneglycol ethers which are temperature sensitive [30].

Amino acids have become increasingly popular in recent years as starting material for surfactants [31-35]. Approximately, one-third of the 20 natural amino acids as well as several non-natural amino acids are utilized for this purpose [36, 37]. An important example of the latter category is sarcosine, also referred to as N-methyl glycine, which constitutes the polar head group in an important type of anionic surfactants [38, 39]. Amino acids based surfactants belong to the class of surfactants with high biodegradability, low toxicity and excellent surface active properties. They possess excellent emulsifying, detergency properties and form fine lather. These surfactants are environment friendly, mild to skin and eyes, and have hard water tolerance. Very
recently, Chandra et al. [40] reviewed synthesis, surface active properties [such as, cmc (critical micelle concentration), surface tension, phase behavior, Kraft temperature, aggregation properties and interfacial adsorption of these surfactants. The biological properties such as antimicrobial activity, aquatic toxicity, biodegradability and hemolytic activity have also been focused. Further, various applications of these surfactants in the area of life sciences, viz. gene transfection, formation of liposomes and drug delivery systems have been reviewed. The structural difference between amino acid surfactant and conventional surfactant is depicted in Fig. 1.7.

Fig. 1.7. Structural difference between amino acid surfactant and conventional surfactant.

2.2. Role of cosurfactant on the formation of microemulsion

Surfactants, due to their diphilic nature, get adsorbed at the oil/water interface to render stability to the dispersion by preventing coalescence. However, the addition of alcohol (cosurfactant) effectively changes the originally unfavorable packing geometry of the surfactant molecules, which in turn, adjust the spontaneous curvature of the film to a negative value (curvature towards aqueous domain) and produces a stable surfactant/alcohol mixed interfacial film. Further, cosurfactants partition themselves among the oil, water and the interface domains depending upon the lipophilicity. By changing alcohol content, imparts an additional advantage to modulate size of the water pools and surface dynamics. Therefore, without a quantitative description of the dependence of the partition equilibria on the system composition under varied physicochemical environments, a full understanding of quaternary microemulsions can not be attained [41, 42]. It is apparent that the presence of surfactant vis-à-vis the interaction of surfactant with cosurfactant, which in turn the accommodation of these amphiphiles at the interface, plays an important role for the formation of these w/o microemulsions. Hence, this phenomenon can be understood by the packing parameter (P) of the molecular geometry [43]. Israelachvili [43]
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defined the dimensionless packing parameter (P) of the molecular geometry as \( v/(a_0 l_c) \). Most of the cationic surfactants cannot satisfy this condition when they are alone in the organic phase. The addition of an alcohol which accommodates in the interfacial region of reverse micelles has an effect of increasing the volume of surfactant tail \( v_c \) and reducing the effective surface area \( a_0 \), thus facilitating the formation of RMs [44]. More precisely, the addition of alcohol (cosurfactant) effectively changes the originally unfavorable packing geometry of the surfactant molecules, which in turn, adjust the spontaneous curvature of the film to a negative value (curvature towards aqueous domain) and produces a stable surfactant/alcohol mixed interfacial film. This imparts an additional advantage to modulate the size of the water pool and surface dynamics by changing alcohol content. Reports on the use of the hydrophilic surfactant (cationic or anionic or nonionic) in combination with alkanol (cosurfactant) as templates for shape and size controlled synthesis of nanoparticles of noble metals and bimetallic nanoparticles [45-47], packaging design of nanomaterials [48], sustained-release drug molecules [49], enzyme activity [50, 51] and morphologies, pore structure of mesoporous silica are available in literature [52]. Recently, Mathew and Juang [44] reported that alcohols play various important role in the formation of inverse microemulsions, such as (i) alcohols act as a weak amphiphile when added into a binary water-in-oil mixture. They act as “co-solvents” in some cases where they partition between the aqueous domain and the amphiphilic film and as “co-surfactant” it dissolves in the amphiphilic film, making co-surfactant mixture more hydrophobic. Medium-chain alcohols are usually considered as co-solvents; (ii) the amount of alcohol added to the microemulsion system is also considered to be critical. At lower concentration of alcohol the water uptake in the organic phase may be almost zero giving rise to Winsor I type system and at very high concentration of alcohols also the water uptake decreases; (iii) for alcohols with hydrocarbon chain longer than \( C_{10} \), the concentration of alcohol required to form RMs eventually start increasing with an increase in the chain length. For a fixed alcohol concentration, the longer the hydrocarbon chains of alcohol, the smaller the water uptake of RMs.

2.3. Stability of Microemulsions

Microemulsions are thermodynamically stable in comparison to emulsions. Emulsions are separated into different phases on standing due to the coalescence of droplets by Ostwald ripening (transfer of materials from small droplets to larger ones), and the process leads to a decrease in interfacial area and hence the free energy of the system. The understanding of the thermodynamic stability of microemulsions was improved considerably with the development of several thermodynamic theories [53-59]. It was considered that the free energy of formation of
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Microemulsions consist of three contributions: (1) interfacial free energy, (2) energy of interaction between the droplets, and (3) entropy of dispersion. These studies lead to the conclusion that microemulsions are thermodynamically stable, as the interfacial tension between oil and water is too low to compensate by the entropy of dispersion. Therefore, an essential requirement for the formation of o/w or w/o droplets and their stability is the attainment of a very low interfacial tension ($\gamma$). Since microemulsions possess a very large interface between oil and water because of the small droplet size, they can be thermodynamically stable if the interfacial tension is so low that the positive interfacial energy (given by $\gamma dA$) can be compensated by negative free energy of mixing ($\Delta G_{\text{mix}}$). If the enthalpy of mixing ($\Delta H_{\text{mix}}$) is assumed to be zero for an ideal solution, the free energy of mixing can be given as,

$$
\Delta G_{\text{mix}} = -T\Delta S_{\text{mix}} = k_B T
$$

(Where $k_B$ is the Boltzman constant and $\Delta S_{\text{mix}}$ is the entropy of mixing).

This follows, $k_B T = \gamma dA = 4\pi r^2 \gamma$.  

where, $r$ is the droplet radius.

So, if $r = 10$ nm, $\gamma$ should be $\sim 0.03$ mN m$^{-1}$. So, to prepare microemulsion, the interfacial tension must reach such an ultralow value. Generally the interfacial tension between oil and water is in the order of $\sim$50 mN m$^{-1}$. The role of surfactant is to reduce this value to such an ultralow value [60, 61]. Except for some double chain ionic surfactants and a few nonionic surfactants, it is not possible to achieve the required interfacial area with the use of a single surfactant. If, however, a second amphiphile is added to the system, the effect of the two surfactants can be additive provided that the adsorption of one does not adversely affect that of the other and such mixed micelle formation does not reduce the available concentration of the surfactant molecules. The second surfactant is generally termed as the co-surfactant. The role of cosurfactant in the formation and stability of microemulsion was reviewed briefly by Mathew and Juang [44]. Besides short chain alcohols, several attempts have been made to use nonionic surfactants, alkanes acids, alkanediols, amines, aldehydes, ketones, butyl lactate, oleic acid etc. as the second surfactant [61-75].

2.4. Geometrical constrains and interfacial curvature of microemulsions

The formation of different phases and microstructures (water-in-oil droplets, oil-in-water droplets, bicontinuous, sponge like, liquid crystalline etc.) in a water-oil-amphiphile system is largely dependent on the amphiphile film separating the two immiscible phases, i.e., oil and water. “Spontaneous curvature” of the interfacial film governs the formation of different kind of microstructures in microemulsion systems at a specified composition of water-oil-amphiphile.
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The surface geometry and the intermolecular forces acting between surfactant molecules set the curvature at the interface [76, 77]. There are also other factors like penetration of oil into the interfacial layer, adsorption of cosurfactant etc. that can modify the curvature of the interface. In a surfactant monolayer, a repulsive hydrophilic force between the head groups of the surfactants exists, and this repulsive force is balanced by the attractive hydrophobic interaction acting at the oil and water interface, steric repulsive forces between the tails of the surfactants and force due to the penetration of oil into the interface [78].

Fig. 1.8. Correlation between packing parameter “P” and structure of self-assembled systems.
A useful concept in determining the interfacial property is the “packing parameter” (P) introduced by Israelachvili et al. [43]. P can be calculated as P = v/(a_o l_c) where, v is the volume and l_c is the length of the hydrophobic tail and a_o is the area of cross section of the surfactant head group. For surfactants with P ~ 1/3, globular structure can be predicted. With P ~ 1/2 and 1, cylindrical and planer layers, respectively can be predicted. Fig. 1.8 represents correlation between packing parameter “P” and structure of self-assembled systems. Although P provides with an idea of the possible structure of the formulation, it should be noted that oil penetration in the hydrocarbon tail of the surfactant layer produces an increase in the apparent hydrophobic volume and subsequently, results in an increase in P. However, co-surfactants are co-adsorbed at the interface and thereby, results in a reduction in P. High amount of ionic surfactant produces a high ionic strength with a subsequent reduction in polar head group area vis-à-vis and reduction in P. Electrolytes and temperature also affects the P values. A correlation between P and structure of self-assembled systems has been presented in Fig. 1.8.

For a mixed surfactant systems, the effective packing parameter (P_{eff}) can be expressed according to the following relation as obtained by Evans and Ninham [79]:

$$P_{eff} = \left[ \frac{(x_A v/a_l) + (x_B v/a_l)}{(x_A + x_B)} \right]$$

(3)

where, x_A and x_B are the mole fractions of surfactant A and B present at the oil/water interface, respectively.

The interfacial curvature and the spontaneous radius of curvature can be obtained for a surfactant in the following manner [80-83]: Consider a surfactant film that can stretch and bend. The surface tension (\gamma) is the first derivative of the film free energy (G_f) with respect to area, i.e.

$$\gamma = \frac{\partial G_f}{\partial A}$$

(4)

The area expansion modulus K_a is given by,

$$K_a = [\left( \frac{\partial^2 G_f}{\partial A^2} \right)_n] \frac{A}{2}$$

(5)

Where, n is the number of surfactant molecules in the film. When the area of the film is changed, the surfactants are equilibrated between the film and bulk.

For a curved surface, the curvature at each point is described by the two principal radii of curvature R_1 and R_2. The mean radius of curvature (H) is given by,
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\[ H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \]  
\[ (6) \]

For spheres, \( R_1 = R_2 = R \) and \( H = 1/R \). For cylinders, \( R_1 = R \), and \( R_2 = \infty \) and \( H = 1/2 \ R \). To assign a sign to the radius of curvature, it is customary to put positive sign for surfaces curved toward oil and negative to surfaces curved toward the polar solvent.

The free energy of the film depends upon the curvature of the film. The free energy per unit area \( (g_f) \) is given by,

\[ g_f = \frac{G_f}{2\pi RL} \]  
\[ (7) \]

where, \( L \) is the length of the cylinder.

For a highly curved surface \( (R \to 0) \), \( g_f \) reaches a high value. Since \( g_f \) cannot arbitrarily reach any small value, the condition for minimum gives,

\[ \frac{\partial g_f}{\partial (1/R)} = 0 \]  
\[ (8) \]

This corresponds to \( H_0 = 1/2 \ R_0 \), where \( H_0 \) is the “spontaneous curvature” of the film.

The bending rigidity (or the bending elastic modulus) \( \kappa \) of the film is given by,

\[ \kappa = \frac{\partial^2 g_f}{\partial^2 (1/R)} \]  
\[ (9) \]

The saddle-play modulus \( \bar{\kappa} \) can be obtained by considering a saddle-like deformation of the planar interface (i.e. \( 1/R_1 = -1/R_2 \))

\[ \bar{\kappa} = -\frac{1}{2} \frac{\partial^2 g_f}{\partial^2 (1/R)} \]  
\[ (10) \]

The area expansion modulus is very large and the area per surfactant molecule remains constant as the film bends. \( g_f \) can be expanded to get the form,

\[ g_f = \gamma + 2\kappa (H-H_0)^2 + \bar{\kappa} K \]  
\[ (11) \]

where, \( K=1/R_1 R_2 \), called the Gaussian curvature.

The curvature free energy thus can be obtained as,

\[ G_f = \int (g_f - \gamma) \, dA = \int \left( 2\kappa (H-H_0)^2 + \bar{\kappa} K \right) \, dA \]  
\[ (12) \]

Many physicochemical properties of microemulsion depend upon the value of \( H_0 \). It is strongly influenced by the nature of the surfactant and also, on the composition of the polar and apolar phases. Oil molecules penetrate into the hydrocarbon chain of the surfactant film. This imposes more curvature towards the polar side. The longer is the hydrocarbon chain length of the oil, smaller is the effect of penetration into the hydrocarbon moiety and hence less is the effect on curvature. Increasing temperature makes the surfactant chain more coiled and hence \( H_0 \) is
increased. For ionic surfactant, addition of electrolyte can alter $H_0$. The higher the electrolyte concentration, the more easily the film curves toward water and consequently, $H_0$ is decreased. Temperature has a strong effect on altering the surfactant curvature for nonionic surfactant. As temperature is increased, water becomes less good solvent for the ethylene oxide monomers and hence water penetration into the palisade layer is decreased at elevated temperature resulting in a decrease in $H_0$. This explains the strong temperature dependence of the phase equilibrium for systems stabilized by nonionic surfactant.

The spontaneous curvature can be tuned by altering the head group and chain length of the surfactant, temperature, nature of the oil, added electrolyte and temperature. The bending elasticity ($\kappa$) depends upon many factors and short chain cosurfactant, oil penetration etc. makes the film more flexible.

Microemulsion can exists in either droplet like structure or in bicontinuous structure. There exist two kinds of droplet structure viz. water-in-oil and oil-in-water structures. In droplet structure the dispersed phase forms the inner core of the droplets. Consider an o/w droplet structure in which oil phase is trapped by a surfactant layer in water continuum. The radius of the hydrocarbon core is denoted by $R_c$, whereas the core plus surfactant head radius is denoted by $R_H$. It can be shown that

$$R_H = \frac{3V_{hc}}{A_{hc}} \tag{13}$$

where, $V_{hc}$ and $A_{hc}$ are the hydrocarbon volume and area respectively of the core. Geometrical consideration shows that,

$$R_c = 3(\phi_o/\phi_s + \alpha) (V_s/a_0) \tag{14}$$

where, $\phi_o$ and $\phi_s$ are the volume fraction of oil and surfactant respectively, $V_s$ is the molecular volume of the surfactant, $a_0$ is the area of each surfactant molecule and $\alpha$ is a fraction in the order of 0.5. A similar expression can be put forward for w/o droplet systems.

When $H_0 \to 1/R_c$, a stable droplet structure is assumed. But, when, $H_0 >> 1/R_c$, the film can relax towards a lower free energy state by decreasing the droplet size and expelling the excess dispersed phase as a separate phase causing phase separation. When, $H_0 \approx 0$, there is no preference between o/w or w/o structure and a bicontinuous structure prevails in which the surfactant film separates a microscopic oil region from a microscopic water region.

2.5. The hydrophilic lipophilic balance (HLB) of surfactants

Attempts have been made to rationalize surfactant behavior in terms of the hydrophile–lipophile balance (HLB) [84], as well as the critical packing parameter (CPP) [43, 85]. Both approaches
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are fairly empirical but can be a useful guide to surfactant selection. Ionic surfactants, for example, sodium dodecyl sulfate, which possesses HLBS greater than 20, often requires the presence of a cosurfactant to reduce the effective HLB to a value within the range required for microemulsion formation. In contrast, the CPP relates the ability of surfactant to form particular aggregates to the geometry of the molecule itself. It must be noted, though, that microemulsions are only obtained under certain carefully defined conditions, and the HLB of the surfactant can only be used as a starting point in the selection of components that will form a microemulsion. Earlier, Griffin [86] proposed to calculate the HLB of a surfactant from its chemical structure and to match that number with the HLB of the oil phase. The system employs certain empirical formulae to calculate the HLB number within a range of 0 to 20. Hydrophilic surfactants that possess high water solubility and generally act as good solubilizing agents, detergents, and stabilizers for O/W emulsions lie at the high end of the scale; at the low end are surfactants with low water solubility, which act as solubilizers of water in oils and are good W/O emulsion stabilizers. The effectiveness of a given surfactant in stabilizing a particular emulsion system depends on the balance between the HLBS of the surfactant and the oil phase involved. Although, the HLB system proposed by Griffin has been useful in most of the general applications by guiding the chemist to a choice of surfactant most suited to individual needs, others have suggested HLB numbers could be calculated based upon polar and non-polar group contribution [87]. Table 1.1 lists the ranges of HLB numbers that have proved most useful for various applications [88].

Table 1.1. HLB ranges and their general areas of application

<table>
<thead>
<tr>
<th>Range</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-10</td>
<td>W/O emulsions</td>
</tr>
<tr>
<td>7-11</td>
<td>Wetting</td>
</tr>
<tr>
<td>11-18</td>
<td>O/W emulsions</td>
</tr>
<tr>
<td>3-15</td>
<td>Detergency</td>
</tr>
<tr>
<td>15-18</td>
<td>Solubilization</td>
</tr>
</tbody>
</table>
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In the past, Griffin introduced a semi-empirical scale (HLB number based on the stability of emulsion), but his original method for determining HLB number was a long and laborious experimental procedure [89]. For nonionic surfactants the type of emulsion changes from o/w to w/o type at a certain temperature [90-94]. Shinoda termed this temperature as the phase inversion temperature (PIT) or HLB temperature ($T_{HLB}$). The HLB number is assigned to a surfactant molecule, whereas the PIT or HLB temperature is a characteristic value of a given system. The more hydrophilic a nonionic surfactant is, the higher the value to which the $T_{HLB}$ or PIT will be shifted. Thus, PIT and HLB number are correlated with each other [95]. Izquierdoa et al. reported the formation of O/W nano-emulsions by the PIT emulsification method for water/mixed nonionic surfactant/oil systems [96].

It has been reported that the hydrophilic-lipophilic (H/L) property [which is defined by the size and water solubility of the surfactant polar head (the hydrophile) relative to the size and oil solubility of the hydrocarbon chain (the lipophile)] of a pure nonionic surfactant for a water/surfactant/oil system is not affected by the change in the water/oil ratio and/or surfactant concentration. A systematic investigation on the H/L property for mixed nonionic surfactants/oil/water system was reported by Kunieda et al. [97]. Further, they explained HLB system on the basis of the phase equilibrium in a series of water/surfactant(s)/oil systems using different types of surfactants, and oils (hydrocarbon and polar) [98]. A correlation between HLB number and HLB temperature for microemulsion systems (including mixed surfactant systems) has been established and an improved HLB system has also been suggested. Recently, stable w/o microemulsions were prepared based on the hydrophilic lipophilic balance (HLB) concept by using nonionic surfactants [99-105]. Very recently, Matsaridou et al. [106] and Fu et al. [107] reported the influence of surfactant HLB on the formation and properties of self-emulsifying microcrystalline cellulose (MCC) pellets, microemulsion reconstitution and also on the stability of water-in-octane nano-emulsion.

HLB of the mixed surfactant systems at the mixed compositions (i.e., $HLB_{mix}$) has been evaluated using the concept of Fu et al. [107]

\[
HLB_{mix} = \frac{(m_A HLB_A + m_B HLB_B)}{(m_A+m_B)}
\]

(15)

where, $m_A$ and $m_B$ are the mass of surfactant A and surfactant B in the mixture, respectively. $HLB_A$ and $HLB_B$ are the hydrophilic-lipophilic balance of surfactant A and surfactant B, respectively. Recently, our group showed the correlation between $HLB_{mix}$ and water solubilization capacity of mixed Brij-58/CTAB/1-Pn/Hp or Dc microemulsions [108].

2.6. Phase Representations

Multicomponent systems consisting of water, oil and amphiphiles can form different phases depending upon the nature and composition of the components and also on the temperature and
pressure. Formulation of microemulsion phase can be different since microemulsion can often coexist with excess oil and/or water or other phases. A map of the locations of microemulsion phases in composition space is known as a “phase diagram”. According to phase rule the degrees of freedom (F) can be related to the number of phases (P) and number of components (C) according to the following relation:

\[ F = C - P + 2 \]  \hspace{1cm} (16)

Generally, while constructing phase diagrams, pressure is kept constant. So the phase equation (16) reduces to,

\[ F = C - P + 1 \]  \hspace{1cm} (17)

So, for a three component system (consisting of surfactant, oil and water), there exists at most three degrees of freedom (temperature and two concentration variables). For such a case phase diagram can be constructed in a three-dimensional phase prism. The degrees of freedom can be reduced into two and phase diagram can be represented in two dimensions.

Fig. 1.9. Phase tetrahedron of a four-component system [water (W)-cosurfactant (CS)-oil (O)-surfactant(S)] illustrating different cuts at (A) constant CS-to-S ratio, (B) constant W-to-O ratio-the fish cut and (C) constant S concentration - the \( \chi \) cut.
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There are many possible ways to convert the phase tetrahedral into two dimensional phase diagrams; the three most popular ways are as follows: 1) a horizontal cut of the phase prism, keeping temperature constant. This will give rise to Gibbs triangle with surfactant, oil and water as the vertices, 2) a cut of constant o/w ratio (the fish cut). This gives rise to a surfactant concentration vs. temperature plot, and 3) a constant amphiphile concentration cut (the $\chi$ cut). This gives rise to $\alpha$ (oil to water ratio) versus temperature plot.

In a four component system, there is atmost four degrees of freedom, and to make a three-dimensional representation, one of the five variables (surfactant, cosurfactant, oil, water and temperature) must be kept constant. Generally temperature is kept constant, and the other four variables are plotted to obtain a three dimensional tetrahedron, where each face of the tetrahedron represents a Gibbs’ triangle with three components as the apices. Different cut of the tetrahedron can be used to represent simpler phase diagrams (Fig. 1.9). Some of these are; 1) constant surfactant to cosurfactant cut which results in a pseudo three-component Gibbs’ triangle. This kind of phase representation provides the information on the extent of monophasic region, which in turn helps to prepare single-phase microemulsion for application, 2) constant oil-water ratio cut (commonly called fish-cut) resulted in a surfactant concentration vs. cosurfactant concentration plot. The main information obtained from this representation is that it provides the optimum amount of surfactant and cosurfactant required to obtain a single phase microemulsion at any specified oil-water ratio. The point at which the three-phase body touches the single-phase body corresponds to these optimum values, and 3) constant amphiphilic concentration cut (known as the $\chi$ cut) [109-111]. Fig. 1.9 depicts phase tetrahedron of model a four component system.

2.7. Mixture of surfactants

In colloid and surface chemistry, there is a continuing interest in research on the supramolecular surfactant assemblies that include micelles, microemulsions, liquid crystals, monolayers, vesicles, etc., with mixed surfactants containing either combination of anionic/cationic or anionic/nonionic or cationic/nonionic surfactants and has been discussed in detail by many pioneering authors [112-115].

When surfactants are added together in water, several physicochemical properties of the mixed system compared to those of the single surfactant system are changed due to the fact that there is a net interaction between the amphiphiles, i.e. due to non-ideal mixing. It is well known that
many amphiphilic self-assemblies and interfaces, biological or synthetic-based, consist of surfactant mixtures. The molecular structure of the surfactant (head groups and tails), the presence of additives (salt, cosurfactant) and experimental variables (pH, temperature) can be manipulated in order to induce changes in interfacial activity and in intra and inter-aggregate forces. The mixed system almost invariably yields enhanced interfacial properties (e.g. decreased CMC higher surface activity) with respect to the individual surfactants, in what is termed *synergism*. However, it is the rich polymorphism in bulk that has, in recent years, attracted increasing interest in experimental investigations and theoretical modeling [116-121].

Mixed surfactant was found superior to the relevant single ones mainly due to the reduction in nonionic surfactant partition and/or sorption to soil as well as the high solubilization capacity of the mixture. Decreasing loss of surfactant due to sorption and the greater apparent solubilization of the mixture will reduce volumes of surfactants needed and thus the capital expenditure and operation cost [122]. The interaction between surfactants in mixtures can produce marked interfacial effects due to change in adsorption as well as in the charge density of the surface. In most cases when different types of surfactants are purposely mixed, what is sought is synergism, i.e. the condition when the properties of the mixture are better than those attainable with the individual components by themselves. Surfactant mixtures of practical interest include like-charge surfactants, for instance mixtures of anionic surfactants, or mixtures of cationic surfactants, but the more common case involves mixture of ionic and nonionic surfactants [123, 124]. The most popular advantage to ionic/nonionic surfactant mixtures is the fact that they result in temperature insensitive microemulsions [125]. Generally, ionic and nonionic surfactants react oppositely with increasing temperature. Ionic surfactants show a hydrophilic shift with increasing temperature, while nonionic surfactants exhibit a lipophilic shift. Therefore, when mixed together in a particular ratio, the two will cancel each other out, resulting in a temperature insensitive microemulsion formulation [125]. Temperature-insensitive microemulsions having a large solubilization capacity are very important not only because of their practical applications in different industrial fields but also for basic studies in the surfactant science field. Earlier, Kunieda et al. [41] Binks et al. [126], and Mitra et al. [127] reported temperature-insensitive microemulsion in single water/sucrose monoalkanoate/hexanol/decane, mixed AOT/n-dodecyl pentaoxyethylene glycol ether (C12E5)/oil and water/CTAB/Brij-58/1-butanol or 1-pentanol/Hp or Dc microemulsion, respectively. Very recently, Rio et al. [128] and Bardhan et al. [108, 129]
formulated temperature-insensitive microemulsion for mixed ionic/nonionic (Aerosol-OT/cyclic ketal alkyl ethoxylate and CTAB/Brij-35 or Brij-58) surfactant systems in either hydrocarbon (Hp or Dc or isooctane) or polar lipophilic (IPM) oils, respectively. Mixtures of oppositely charged surfactants (i.e., anionic-cationic which also known as catanionic surfactant mixtures) usually exhibit large synergistic effects (i.e., mix non-ideally). Mixing oppositely charged surfactants reduces the monomer surfactant concentration in the solution (i.e., increase the surface activity). On the other hand, due to absence of electrostatic attractive interactions in mixtures of like-charged surfactants, the effects (if any) is not expected to be as large as for a mixtures of oppositely charged surfactants [130]. Mixtures of nonionic surfactants tend to behave ideally [131]. This means, no synergistic effect is to be expected in terms of the physicochemical properties (viz. solubilization behavior, cloud point etc.). A typical feature of the adsorption of ionic (anionic or cationic)-nonionic surfactant mixtures is the synergism or anti-synergism (antagonism) at oil/water interfaces. For example, the adsorption of one surfactant is either enhanced or retarded by the addition of a small amount of the other (second) surfactant. In many applications, surfactant mixtures in particular anionic-nonionic mixtures often give rise to enhanced overall performance over single component systems. For example, anionic-nonionic surfactant mixtures might show advantageous solubilization behavior, exhibiting cloud points higher than those of the single nonionic surfactant, along with Kraft points lower than those of the single anionic surfactant. Synergism in solubilization behavior using mixed surfactant systems has been discussed in details in the latter section. Mixed surfactants could be employed over a wide range of temperature, salinity, and hardness conditions than the individual surfactants [132, 133]. Consequently, surfactant mixtures are employed in a wide variety of practical applications such as surfactant enhanced aquifer remediation (SEAR), enhanced oil recovery (EOR), drilling mud formulation, detergency, and waste water treatment, to name just a few [134].

3.0. Methods to characterize microemulsions

Microemulsions have been studied using a great variety of techniques. Due to their complexity, namely the variety of structures and components involved in these systems, as well as the limitations associated with each technique, the characterization of microemulsions is rather a difficult task. On the other hand, microemulsions are technologically important complex fluids. In many applications, they are required to accommodate functional additives such as drugs,
biomolecules (e.g., enzymes and proteins), polymers, and nanoparticles etc., which increase the complexity of the systems further. Therefore, complementary studies using a combination of techniques are usually required to obtain a comprehensive view of the physicochemical properties and structure of microemulsions [135, 136]. Some of these techniques, are presented, in brief, to identify and characterize microemulsions in the following section:

3.1. **Phase behavior of microemulsions**

Phase studies are inevitable for the basic understanding of general phase behavior and kinetics of the structural changes of a system. This is not only of fundamental interest but also is very important for industrial and technological processes. Phase diagrams for microemulsions are quite complex since there are at least three components; water, oil and surfactant. These structured fluids have a readily deformable surfactant interface, which bend either towards water or oil or both. Wide range of surfactants can produce microemulsion. Formulations of microemulsion phases can be difficult since microemulsions often coexist with other phases such as excess oil and/or water, and are often too close to lyotropic liquid crystalline phases. A map of the locations of different phases in composition space is known as a “phase diagram” of microemulsions. A pictorial representation of “phase diagram” (as a model) considering different phases in composition (of water, surfactant and oil mixtures) has been depicted in Fig. 1.10.

![Fig. 1.10. Pictorial representation of “phase diagram” of water, surfactant and oil mixtures.](image)
The phase behavior of microemulsions depends on chemical structures of oils and surfactants, temperature, pressure and additives such as salt and polymers i.e. the “phase behavior” is essential in the study of surfactant systems. Earlier, Moulik and coworkers [137] were carried out extensive studies on phase behavior systems containing surfactant, cosurfactant, oil and water using a three dimensional (Gibbs triangle) or tetrahedral representation. The ingredients used were Brij-92 [polyoxyethylene (2) oleyl ether], Brij-52 [polyoxyethylene (2) cetyl ether], Brij-30 [polyoxyethylene (4) lauryl ether], and Tween-20 [polyoxyethylene sorbitan monolaurate] as surfactants, and ricebran, isopropyl myristate (IPM), eucalyptus, coconut as oils. Phase volumes of the heterogeneous combinations were estimated to understand the mixing efficiency of the combinations. The effect of temperature on these volumes of multiphasic compositions was also investigated. In addition, the effect of additives (sodium chloride, bile salts and urea) on the physicochemical features of these systems was examined. In tune with these studies, Paul and his coworker [66, 138-140] reported phase characteristics of plant oils (saffola, eucalyptus, and clove oils) derived microemulsions. Further, Sjoblom et al. [141] reviewed the progress in understanding microemulsion structure on a molecular level as well as from extensive experimental studies of phase diagrams of both ionic and nonionic surfactants. In the contribution of Hellweg [142], new developments in the investigation and understanding of complicated phase behavior and the fascinating microstructures encountered in microemulsion forming systems was outlined. Very recently, several authors were constructed the phase diagrams of the multicomponent microemulsion systems comprising of four or five components, surfactant(s), cosurfactant, oil and water, where more than one amphiphile were used [109, 127, 129, 139, 143-151].

3.2. Electrical conductivity
Electrical conductance study is perhaps the simplest study to perform, and the equipment is less expensive. The results obtained from conductivity study do not provide a straightforward picture of the microstructure of microemulsions, but transition from water continuous to oil continuous microemulsion can easily be obtained from such measurements, since water continuous formulation usually shows higher conductance than the oil-continuous formulations. In particular, for the w/o microemulsion systems, transition from discrete droplet structure to connected droplet or bicontinuous structure can be envisaged from sharp increase in conductance.
value (100-1000 times increase) with increasing volume fraction of the polar solvent or temperature. Such phenomenon is called “conductance percolation”.

The low conductance in dilute w/o microemulsion has been explained on the basis of charge fluctuation model [152, 153]. But at a certain volume fraction of the dispersed polar phase and/or after reaching a certain temperature, a sudden increase (100 to 1000 times) in conductance was observed for these systems. This phenomenon is called “percolation in conductance”. Two types of percolation phenomenon have been recognized, viz. “static percolation” [154, 155] in which a connected droplet network can be assumed; “dynamic percolation” [156, 157] in which droplets can approach their neighbors by diffusion and transfer charge to augment sharp rise in conductance. Such a conductance manifestation can occur after a threshold dispersant concentration at a constant temperature (called the volume-induced percolation) as well as after a threshold temperature at a constant dispersant concentration (called the temperature-induced percolation).

The enhancement in conductance can be attributed to two probable mechanisms, namely the “hopping” of ions from droplet to droplet beyond a threshold volume fraction of the dispersed phase is reached [158, 159] and/or by exchange of droplet contents during droplet collision known as “transient-fusion-mass transfer-fission” mechanism [160-163]. Mays [164] suggested that the “transient-fusion-mass transfer-fission” process plays the major role in conductance percolation than the hopping mechanism. Over the past decade, a number of studies were carried out on the effects of experimental conditions on the electrical conductivity of single and mixed surfactant reverse micelles based on hydrocarbon and polar lipophilic oils in absence and presence of additives of different physicochemical properties by several researchers [129, 165-177].

Bumajdad et al. [119, 178] measured the electrical conductivity of D$_2$O-in-n-heptane microemulsions stabilized by cationic/nonionic surfactant mixtures (DDAB, and either the C$_x$E$_y$ non-ionic surfactants, or polymeric nonionic surfactants of the type PEO-PPO-PEO, Pluronic®) as a function of D$_2$O content, surfactant concentration, and surfactant mixture composition.

The temperature-induced percolation of RMs in the post-percolation stage at a constant $\omega$ obeyed the scaling law of the form, [179]:

$$\sigma = P (T-T_p)^n$$  \hspace{1cm} (18)
where, $T$ is the temperature corresponding to the specific conductance $\sigma$, $T_p$ is the threshold temperature of percolation, $n$ is a critical exponent, and $P$ is a constant. $T_p$ is evaluated from the differential plot of $(\Delta\sigma/(\sigma\Delta T))$ vs. $T$. To evaluate $P$ and $n$, the logarithmic form of the equation is used:

$$\ln \sigma = \ln P + n[\ln (T - T_p)]$$

(19)

The graphical plot of $\ln (\sigma)$ vs. $\ln [(T-T_p)]$ was processed by least-squares analysis, and $n$ and $\ln P$ were obtained from the slope and the intercept, respectively. From theoretical point of view, two approaches were proposed for the mechanism leading to percolation; one, in which the static percolation model attributes the phenomenon to the appearance of bicontinuous oil and water structure, and the other, in which the dynamic percolation model considers the attractive interactions between water droplets and the formation of clusters due to Brownian motion. The difference between the static and dynamic percolation is reflected in terms of deviation in the value of the exponent $n$ from the predicted value [180]. According to static theory [181, 182], the value of critical exponent in the region above percolation should be $n = 1.6$. However, the dynamic nature makes the value of exponent higher than those predicted for static case [183, 184].

The effect of temperature on conductance has three distinct stages (pre-percolation, percolation and post-percolation). Interfacial deformation, fusion, mass transfer, and fission models were considered by different authors to rationalize the clustering process for percolation of conductance in terms of activation energy, $E_p/\text{kJ mol}^{-1}$. The activation energy, $E_p/\text{kJ mol}^{-1}$ for percolation of conductance in mixed RMs was estimated on the basis of Arrhenius form of relation [171, 185],

$$\sigma = A \exp(-E_p/RT)$$

(20)

or,

$$\ln \sigma = \ln A - E_p/RT$$

(21)

where, $A$ is the constant, $\sigma$ is the conductance and $R$ and $T$ have their usual significance. $E_p$ value can be obtained from the slope of the linear portion of the plot of $\ln (\sigma)$ vs. $(1/T)$. In general, $E_p$ depends on two different contributions: the electrostatic interactions among the charged droplets and an attractive interaction resulting in the interdigitation of the surfactant tail and the solvent molecule, whose effect should depend on the distance between the particle surfaces [174, 175].
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3.3. Viscosity measurement

The viscosity of a solution can give first hand information on its internal consistency; in case of macromolecular solutions and colloidal dispersions, an understanding of the overall geometry of the dispersed entities can as well as be obtained. In the field of application, fluidity of a medium plays an important role. For localized applications, low fluidity is wanted; whereas for solubilization, spreading and reaction study, it is advantageous to have high fluidity. The knowledge of colloidal dispersions (e.g., micelles or microemulsions) thus enjoys both fundamental and application importance. Thus, an overview on the studies of the viscous behaviors of microemulsions with reference to internal structure, data correlation, flow patterns, additive influence etc. from the view point of applications (viz. nanoparticle synthesis, reaction medium etc.) was reported by Paul et al. [186]. Microemulsions have varied flow behaviors, for example, lamellar (Newtonian) and non-lamellar (non-Newtonian). Low viscous microemulsions show Newtonian behavior. The W-III and the bicontinuous types are usually non-Newtonian in nature and they can show plasticity [187].

The phenomenon of percolation in microemulsion is associated with droplet clustering and fusion, i.e. internal structure changes and hence it gets reflected in viscosity. A pronounced similarity in the behavior of self-diffusion, specific conductivity and viscosity of sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/isoctane/water microemulsion was reported by Eicke et al. [188]. The increase in viscosity with temperature has been attributed to the phenomenon of increased clustering of the droplets [189]. The structural inversion from w/o to o/w type of microemulsion has been studied from viscosity measurement [190]. Paul et al. [186] reviewed the viscosity behaviors of microemulsion systems and critically reviewed different equations available for determination of microstructures. Several authors reported that viscosity measurements can contribute significantly in understanding of the inter-micellar interaction in single as well as mixed surfactant microemulsion systems [120, 150, 191-195].

3.4. Light scattering techniques

Among the light scattering techniques, dynamic light scattering (DLS), also known as photon correlation spectroscopy, can be used to analyze droplet size of microemulsion via determination of hydrodynamic radius which can be extracted from measurements of the diffusion constants of diluted dispersed phase (droplets) undergoing Brownian motion. The technique employs relatively simple equipment and involves very short experimental times [136]. This technique
provides the determination of z-average diffusion coefficients (D). If interparticle interaction is assumed to be absent in a system, the hydrodynamic radius of particles (droplets) ($R_H$) can be obtained from the Stokes-Einstein equation:

$$D = \frac{k_B T}{6 \pi \eta R_H}$$  \hspace{1cm} (22)

where, $k_B$ is Boltzmann constant, $T$ is absolute temperature, $\eta$ is the viscosity of the medium. The DLS technique is useful to characterize size and size distribution of microemulsion droplets, as it monitors the collective diffusive motion in colloidal structured systems, provided simple diffusion is the sole mechanism responsible for the variation of the scattered intensity [192, 196]. Polydispersity index (PDI) is another important parameter for evaluation of measurement from DLS experiment. The ratio $SE/d_h$, where SE is the standard error in $d_h$, is called PDI [197]. For a mono-dispersed sample, the value is taken to be less than 0.08; whereas the value ranges from 0.08-0.7 is considered as a mid-range polydispersity [198]. Several authors have significantly contributed to the understanding of various interactions in the microemulsion droplet core by measuring the droplet size [176, 177, 192, 199-202].

The interaction between droplets can be investigated by employing Static Light Scattering (SLS) technique, wherein the intensity of scattered light is generally measured at various angles for different concentrations of microemulsion droplets. At sufficiently low concentrations, the Rayleigh approximation is used provided the particles are small enough [203]. Warisnoicharoen et al. [204] investigated the structure of dilute 3-component nonionic oil-in-water microemulsions formulated with either a polyoxyethylene surfactant ($C_{18:1}E_{10}$ or $C_{12}E_{10}$) or the alkylamine-N-oxide surfactant, DDAO ($C_{12}AO$), and containing either a triglyceride or an ethyl ester oil using static light-scattering technique.

Earlier, Liu et al. [205] reported the droplet size of reverse micelles formed by AOT-Brij mixed surfactants in hydrocarbon oils. Very recently, Das et al. [206], Fanun et al. [100] and Bardhan et al. [108, 129] also measured the droplet diameter of mixed anionic or cationic or nonionic/nonionic microemulsion systems stabilized in hydrocarbons and polar lipophilic oils.

### 3.5 Small angle scattering techniques

In small angle neutron scattering (SANS), neutrons from a reactor source are scattered by the atomic nuclei of the sample. An advantage of the use of neutrons is that neutrons are non-destructive, relative to X-rays, and hence radiation damage rarely occurs in a neutron based experiment. On the other hand, the relatively slow data acquisition times and necessity for a
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powerful neutron source for neutron scattering experiments limits their applicability in the study of dynamic phenomena [136]. Radiation wavelength of X-rays (1-10 Å) is smaller than the typical structural length scale in microemulsion. Thus small angle scattering with X-rays can be very useful to determine the size and shapes of microemulsion droplets from the magnitude and angular dependency of the scattered intensity [150, 176, 207]. Using synchrotron radiation sources, information about a wide range of systems can be obtained with this technique, including those systems in which the surfactant molecules are poor X-ray scatterers [208]. SANS also provides relevant information about structure of microemulsions [74, 191, 209-211]. Normal hydrogen and deuterium have significantly different scattering lengths. Thus scattering contrast between heavy water and hydrocarbon is very high. By varying the contrast and even matching the contrast between different components of a mixture, selective portions can be highlighted. By matching the scattering length densities of water and oil, structure of both droplet-type and bicontinuous microemulsions can be established [212, 213].

Earlier, Bumajdad and his coworkers [214-216] summarized the effects of surfactant blending on microstructural properties (e.g., size and shape) as a direct consequence of mixing and physicochemical interactions in interfacial films using SANS technique. The structure and surfactant film composition of water-in-n-heptane microemulsions stabilized by DDAB/C_{12}E_{5} mixture were studied by Giustini et al. [217] using SANS technique. In another study, Bumajdad et al. [218] also reported the structure of and film properties of a zwitterionic surfactant-alcohol stabilized microemulsion system by SANS technique. Very recently, the physicochemical behavior of the mixed surfactant system consisting of the ionic surfactant AOT, and the two-tailed cyclic ketal alkyl ethoxylate (2-tridecyl, 2-ethyl-1,3-dioxolan-4-yl) methoxy]-O'-methoxy poly(ethylene glycol)], or CK-2,13, in water/isoctane water-in-oil (w/o) microemulsion systems enriched in AOT was investigated by SANS to understand the arrangement of the two surfactants at the interface vis-à-vis the behavior and properties of the microemulsion systems to enable applications [74]. Kljajic et al. [150] also studied the structural aspects of mixed AOT/sodium bis(amyl) sulfosuccinate (DAS) by small-angle X-ray scattering (SAXS) technique in heptane. Fanun [194, 219] also reported the size, shape, and internal structure of colloidal particles using SAXS technique to understand the microstructure parameters (i.e. periodicity, and correlation length) of the water + propylene glycol/mixed nonionic surfactants/peppermint oil + ethanol and water/sucrose laurate/ethoxylated mono-di-glyceride/R (+)-limonene U-type microemulsions.
3.6. Nuclear magnetic resonance (NMR)

This experimental technique provides important information about the microstructure of microemulsions. Molecular self-diffusion coefficients of each component (water, oil and surfactant) can be determined within the same experiment and information can be obtained by comparing the diffusion coefficients [191, 220-232].

Recently, El Seoud and his coworkers also studied solubilization of formamide and 1,2,3-propanetriol, PT, and also aqueous solution of formamide and PT in reverse aggregates of AOT, in Hp or isooctane by $^1$H NMR spectroscopy [233, 234]. A review article focusing a variety of NMR methods applied to different surfactant aggregates is available in literature. Spin relaxation and self diffusion experiments yielded both structural and dynamical information [235].

Generally, radioactive labeling technique is employed to obtain information on the mobility of the components. Earlier, this technique comes out to be time consuming and use of labeled molecules in a multicomponent system seems to be impractical [236]. But, the Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique, in which magnetic field gradients are applied to the sample, allows simultaneous and rapid determination of the self-diffusion coefficients (in the range of $10^{-9}$ to $10^{-12}$ m$^2$s$^{-1}$), of many components [237, 238]. For an o/w droplet structure, the surfactant and oil diffuse as droplets and hence, possesses the same diffusion coefficient (in the order of $10^{-11}$ m$^2$s$^{-1}$), which in turn is lower than that of water (in the order of $10^{-9}$ m$^2$s$^{-1}$). For a w/o droplet structure, a reverse phenomenon was observed and water has a lower self-diffusion coefficient in comparison to the continuous oil phase. In bicontinuous microemulsions the diffusion coefficients of both water and oil are high (of the order $10^{-9}$ m$^2$s$^{-1}$) and that of surfactant lies in between the value of non-associated surfactant molecules and the value for droplet-type structure (of the order of $10^{-10}$ m$^2$s$^{-1}$). Hydrodynamic radius of microemulsion droplets can also be measured from self-diffusion data. The diffusion co-efficients of oil and water are slightly reduced than the pure solvents due to the obstruction imposed by the opposite domain.

Earlier, the structure and surfactant film composition of water-in-n-heptane microemulsions supported by DDAB/C$_{12}$E$_5$ mixture was studied by Bumajdad et al. [215] and Giustini et al. [217] using pulsed-field gradient NMR (PFG-NMR). Li et al. [239] also studied the microstructure of mixed reverse micelles stabilized with surfactants of AOT and/or NaDEHP by means of proton nuclear magnetic resonance ($^1$H NMR) measurement. The chemical shift in $^1$H
NMR of water solubilized in the reverse micelles was changed concomitantly with both water content (\(\omega_0\), the number of solubilized water molecules per surfactant molecule) and relative content of surfactants (AOT and NaDEHP). Fanun [101, 105, 193, 219, 240] also characterized mixed nonionic surfactant microemulsion systems using PGSE NMR. Wolf et al. [241] reported PFG-NMR self-diffusion measurements in the single phase channels of a microemulsion system with an anionic–nonionic surfactant mixture. Very recently, Bumajdad et al. [227] studied the structure of solubilized water in water-in-n-heptane aggregates by mixing single and double tailed surfactants namely didodecyldimethylammonium chloride/dodecyltrimethylammonium chloride (DDAC/DTAC) or didodecyldimethylammonium bromide/dodecyltrimethylammonium bromide (DDAB/DTAB) by \(^1\)H NMR technique.

3.7. Fourier transform infrared spectroscopy (FTIR) measurements

The knowledge about hydration of surfactants in microemulsions/reverse micelles is helpful for understanding of dynamics of physicochemical processes (local interactions in the vicinity of water molecules in reverse micelles). Further, it is also helpful and prospective for applications in biological and chemical reactions occurring in w/o microemulsions/reverse micelles [242]. In order to get a clear understanding of various interactions in the droplet core, including the type of H-bonding which is operative within the water pool, an excellent and non-invasive technique viz., FTIR has been introduced. Several authors significantly contributed to the understanding of the water dynamics in single and mixed surfactant derived w/o microemulsion systems by studying the state of water using FTIR method [175, 227, 239, 243-250]. The characteristics of the water molecules confined inside the water pool depend strongly on both water content and the nature or type of the surfactant head group [206]. A detailed observation on the dynamics and nature of encapsulated water in AOT reverse micelles with the large variation in water content was reported by Piletic et al. [245] using linear and nonlinear infrared spectroscopy. Zhou et al. [247] reported on the characteristic features of the solubilized water by employing FTIR technique, and the results were explained on the basis of the models that pictures the water present in “layers” of different structures. For example, in the two-layer model [251, 252], water is present in two layers: the first contains water molecules that interact strongly with the surfactant head-group, this is hydration or bound water, with different physical properties (organization, mobility, microviscosity, conductivity, etc.) from those of bulk water [253, 254]. The second type possesses physical properties similar to those in bulk water and hence called
(bulk-like). In the three-layer model [255], the water located at the interface is considered to be of two types, trapped water located between the chains of the surfactant and water that solvates the head-groups [256]; a four-layer model has also been advanced [248, 257, 258]. The suggested four types of water as follows: $W_{\text{bound}}$ (water molecules tightly bound to the surfactant head-group); $W_{\text{intermediate}}$ (distorted H-bonded water species); bulk-like water, $W_{\text{bulk-like}}$; interfacial water, $W_{\text{interfacial}}$, (lies between hydrophobic interfacial tails, which is unfavourable environment for water). This can be water in-transit during the breakdown and fusion of the droplet, which is a well-known phenomenon in microemulsion. Earlier, Correa and his coworkers investigated the states of solubilized formamide or PT or their aqueous solution by FTIR spectroscopy in AOT/Hp or isoctane based nonaqueous RMs [233, 234]. Further, Gonzalez-Blanco and Velazquez [259] investigated the effect of addition of two different poly (vinylpyrrolidone) on the structure of AOT/isoctane w/o microemulsion using FTIR. FTIR technique was also applied to water-in-sCO$_2$ microemulsion stabilized by a mixture of fluorinated surfactants including fluorinated AOT-analogue surfactant by studying the stretching and bending bands of water and S=O stretching band of the surfactant over the wide range of water/CO$_2$ ratios. The number of water molecules in CO$_2$ as dissolved, associated with the core, or at the water-surfactant interface as a function of water content in the system was determined [260]. Very recently, Bumajdad et al. [227] reported on the state of solubilized water in w/o microemulsions stabilized by mixtures of single- and double-tail quaternary ammonium cationic surfactants, namely (DDAC/DTAC) or (DDAB/DTAB) by employing two noninvasive techniques, FT-IR and $^1$HNMR. The results indicated that water appears to be present as a single nano-phase (or one pseudo-phase), rather than coexisting of structurally different water layers, and the properties of such one-pseudo-phase changed continuously as a result of increasing water content ($\omega$). Further, no significant effect was found to affect the state of solubilized water on account of changing counter-ion (Br$^-$ or Cl$^-$), in spite of the known difference in the dissociation of these counter-ions from micellar aggregates. Very recently, Das and Mitra [261] characterized the state of water in a biocompatible mixed lecithin/TX-100/butyl lactate/IPM/water microemulsion by FTIR technique. They found three different types of water molecules in these systems, and their relative content changes with the interfacial composition as well as total water content in the system. However, Hayes et al. [74] demonstrated that an increase in the CK-2,13 content of the total surfactant led to an increase in water molecules localized near the
Na\(^+\) counter ion of AOT from the analysis of the -OH stretching region of the FTIR spectrum for mixed surfactant w/o microemulsion. It was suggested that the water molecules of hydration for the ethoxylate group reside near AOT's counter ion, resulting in increased dissociation of the counter ion, hence to an increased hydrophilicity for AOT.

### 3.8. Electron Microscopy

Electron microscopy can provide straightforward structural information. Images showing clear evidence of the microstructure have been obtained [81]. Both droplet and bicontinuous structures of microemulsion systems can be seen through “freeze fracture” technique [262-26]. Another technique, Cryo scanning electron microscopy (Cryo-SEM) is a powerful method for visualizing the structure of microemulsions. Recently, Koetz and his coworkers [267, 268] investigated the structure of the bicontinuous microemulsion by cryo-high resolution scanning electron microscopy. Fanun [101] also formulated and characterized biocompatible microemulsions based on mixed surfactants (sucrose laurate/ethoxylated mono -di-glyceride) and peppermint oil by cryogenic-transmission electron microscopy (Cryo-TEM), which provides a measurement of the droplet dimensions of these systems. Recently, Wolf et al. [269] also reported the nanostructures in a novel microemulsion system of silicone oil or decane, water and a surfactant mixture of an anionic [calcium dodecyl sulfate Ca(DS\(_2\))] and a nonionic surfactant (iso-tridecyl-triethyleneglycolether, abbreviated as IT 3) by Cryo-TEM and Freeze-Fracture Transmission Electron Microscopy (FF-TEM) imaging.

### 3.9. Solubilization in reverse micelle

A unique feature of reverse micellar systems (RMs) is their ability to solubilize a wide class of ionic, polar, apolar and amphiphilic substances. The existence of multiple domains in reverse micelles (e.g. apolar bulk solvent, the oriented alkyl chains of the surfactant, and the hydrophilic head group region of the reverse micelles) help to solubilize such a wide range of substances. Ionic and polar substances are hosted in the micellar core, apolar substances are solubilized in the bulk apolar solvent, whereas amphiphilic substances are partitioned between the bulk apolar solvent and the domain comprising the alkyl chains and the surfactant polar heads, i.e. the palisade layer [270]. The water molecules solubilized in the interior of the water pool have properties different from those of bulk water. This makes reverse micellar systems potentially useful for various technological applications [141, 271-273] and biologically important systems [274-276]. Earlier, Maitra and Patanjali [277] suggested that the surfactant monolayer of w/o
microemulsion droplet can be used well as a model for biological membranes for studies of solubilization of extramembraneous components from the solubilization study of cholesterol in the monomolecular hydrophobic shell of AOT reverse micellar droplet. Further, Silber et al. [242] described the methodologies that can be employed to obtain distribution constants of the solute between the surrounding solvent and the micellar interface, and discuss literature data obtained in RMs. The localization of solutes associated to the RMs, as well as the effects promoted by solute incorporation on reverse micellar properties was also discussed. Solubilization of water in RMs has been found to be dependent on various factors such as the rigidity of the interfacial film, which in turn depends upon the size of the polar head group and hydrocarbon moiety of the surfactant, the composition, the type of oils, the presence of electrolyte, the nature and valence of the counter ion, the temperature, etc. [278, 279]. Kon-No et al. [279-281] first reported on the solubilization behavior of reverse micellar systems using surfactants such as dodecylammonium carboxylate, butyldodecyldimethylammonium bromide, polyoxyethylene nonylphenyl ether, and sodium bis(2-ethylhexyl) sulfosuccinate (AOT) in organic solvents at different temperatures in the absence and in the presence of electrolytes of different charge types. Modification of the interface by blending of surfactants brings about considerable changes in the elastic rigidity of the interfacial film. Earlier studies with RMs involving more than one surfactant using solubilization identify significant modification of the physicochemical properties of the interface and consequently of the water structure in reverse micellar/microemulsion systems in comparison to the corresponding single surfactant systems. These findings summarize that the effects of surfactant blending on solubilization and other physical properties are a direct consequence of mixing and physicochemical interactions in interfacial films. In a recent study, Das et al. [206] showed the mixed AOT/polyoxyethylene (5) nonylphenyl ether (Igepal-520), cyclohexane (Cy) systems exhibit a considerable synergism in the water solubilization capacity wherein maximum solubilization capacity initially increases with increasing Igepal content to pass through a maximum and beyond which it decreases. Such synergistic solubilization behavior has previously been reported in a few mixed surfactant systems [74, 82, 108, 151, 161, 205, 215, 216, 282-286]. Earlier, Li et al. [161] investigated the influence of two typical additives, long chain organic molecule, bis (2-ethylhexyl) phosphoric acid (HDEHP) and inorganic electrolyte (NaCl) on the water solubilization capacity of AOT and NaDEHP in n-heptane solutions. The effects of the variable (additives, water content and
temperature) on the water solubilization capacity and structure of oil-water interface were also been examined by measuring the electrical conductivity of these systems. Mitra and Paul [284] also reported the effect of NaCl and temperature on the water solubilization behavior of AOT and nonionic surfactants (Brij-30, Brij-35, Brij-52, Brij-56, Brij-58, Brij-76, Tween-20, Tween-40, Span-20, Span-40, Span-60, Span-80) mixed reverse micellar systems stabilized in IPM oil. Eastoe and his co-worker reviewed the studies emphasize on controlling and enhancing the stability with respect to temperature and solubilization power (and hence the efficiency) of mixed surfactant microemulsions. Further, studies concerning the effect of mixture of surfactants on the natural curvature, droplet size and droplet shape was discussed. Also, the relation between microscopic properties (such as interfacial structure and composition) and macroscopic phase behavior was dealt in depth [130].

3.10. Dilution method

3.10.1. Interfacial composition of microemulsion

It is considered that in the formation of water-in-oil (w/o) microemulsion using surfactant/cosurfactant, all the surfactant molecules reside at the interface and the cosurfactant molecules are distributed between the oil and the interface depending upon their solubility in water. At fixed water to surfactant composition, a threshold amount of cosurfactant is required for the formation of stable microemulsion and the size of the dispersed droplets in the dispersion is controlled by the cosurfactant composition. For the estimation of the distribution of cosurfactant between the interfacial plane and the bulk oil phase, different methods have been used, viz. SANS, SAXS and DLS [287, 288], conductivity [289], interfacial tension [290].

To understand chemical reactivity of a single or mixed species either inside the water pool or at the interface of a quaternary w/o microemulsion, the detailed knowledge on (i) interfacial composition such as relative population of surfactant(s) and cosurfactant at the oil/water interface, (ii) the distribution of cosurfactant between the interface and bulk oil phase vis-à-vis the thermodynamics of this transfer process of cosurfactant, (iii) the number and the size of the droplets and (iv) physicochemical characteristics of the entrapped water. These parameters are strongly influenced by the type and alkyl chain length of oil and the amphiphiles (surfactant or cosurfactant) [289]. All these parameters [except (iv), which can be determined from Fourier transform infrared spectroscopy and FTIR measurements] [246]), can be evaluated (without using sophisticated instruments) from the dilution method which is a pioneering work of
Bowcott and Schulman [291]. This simple but elegant method is accomplished by adding oil at a constant water and surfactant level to destabilize an otherwise stable w/o microemulsion and then restabilizing it by adding a requisite amount of cosurfactant (alcohol) with constant composition of interface and continuous phase. For these reasons, the dilution method (which is commonly represents the Schulman’s cosurfactant titration of the oil/water interface and an understanding of the interfacial cosurfactant and surfactant composition as well as distribution of the cosurfactant between the interface and oil can quantitatively account for the thermodynamic stability of microemulsion) has been widely used by a number of workers to estimate the parameters involved in the transfer of alcohol from the bulk oil to the interface [108, 127, 129, 175, 292-306]. A pictorial representation of dilution method or Schulman’s cosurfactant titration has been shown in Fig. 1.11.

![Schulman's titration](image)

**Fig. 1.11.** Pictorial representation of dilution method or Schulman’s cosurfactant titration. $2\phi$ indicates biphasic composition.

Though Gu et al. [307], criticized the method on the basis of energetic and distribution parameters from calorimetric data, it has been confirmed to be correct by Moulik et al. [308] and Palazzo et al. [309] later on. Palazzo et al. [309] further pointed out that this method has applications in scattering and diffusion studies, because it provides extrapolation to single-particle properties by reducing inter-particle interactions of the microemulsion system without changing its composition.
3.10.2. Thermodynamics of transfer of cosurfactant from the oil phase to the interface on the basis of the dilution method

In w/o microemulsions, nano water droplets are stabilized by amphiphiles in oil continuum. Most of the cases, the surfactants and the cosurfactant are the stabilizers for the water droplets in oil medium. In the matter of understanding the structure of w/o microemulsions, the role of the cosurfactant on the stabilization of the interface between water and oil and the component population in the interfacial region relative to that in the internal oil phase, along with the thermodynamics of the formation of the interface have been stressed. It is normally considered that the total surfactant resides at the interface and the cosurfactant remains distributed in the water, interface, and oil [296]. The cosurfactants higher than butanol essentially remain partitioned between the interface and oil because of their negligible solubility in water. The distribution or partition of the cosurfactants between the interfacial region and the oil phase depends on a number of factors, namely, their hydrophobic/hydrophilic molecular nature, the nature of the oil, interfacial forces, the interaction with the surfactant, the system composition, and the thermal conditions [308]. The distribution constant, $K_d$, and its temperature dependence have been processed to derive Gibbs free energy change ($\Delta G^0_t$), standard enthalpy change ($\Delta H^0_t$) and standard entropy change ($\Delta S^0_t$) of the cosurfactant transfer process from oil to the interface. Reports on the evaluation of thermodynamic parameters for w/o microemulsions stabilized in hydrocarbons and polar lipophilic oil using single surfactant are available in literature [175, 295-304, 308]. However, analysis of the transfer of cosurfactant from oleic phase to the interface of mixed ionic/nonionic surfactant w/o microemulsions using dilution experiments from thermodynamic point of view is seldom reported. Recently, Mitra et al. [127] and Bardhan et al. [108, 129, 306] reported the details thermodynamic studies on the formation of mixed $C_n$TAB/$C_{18}E_{20}$ (where, $n = 12\rightarrow 18$) and CTAB/Brij-35 microemulsion stabilized in either hydrocarbon (Hp or Dc) or polar lipophilic (IPM) oil using dilution method.

4.0. Types of microemulsions

4.1. Biocompatible microemulsions

The 20th century has witnessed a remarkable growth in drug discovery, development and use. But towards the end of the century, toxic side effects of the drugs became apparent in addition to their therapeutic effects, due to several reasons. Over the past few decades colloidal systems have been explored as potential delivery systems [310], because of their compartmentalized
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hydrophobic and hydrophilic domains (cavities), where both polar and non-polar molecules could be encapsulated and stabilized.

Several advantageous physicochemical properties (viz. transparency, low viscosity, thermodynamic stability and high solubilization capacity) have enabled microemulsion systems to be used in many biological and biotechnological processes as drug delivery vehicles, pharmaceuticals etc. [12, 311-313]. Microemulsion formulations are also claimed to improve bioavailability and the reproducibility of the plasma profile of peptides administrated by the oral route [314]. Earlier, Moulik and his coworkers [66, 137, 138, 311, 315] reported a series of studies on the formulation and development of multicomponent systems containing biologically occurring components (with biocompatibility and non-toxicity) and their stability, physicochemical properties. The size, polydispersity and diffusion coefficients of droplets of different monophasic microemulsion compositions have been determined at different temperatures by the DLS method. The energetic parameters (enthalpy, free energy and entropy) of the microemulsification process have been estimated calorimetrically. The active ingredients used in these systems were Tween, Brij and sodium oleate as surfactants, ethanol, and isopropyl alcohol, cinnamic alcohol as cosurfactants, and eucalyptus, coconut, ricebran, clove, isopropyl myristate, saffola as oils. A series of publications on the formation of microemulsions using biocompatible and nontoxic ingredients (both surfactants and oils) for prospective applications in pharmaceuticals, cosmetic, food, toilets etc. have been reported by several authors and our group also [12, 41, 139, 316-321]. The potential application of highly biocompatible w/o microemulsions to the food, cosmetic and pharmaceutical industry as solubilization media of hydrophilic, hydrophobic, and amphiphilic functional materials, has been growing interest during the past few years [322-324]. Formulation and characterization of nontoxic microemulsion formulations based on biological amphiphiles and different oils were studied and also reviewed over a decade [313, 325-329]. Saha et al. [71] recently formulated and characterized an edible microemulsion composed of Tween-80/butyl lactate/IPM/water and they reported that these systems could act as a potential drug delivery system and nontoxic nanotemplate for other applications. Fanun reported alcohol free microemulsions using peppermint oil and sugar esters, ethoxylated mono-di-glyceride, and polyoxyethylene sorbitan monooleate for food, cosmetics and pharmaceutical applications [83]. Water solubilization, microstructure, diffusion, viscous and conductive flow parameters and diclofenac solubilization studies were investigated on
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systems based on mixed nonionic biocompatible surfactants and IPM [82, 105, 110, 115, 219, 330, 331]. Earlier, Watnasirichaikul et al. [332] prepared poly(ethyl 2-cyanoacrylate) nanocapsules containing insulin by interfacial polymerization of spontaneously forming biocompatible microemulsions composed of mixture of surfactants (polysorbate 80 and sorbitan mono-oleate) and mixture of medium chain glycerides (caprylic/capric triglycerides and mono-/diglycerides). Acosta et al. [333] studied the formulation of biocompatible microemulsions using lecithin as major surfactant and biocompatible linker molecules (hexyl polyglucoside as the hydrophilic linker and sorbitan monoleate as the lipophilic linker) and IPM as oil medium. They found that these biocompatible systems could be used as potential substitutes for chlorinated solvents in dry cleaning applications and as solvent delivery systems for pharmaceutical applications. Papadimitriou et al. [334] prepared microemulsions composed of olive oil, as the continuous oil phase, water as the dispersed phase, and a mixture of lecithin-propanol as the emulsifier and investigated as potential biocompatible media for biotransformation. In another study, they concluded that w/o biocompatible microemulsions formulated with R-(+)-limonene, water, and a mixture of lecithin and 1-propanol as emulsifiers can be used as reaction media for the effectuation of esterification reactions catalyzed by lipase [73]. Sharma and Warr [335] and Montalvo et al. [336] studied the phase behavior and microstructure of Tween-80/d-limonene or perfluoromethyldecalin (PFMD) and lecithin/PEG 40 monostearate/water systems, respectively. Recently, Nguyen et al. [337] and Wilk et al. [75] also formulated and characterized biocompatible microemulsions using biosurfactant mixtures of lecithin, rhamnolipid, sophorolipid with IPM, limonene and N-dodecyl-N,N-bis[(3-D-aldonylamido)propyl]amines C_{12}-DX (glucosyl GA or lactobionyl LA), iso-butanol, hydrophilic (diethylene glycol monoethyl ether DGME) or hydrophobic (iso-octane) oils, respectively. However, reports on formulation and charatirization of biocompatible microemulsions using mixed surfactants are available in literature and a few of them are summarized here.

Pharmaceutically usable microemulsion systems were prepared from water and isopropyl myristate with a constant amount of Tween 40 and Imwitor 308 at a mass ratio of 1 by Podlogar et al. [338]. Type and structure of these microemulsions were examined by measuring density, surface tension, viscometry, electrical conductivity, differential scanning calorimetry (DSC) and SAXS, and also the degree of agreement between the techniques was assessed. Liu and his coworkers [339, 340] investigated the influence of structure and composition of biocompatible
microemulsions (AOT/Tween85/isopropyl myristate/water) on their transdermal delivery potential of a lipophilic model drug (Cyclosporin A), and compared the drug delivery potential of these microemulsions to the suspension of drug in normal saline containing 20% ethanol and also a potentially improved skin bioavailability of CysA was designed in these systems. Lv and coworkers [341] also investigated biocompatible mixed Span-20/Tween-20/IPM/water RMs as potential drug delivery systems for effective component of eye drops, i.e., chloramphenicol. Yanyu et al. [342] also investigated the influence of the structure and the composition of water/Aerosol-OT (AOT)-Tween-85/isopropylmyristate (IPM) microemulsion system on transdermal delivery of 5-fluorouracil (5-FU). Fanun et al. [100] formulated and structurally characterized new U-type bioinspired microemulsions by using mixed safe surfactants (sucrose laurate and ethoxylated mono-di-glyceride) and mixed oils (IPM and peppermint oil) in absence of harmful cosurfactants and was used as transdermal carriers of compounds with medicinal interest such as cephalaxin. Very recently, Tzika et al. [343] carried out oxidative enzymatic reactions using horseradish peroxidase (HRP) in a model biomimetic system composed of lecithin/1-propanol/olive oil/water using gallic acid, octyl gallate, 2,2’-azino-bis[3-ethylbenzo-thiazoline-6-sulfonic acid. Kunz et al. [344, 345] also formulated highly water dilutable green microemulsion comprising of water/sodium oleate/citronellol/limonene and using ethanol as cosolvent in same system. In another report, they also showed a strategy of formulating highly and fully water dilutable sustainable microemulsions with dibasic esters (succinate C2, glutarate C3 and adipate C4) as oil pseudophase, SDS and 1-Pn as surfactant and cosurfactant, respectively [346]. Bauduin et al. [347] also formulated low toxic and temperature sensitive anionic SDS/dodecane microemulsion by combining with short propyleneglycol mono alkyl ethers (CnPOM, n = 3, m = 1 and n = 4, m = 2, 3) as cosurfactant. Das and Mitra [261] and our group [321] formulated and characterized biocompatible mixed lecithin/TX-100/butyl lactate/IPM/water and AOT/Tween-85/EO or IPM or IPP/water microemulsions/RMs, respectively by phase behavior, solubilization capacity, conductivity, viscosity and FTIR techniques. In recent years, biodiesel has gained importance as green solvents [348, 349]. Biodiesel is obtained by transesterification of vegetable oils with monohydric alcohols, commonly methanol, to give the corresponding mono-alkyl esters (FAME) [350]. During last decade, Biodiesel and FAME used as green solvents in the formulation of microemulsions, as reported by several
authors [351-356]. However, some research groups also reported the formulation and characterization of nontoxic microemulsions based on different kinds of renewable feedstock oils [323, 357-359].

4.2. Food grade microemulsions
Sucrose esters form a class of surfactants with the important properties of being biodegradable, nontoxic and capable of forming temperature-insensitive microemulsions. Such microemulsions have been reported to suit a variety of food-based and pharmaceutical applications. Garti and his coworkers [49, 360-368] published a series of papers depicting the phase behaviors and microstructures of such kind of food grade microemulsions. They used polyol nonionic surfactants like sucrose stearate (S-1570), sucrose laurate (L-1695), sucrose palmitate (P-1570), sucrose monooleate (O-1570) etc. or Tweens to stabilize these systems. Oils used were either hydrocarbon oils like dodecane, hexadecane etc. or triglycerides. These surfactants do not form microemulsion by its own, so a fourth component (cosurfactant, generally small chain alcohols) was added into these systems to form microemulsions. Alcohols used were ethanol, n-propanol, n-butanol, n-pentanol. Considerable monophasic regions were obtained with these systems, and the extent of monophasic regions were observed to be dependent on the types of oil and cosurfactant used. The MCT (medium chain triglycerides) based systems were found to be temperature insensitive. The structural features of these systems were carried out using viscosity, SANS, PGSE NMR and SAXS studies. Campo et al. [147] also studied the structural characterization of a five-component food-grade microemulsion containing Tween 80, R(+-)-limonene, ethanol, glycerol, and water using SANS, viscosity, electrical conductivity and DLS techniques. Corswanto et al. [220, 221, 369, 370] reported food grade microemulsions prepared from extracted cereal lipids and liquid mixtures of mono- and triglycerides, and corresponding L3 (sponge phase) and L2 structures respectively, were established. Flanagan et al. [323] presented an overview of the structure and phase behavior of microemulsions, methods of microemulsion formation, and techniques used for characterization and also delineated through an comprehensive review of previous work on both food grade microemulsion systems and non-food grade systems of specific food interest. Engström and Larsson [371] and Dungan [372] also reviewed the prospective application of microemulsion in foods in detail. Recently, several authors were also paid attention to formulate and characterize the food grade microemulsions [373-375].

4.3. Nonaqueous microemulsions
The first reports of microemulsions in which water were substituted by polar solvents like glycerol and formamide appeared in 1984 [376-378]. These nonaqueous microemulsions are
essentially oil continuous [379] and have attracted attention from the viewpoints of both theoretical (thermodynamics, particle interactions) and practical (potential use as novel reaction media) [378]. Further, these systems are also interesting due to their applications in a large number of fields, such as cosmetics, solar energy conversion, semiconductors and microcolloids whose sizes can be controlled by that of the droplets used in their formation [380]. A number of distinct advantages of the nonaqueous microemulsion systems over the aqueous ones in particular, because they can be used as good reaction media, for example, organic reactions like esterification [381] and polymerization [382]. Nonaqueous systems often show much larger stability regions of isotropic solutions as compared to the analogous aqueous systems [377]. A large variety of different surfactants can be used to produce nonaqueous microemulsions. For example, a long chain phosphonium halide could be incorporated in nonaqueous microemulsions, while the corresponding attempts to prepare aqueous microemulsions with this surfactant were unsuccessful [383].

In recent years, much attention has been focused on the formulation, phase determination and structural characterization of microemulsion systems stabilized by nonaqueous (polar) solvents like formamide (FA), dimethyl formamide (DMF), dimethyl acetamide (DMA), ethylene glycol (EG), propylene glycol (PG), and glycerol (Gy) [384-392].

Very recently, Correa et al. [393] reviewed the self-assembly of amphiphiles in absence of water and subsequently, microemulsions and reverse micelles in nonaqueous polar solvents (viz. glycerol and other polyols, formamide, dimethyl formamide, dimethylacetamide) while sequestering a polar nonaqueous core. Further, they demonstrated these systems as interesting nanoreactors for heterogeneous chemistry, templates for nanoparticles, models for biological membranes. In addition, RTILs in reverse micelles have been reviewed. Recently, Sarkar and co-workers reported nonaqueous reverse micelles (for example, MeOH/AOT/Hp and EG/AOT/Hp) as media for the formation of silver nanoparticles [394, 395].

5.0. Use of microemulsions

The outstanding properties of microemulsions, such as high capacity to solubilize water and oil, low interfacial tension, large interfacial area, spontaneous formation and fine microstructures render them excellent candidates for a variety of applications, which have been summarized in several review papers and books [13, 14, 396-400]. However, most important and promising applications, as described in literature, are summarized in order to demonstrate the significance and potential of the microemulsion systems.

5.1. Advantages and disadvantages
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It can be noted that that microemulsions possess both advantages and disadvantages. Because of the ease of microemulsion preparation, drugs that are thermo-labile are easily incorporated without the risk of degradation [401]. In drug delivery, microemulsion ultimately increases the surface area of drugs, which improves their solubilization and permeation behavior. The rate of penetration of drug is much faster in microemulsions than other drug delivery vehicles and also microemulsions possess several advantages in terms of controlled drug release rates, slow degradation, and target specificity [401]. Microemulsions have a higher solubilization capacity for hydrophilic and hydrophobic compounds compared to simple micellar solutions. Because of their thermodynamic stability, they are more favorable than regular emulsions since microemulsions can be prepared with very little energy input and have a long shelf life [402]. Microemulsions possess some disadvantages as well. Formation of microemulsions generally requires large amounts of surfactant(s) and/or cosurfactant(s). All of these are generally irritating at high concentrations [401]. Many experimental conditions, such as temperature and pH, influence the stability of microemulsions as well. The effects of these factors must be examined for each potential formulation.

5.2. Applications in various fields

From the extensive review reports of Paul and Moulik [397], Moulik and Rakshit [13], Stubenrauch [403-406], Sjoblom et al. [407], Paul and Moulik [408], and De and Maitra [409], some applications of microemulsion systems have been mentioned here in brief. Microemulsions can function as a liquid membrane. They have been found to be efficient in the extraction of heavy metals (e.g., Hg) involving oleic acid from contaminated water, and used as fuels, lubricants, cutting oils and corrosion inhibitors for several decades. Paint formulations using microemulsions have shown higher scrub resistance, better color intensity and more stain resistance than those prepared by emulsions. Due to their characteristic properties, microemulsions are promising systems for detergency purposes over traditionally-used organic solvents, as they can solubilize polar (e.g., salt, pigment, protein) and non-polar soil components (e.g., grease, oil). In many cosmetic applications such as skin care products, emulsions are widely used with water as the continuous phase. The dispersed phase, lipophilic or hydrophilic (o/w or w/o type) can act as a potential reservoir of lipophilic or hydrophilic drugs that can be partitioned between the dispersed and the continuous phases. The unique properties of microemulsions have been utilized to produce microemulsion-gel glasses and microporous media with high surface area. Recently, interest on microemulsions is being focused for various applications in biotechnology, viz., enzymatic reactions, immobilization of proteins and bioseparation. Due to varied consistencies and microstructures, microemulsions have been
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considered as useful reaction media for a variety of chemical reactions. The major types of reactions studied in microemulsions comprise formation of inorganic particles (nanoparticles), polymerization, photochemical, electrochemical and electrocatalytic and organic synthesis. Numerous nanostructured materials, ranging from metallic catalysts [410-413] to semiconductor quantum dots [414, 415] to various ceramic materials [416-418], silica and gold coated nanoparticles [419, 420], latexes and polymer composites [421, 422], double-layered nanoparticles [423], bimetallic nanoparticles [406] and even superconducting and magnetic materials [424] have been synthesized and characterized in reverse micelles. Earlier, Shah and his coworkers reviewed several articles pertaining to the synthesis of nanoparticles in microemulsions and described in some detail our research efforts in the past decade in the field of synthesis of nanoparticles of silver halides, superconductors and magnetic materials using w/o microemulsions as nano-reactors [425]. In another review, Lo’pez-Quintela and his coworkers [426] showed the use of microemulsions for the preparation of nanoparticles, and also as interesting organic reaction media. Recently, Hegde et al. [427] and Fawzia et al. [428] reviewed the prospective application of microemulsion in ocular drug delivery systems. The review article of Holmberg [429] and Garcia-Rio et al. [430] demonstrated that microemulsions could be a useful for (i) overcoming reactant incompatibility, (ii) speeding up reactions of one polar and other apolar reactants (microemulsion catalysis), and (iii) inducing regiospecificity in organic reactions.

![Fig. 1.12. Schematic representation of nanoparticle synthesis by using microemulsions.](image)

About three decades ago, Boutonnet et al. [431] showed that metallic nanoparticles (Pt, Pd, Rh, Ir) could be obtained by simple mixing of two w/o microemulsions, one containing a salt, or a complex of the metal and the other containing a reducing agent, such as boron hydride, or
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hydrazine. The microemulsion systems were water/CTAB/octanol and water/C_{12}E_{5}/hexane or hexadecane. A schematic representation of nanoparticle synthesis by using microemulsion as template has been shown in Fig. 1.12.

In recent years, several authors presented review of the literature in the field of nanoparticle synthesis and characterization of several metals in the inverse microemulsion systems [432-435]. Nagy and his co-workers reported the preparation of inorganic nanoparticles - Ni_{2}B, Pt, Au, Pt-Au, Ag-halides and also the synthesis of organic nanoparticles – cholesterol, rhovanil, and rhodiarome in different microemulsions (AOT/heptane/water, TX-100/decanol/water, CTAB/hexanol/water) by direct precipitation of active compound in aqueous core with a prospect of using these systems in transdermal drug delivery [436]. Further, they emphasized on the mechanism of formation of the inorganic and organic particles. In the case of organic NPs, all the particles sought to follow the thermodynamic stabilization (with the exception of the cholesterol synthesized in the AOT/heptane microemulsion) and argued that a specific interaction between the surfactant and NPs might be responsible for this phenomenon [436]. Recently, a novel and straightforward approach of metal (Pt, Pd and Rh) and metal oxide [cerium (IV) oxide with cubic type crystalline structure] nanoparticle synthesis was reported by Solans et al. [437] by nonionic surfactant-based oil-in-water (o/w) microemulsion reaction method. The ability of amphiphilic compounds to self-assemble into well-defined structures can be taken advantage of the synthesis of inorganic materials with nanometer dimensions. The principle, which is biomimetic in character, was discussed with special attention on three areas: microemulsion-based synthesis of nanoparticles, preparation of mesoporous materials from surfactant templates, and surfactant-mediated crystallization [438]. In a review, Eastoe et al. [439] described synthesis of nanoparticles in microemulsions, which is an area of considerable current interest. They broadly divided the article into two sections defined by the nature of the host microemulsion reaction medium as well as wide variety of materials synthesized by w/o microemulsions methods for more than two decades. Control parameters were elucidated for influencing both nanoparticle concentration and morphology, allowing for tailored syntheses with various applications. More recently, the ability to synthesize nanoparticles in water/supercritical fluid microemulsions was realized. This method promises to be a highly useful route for controlled nanoparticle synthesis due to the added control variables afforded by tuneability of the solvent quality (density) through pressure and temperature. This review
presents the current state-of-the-art in both fields. The evolution of the microemulsion technique (theoretical/simulation results) was reviewed by López-Quintela with special emphasis in the mechanism of control of particle size, viz. the control by the proper microemulsions and also the control by the surfactant adsorption (capping). The kinetics of the particle formation, the possibility of the preparation of the particle formation, as well as coating core-shell and ‘onion-like’ structures with a precise size control, and the use of microemulsions to produce fine ceramics and finally their use in the preparation of super-lattices were addressed [440]. Tojo and his coworkers reviewed a series of papers on computer simulation study on the synthesis of nanoparticles in microemulsions [441-445]. Harada and his coworkers [446] also prepared colloidal dispersions of silver (Ag) particles by the photoreduction of silver perchlorate (AgClO₄) in water/AOT/benzene water-in-oil (w/o) microemulsions. Recently, Chaudhuri and Paria [447] reported an in-depth review of synthesis mechanism and applications of core/shell nanoparticles by microemulsion synthesis media. Dominguez et al. [448] discussed the synthesis of inorganic nanoparticles [metallic (Pt, Pd, Rh, Ag), single metal oxides (CeO₂, ZrO₂, TiO₂, Fe₂O₃), mixed and doped metal oxides (Ce₀.₅Zr₀.₅O₂, Ce₀.₉₉Eu₀.₀₁O₂, Zr₀.₉₉Eu₀.₀₁O₂, and Fe₂Mn₀.₅Zn₀.₅O₄), semiconductors (PbS, CdS, Ag₂S, ZnS, CdSe, PbSe, Ag₂Se), fluorides (CaF₂, YF₃, NdF₃, PrF₃), phosphates (CePO₄, HoPO₄), and chromates (BaCrO₄ and PbCrO₄)] using oil-in-water (O/W) microemulsions as confined reaction media. In a review report, Goshen and Magdassi [449] reviewed the methods employed to produce organic nanomaterials from microemulsions, and also provided an outlook on particle design possibilities that can be achieved by various techniques.

Enzymatic oxidation of oleuropein, the most abundant olive phenolic compound, in the restricted aqueous environment of olive oil based microemulsion composed of lecithin and 1-propanol was reported by Papadimitriou et al. [450]. Xenakis and his coworkers also reported a series of papers on lipase containing microemulsions [451]. Kunz and his coworkers [452] also reported the activity of the enzyme horse radish peroxidase (HRP) in single surfactant based reverse microemulsions, composed of sodium dodecylsulfate (SDS), n-dodecane, aqueous buffer, and alcohols of the homologous series 1-butanol to 1-octanol. Sawada and Ueda [453] also investigated the activity of proteases α-chymotrypsin and subtilisin in Tween-85/isopropyl alcohol/n-hexane/water and TX-100/cyclohexane/water RMs. They found that the activity of enzymes in the Tween-85 based RMs was similar to that of aqueous systems but TX-100 based
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RMs did not show any enzyme activity. Recently, Fanun [454] reported an in-depth review on the prospective use of microemulsions as delivery systems. In the field of drug carriers, microemulsion medium has been found to be a good proposition, and can be applied to a wide variety of dosage forms including oral, topical, ocular, parenteral, periodontal, buccal, and nasal formulations [455]. In addition, microemulsion systems have applications in cancer therapy [456], as decontamination media for chemical weapons and toxic industrial chemicals [457], and petroleum-processing technologies [458].

5.3. Application of mixed surfactant microemulsions

5.3.1 Nanoparticle synthesis

Earlier, Gan et al. [459] synthesized nanometer silica particles (5–20 nm) from sodium orthosilicate in mixed polyoxyethylene (5) nonylphenol ether (NP-5)/polyoxyethylene (9) nonylphenol ether (NP-9)/cyclohexane microemulsion. Also, Ingelsten et al. [460] studied the effect of surfactant type and temperature on the kinetics of the formation of platinum nanoparticles in w/o mixed microemulsions composed of mixtures of poly(ethylene glycol)monododecyl ethers (C_{12}E_{4}, C_{12}E_{5}, C_{12}E_{6}), and AOT in Hp. Bumajdad and his coworkers reported the synthesis of pure ceria powders, CeO_{2}, in Hp-microemulsified aqueous solution of CeCl_{3} or Ce(NO_{3})_{3} stabilized by AOT, DDAB, or DDAB/Brij-35 surfactant mixtures [461]. Also, Hada et al. [462], Chiang [463] and Bumajdad et al. [464] employed novel water-in-oil microemulsion systems stabilized by mixed surfactants (anionic or cationic/nonionic) for the synthesis of titania (TiO_{2}), gold (Au) and iron oxide-hydroxide [α-Fe_{2}O_{3}; Fe(OH)_{3}] nanoparticles, respectively. Morphology of CdS nanocrystals synthesized in mixed AOT and zwitterionic phospholipid, L-alpha-phosphatidylycholine (lecithin) in isoctane microemulsion was reported by Simmons et al. [465]. In contrast, conventional spherical CdS quantum dots were also obtained from the AOT water-in-oil microemulsion system [465]. Bagwe et al. [466] reported the effects of intermicellar exchange rate on absorption spectra and particle size of silver (Ag) nanoparticles synthesized in AOT/Hp RMs, and the rate was varied by changing the organic solvent (decane, Dc and cyclohexane, Cy), surfactant (SDS, NP-5 and DTAB) and organic additives (benzyl alcohol and toluene). An interesting and potentially useful effect was observed by the addition of a small amount of a nonionic surfactant (NP-5) that particle size significantly reduces. Yunxia et al. [467] investigated water solubilization capacity and the properties of confined water molecules in the pool of an
environmental friendly mixed surfactant microemulsions composed of Span-80, Tween-60, 1-butanol, isooctane and used as nanoreactors to synthesize iron nanoparticles, which can be used in environmental contaminants remediation. The properties of the synthetic iron nanoparticles were characterized and analyzed. In another study, Chiang et al. [468] demonstrated that mixed AOT/Span-80/isooctane reverse micelles are good candidates to be used as nanoreactors for formation of shape-controlled high-quality colloidal nanocrystals and nanowires under mild conditions. Manipulation of the rate of nucleation and subsequent growth of the Au in the mixed reverse micelles induces drastic changes in the particle shape and structure. Further they demonstrated that control of the nucleation and growth kinetics of the Au in the mixed reverse micelles can be used to vary the shapes of the resulting particles from a nearly spherical morphology to cylinders, trigons and cubics. Spirin and his coworkers described the mechanisms of the formation and stabilization of gold nanoparticles in reverse micelles based on mixed Triton X-100 (TX-100) and Aerosol OT (AOT) surfactants in n-hexane or cyclohexane [469]. Stubenrauch and coworkers [470] synthesized platinum (Pt), bismuth (Bi), and lead (Pb) nanoparticles by using w/o mixed AOT/SDS/1-butanol/n-decane/water microemulsions in presence of the salt (either the metal precursor [H₂PtCl₆, Bi(NO₃)₃, or Pb(NO₃)₂] or the reducing agent [NaBH₄]) as templates. The size and the structure of the resulting nanoparticles were found to depend on the size and structure of the templating microemulsion.

Recently, Bumajdad et al. [471] showed that mixing di-n-didodecyltrimethylammonium bromide (DDAB) with small amounts of the non-ionic Brij-35 [polyoxyethylene (23) dodecyl ether, C₁₂E₂₃], gives rise to microemulsions offering an excellent environment for prospective synthesis of nanoparticles. They argued as follows. The optimized DDAB/Brij-35 microemulsions have the advantages of both low thermal sensitivity and stability toward added inorganic salt type and concentration. The positive head-group charge of the cationic surfactant ensures bulk solubility of the inorganic metal cation, whereas the long polyethylene oxide chain of the non-ionic surfactant facilitates increased steric stability.

5.3.2. Enzyme activity and drug delivery

Shioi et al. [472] reported the enzyme activity of α-chymotrypsin (CT) in RMs composed of CᵩEᵣ and/or AOT using an oil-soluble substrate, 2-naphthylacetate and compared the activity in CᵩEᵣ/AOT stabilized RMs with the AOT stabilized RMs. The activity of CT in mixed RMs remained almost unchanged after one week, whereas in the single system, the activity decreased
substantially with time. Das and co-workers [473] investigated on the modification of the anisotropic interface of cationic (CTAB) w/o microemulsions by the addition of different types of non-ionic surfactants (Brij-30 or Brij-92 or Tween-20 or Tween-80) at different physicochemical conditions and the catalytic efficiency of surface active enzymes, *Chromobacterium viscosum* (CV) lipase and *horseradish peroxidase* (HRP) was influenced by the mixed systems. n-hexanol and isooctane were used as cosurfactant and oil. The activity of CV-*lipase* in these systems improved maximum up to ~200% with increasing content of non-ionic surfactants compared to that in pure CTAB RMs. It was argued that the reduced positive charge density (due to addition of nonionic surfactant) as well as plummeted n-hexanol content (a competitive inhibitor of lipase) at the interfacial region of cationic w/o microemulsions. Chen and Liao [474] studied the stability of *yeast alcohol dehydrogenase* (YADH) in AOT/Brij-30 mixed reverse micelles. The activity of YADH in mixed reverse micelles was affected by the hydrophobic and electrostatic interactions between enzyme and surfactants, the reverse micellar size, and the bound degree of water molecules. From the measurements of the hydrodynamic diameter (droplet size) and its distribution via dynamic light scattering, they suggested that the structure of mixed reverse micelles and the stability of YADH were determined by four important factors, which include the surface charge density, bound water, reverse micellar size, and the entrapment of water by hydrophilic-hydrophilic interaction of AOT and Brij-30. An optimal reverse micellar size and the decrease of the other two factors (i.e., the hydrophobic and electrostatic interactions between enzyme and surfactants, and the bound degree of water molecules) would lead to the enhancement of enzyme activity. Earlier, Hossain et al. [475] and Hayashi et al. [476] reported an enhancement in the activity of *Chromobacterium viscosum* (CV) lipase entrapped in AOT/Tween-85/isoctane RMs by the addition of olive oil as a water-insoluble substrate and short chain methoxypolyethylene glycols (MPEGs). Fan et al. [477] also showed an enhancement in the activity of *papain* in mixed AOT or TTAB/Tween-80/isoctane RMs. Recently, Hemavathi et al. [478] reported that mixed AOT/Tween-20 or Tween-80 or Tween-85 or TX-100/isoctane RMs can be used for the extraction and primary purification of β-glucosidase from the aqueous extract of barley (*Hordeum vulgare*). Liu et al. [339, 340] reported the influence of structure and composition of mixed AOT/Tween-85 stabilized in IPM on transdermal delivery potential of a lipophilic model drug, *Cyclosporin A* (*CysA*). Further a potentially improved skin bioavailability of *CysA* in these systems was designed. Recently,
Yanyu et al. [342] investigated the influence of the structure and the composition in water/AOT/Tween-85/IPM microemulsion system on transdermal delivery of 5-fluorouracil (5-FU). Kunz and his coworkers also reported the activity of the enzyme horse radish peroxidase (HRP) in mixed RMs, composed of anionic, SDS, cationic, DTAB (known as, catanionic microemulsion), n-hexanol, citrate buffer, n-dodecane [479]. Under the perspective of potential pharmaceutical applications, Kalaitzaki et al. [480] prepared new surfactant-rich biocompatible microemulsions based on single and mixed surfactant (isopropyl palmitate/Triton X-100/water + propylene glycol, Miglyol 818/Triton X-100/water + propylene glycol and isopropyl palmitate/Triton X-100 + Span 20/water + propylene glycol) and used as novel carriers of methylxanthine drugs (e.g., theophylline and theobromine) after characterization of their structures. Moghimipour et al. [143] also formulated and characterized a microemulsion system by mixing of appropriate amount of surfactant including Tween-80 and Span-80, propylene glycol (PG) as co-surfactant and oil phase including Labrafac PG – transcutol P as a topical delivery system of naproxen for relief of symptoms of rheumatoid arthritis, osteoarthritis and treatment of dysmenorrheal.

Chaladze et al. [481] used reverse micellar mobile phases based on mixed Brij-30 and AOT surfactants in high-performance liquid chromatography (HPLC). Mehta et al. [482] also exploited the added degree of compositional freedom provided by mixed AOT/lecithin surfactants in isoctane reverse microemulsion to demonstrate that a small amount of added lecithin significantly enhances the solubility of organodiselenides over that in single AOT reverse microemulsion alone. The information obtained from these experiments was used to design a novel synthetic route for preparing 4-chloro-2-(naphthalen-2-ylselenyl) pyrimidine (i.e., organochalcogen) in reverse microemulsion.

**6.0. Ionic liquid based microemulsions**

**6.1. Background**

In the field of colloid and interface science, the self-assembling phenomena of amphiphilic molecules, (e.g., anionic, nonionic, zwitterionic surfactants in single or mixed state) in ionic liquids are currently an attractive and useful research area, because an understanding of the self-assembling mechanism is of much interest and importance from academic point of view as well. In addition, the aggregate formed from amphiphilic compounds (with special reference to microemulsions/reverse micelles) would be able to solubilize substances essentially insoluble in
ionic liquids, which should widen knowledge on both fundamental aspects and the application fields of ionic liquid based microemulsions/RMs, for example, metal nanomaterials, biological extraction, enzymatic reactions, drug delivery etc. [483]. However, the tremendous potential of microemulsions as modern colloidal carriers for topical and transdermal drug delivery are well recognized [484]. Microemulsions, a system of water, oil and amphiphiles, provide a variety of advantages for pharmaceutical use, such as nanometer-sized aggregations, long-term stability, biocompatibility, straightforward preparation and high solubilization capacity for drug molecules and enhanced drug delivery [311, 485, 486]. Oil soluble drugs can be formulated in oil-in-water microemulsions whereas, water soluble ones are better suited for water-in-oil microemulsions. However, drug delivery using such self-assembled systems yet to fully achieve its potential for a large number of drugs, which are insoluble or sparingly soluble in water and most pharmaceutical grade organic liquids. These limitations demand the need for alternative solvents that can be used as a polar core in microemulsions for the solubilization of sparingly soluble drug molecules. Considering these aspects, ionic liquids (ILs) could be good candidates because they are capable of dissolution of various poorly soluble compounds including drug molecules [487, 488]. Room temperature ionic liquids (RTILs), an interesting class of tunable and designer solvents, have been extensively used as a “green” replacement for toxic, hazardous, flammable and highly volatile organic solvents (VOSs) [489-491]. However, ILs are not as green as was initially considered. The environmental, health, and safety impact of ILs have been a growing concern; evaluation of their toxicity levels is being considered important [492]. ILs with long alkyl side chains are found to be cytotoxic. Hexafluorophosphate and tetrafluoroborate based ILs can undergo thermal and chemical decomposition and in acids can undergo hydrolysis forming HF at low temperature [493]. Further, microemulsions in which water is replaced by a polar solvent have attracted great interest and in this context, the most common solvents used include formamide, dimethylformamide, dimethylacetamide, ethylene glycol, propylene glycol, and glycerol [376, 393]. Nonaqueous microemulsions have been discussed at length in a previous section (Sec. 4.3). The reason behind this interest is that there are a large number of organic reactions for which contact with water needs to be avoided. In this regard, nonaqueous ionic liquid (IL) microemulsions where the ILs are sequestrated by non-ionic surfactants represent an interesting
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area since they provide hydrophobic or hydrophilic microdomains that expand the potential uses of the ILs in microheterogeneous systems [494].

6.2. Definition, types, and properties of ILs

Ionic liquids (ILs) are class of novel solvents (or, special class of salts) with low melting points of below 100°C, and composed entirely of organic cations (e.g., imidazolium, pyridinium etc.) and inorganic/organic anions [e.g., chloride (Cl\(^{-}\)), bromide (Br\(^{-}\)) and tetrafluoroborate (BF\(_4\)\(^{-}\)), hexfluorophosphate (PF\(_6\)\(^{-}\)), bis(trifluoromethylsulfonyl)imide (Tf\(_2\)N\(^{-}\)) etc.]. A schematic representation of different cations and anions in ILs is presented below. The definition of ionic liquids allows distinguishing them from a classical molten salt. A molten salt is mostly a high-melting, highly viscous and very corrosive substance while ionic liquids are already liquid at lower temperatures (< 100°C) and exhibit in most cases relatively low viscosities [483]. Conventional ILs typically contain bulky organic cations with a low degree of symmetry such as imidazolium, pyrrolidinium, tetraalkyl phosphonium, trialkyl sulfonium or quaternary ammonium (Fig. 1.13). These cations hinder the regular packing in a crystal lattice. Consequently, the solid crystalline state becomes energetically less favorable, leading to low melting points [495]. This effect enhances further by the implementation of an anion with a delocalized charge, resulting in decreased interionic interactions [496]. Depending on the combination of the cation and the anion, ILs can have either hydrophilic or hydrophobic character. The most frequently investigated ILs are based on imidazolium cations. Anions such as halides, acetate, nitrate and ethylsulfate form hydrophilic ILs, while anions such as bis(trifluoromethylsulfonyl)imide (Tf\(_2\)N\(^{-}\)) and hexafluorophosphate (PF\(_6\)\(^{-}\)) lead to hydrophobic ILs (Fig. 1.13). Greaves and Drummond presented a comprehensive overview about the use of ILs as amphiphilic self-assembly media including micelle and liquid crystalline mesophase formation, there use as a solvent phase in microemulsions and emulsions, and the emerging field of nano structured inorganic materials synthesis [497].

In the field of colloid and interface science, the synthesis of biocompatible ionic liquids is attractive research area now a day, since these ILs have prospect in polymerization to eliminate toxic initiators and solvents, in the preparation of mesoporous biocompatible silica aerogel and biodegradation of dyes and also as nutrients for growing bacteria, etc. [498-500]. Biocompatible ionic liquids have been dealt briefly in a subsequent section.
Fig. 1.13: A schematic representation of different cations and anions in commonly used ILs. Room temperature ionic liquids (RTILs) are important for their convenient physicochemical properties like low vapour pressure, low melting point (< 100°C) [501], non-flammability [502], high conductivity [503], good thermal and chemical stability [504], tunable solubility [505] etc.
6.3. Biocompatible ionic liquids (Bio-ILs)

Initially, many authors have highlighted ILs as a state-of-the-art approach to sustainability [489, 506, 507]. However, recent research studies have demonstrated that some ILs can be toxic [508] and non-biodegradable [509]. The studies of environmental fate and toxicity of ILs have shown that the ILs commonly used to date are toxic in nature and their toxicities vary considerably across organisms and trophic levels. In general, toxicity of ionic liquids depends on both cation and anion. Although the type of anion exerts some impact on the overall toxicity, the effect of the cation alkyl chain length is the most pronounced [492]. Although a large number of ILs have been prepared and characterized to date, most of them were based on halogenated counter anions such as hexafluorophosphate (PF$_6^-$), tetrafluoroborate (BF$_4^-$), bis(trifluoromethanesulfonyl)amide [(CF$_3$SO$_2$)$_2$N$^-$] and their derivatives. Halogens are effective in reducing negative charge density due to their electron withdrawing effect. Although these halide-containing anions are low melting point ILs, their applications are limited by toxicological, ecological and economic issues. An alternative approach which is capable of overcoming these drawbacks is the development of ILs from components having well characterized biodegradable and toxicological properties [510]. One of the strategies to design low toxicity and highly biodegradable ionic liquids is to use cations and anions based on biomolecules, such as those based on natural amino acids [511], lactic acid [512], choline [513, 514] and fructose [515]. Another alternative is to introduce the ether- and hydroxyl-functionalization on cation moiety which typically reduces the toxicity. Also the ester group in the side chain can induce a higher biodegradability [516]. The synthesis, characterization and toxicological assessment of a new group of environmentally benign ionic liquids were reported by Petkovic et al. [517]. Focusing on the toxic effect of the anion, the ionic liquids were designed by combining the benign cholinium cation, [NMe$_3$(CH$_2$CH$_2$OH)]$^+$, with a range of linear alkanoate anions ([C$_n$H$_{2n+1}$CO$_2^-$], $n = 1$-9), as well as two structural isomers ($n = 3$ or 4). The toxicity of these ionic liquids was evaluated using filamentous fungi as model eukaryotic organisms. Surprisingly, most of the tested species showed active growth in media containing extremely high ionic liquid concentrations, up to molar ranges in some cases. The biodegradability of these ionic liquids was assessed, and new biotechnological applications were proposed, e.g., as solvents for biopolymers. Very recently, Isik et al. [518] synthesized cholinium based IL methacrylic monomers having halide, lactate and acetate counter ions and characterized by different techniques. Ionic liquids combining cholinium cation with nonhazardous anions
showed biocompatibility, excellent biodegradability, and low toxicity [519]. In recent years, some reports have demonstrated that nontoxic ionic liquids could be produced by selecting biocompatible organic cations and inorganic anions [520, 521]. Amino acids, as one of the most abundant biomaterials in nature, are known to be nontoxic, biodegradable, and biocompatible. They are excellent feedstocks for the synthesis of ILs, owing to their reasonable cost and environment-friendly characteristics. In 2005, a group of amino acid ionic liquids (AAILs) was first synthesized by Fukumoto’s group from 20 natural amino acids [511]. Since then, many ILs in which the forming anion comes from a natural product are reported by many groups [522-525]. Also, Trivedi et al. [526] synthesized novel highly biodegradable amino acid-based ionic liquid surfactants (AAILs) [viz. (ProC$_3$LS), L-alanine isopropylester lauryl sulphate (AlaC$_3$LS), L-glycine isopropylester lauryl sulphate (GlyC$_3$LS) etc.] with better surface activity than conventional surfactants, and demonstrated their potential application in areas such as the mitigation of harmful algal blooms from sea water and the shape and size-specific synthesis of nanomaterials. Their high solubility in water, biodegradability, and nontoxic features make them attractive for biological and drug-delivery applications. They opined that these kinds of surfactants also have the ability to bind metallic nanoparticles, based on which biomolecular devices may be fabricated. In another study, they also investigated density, speed of sound, refractive index as a function of temperature and aggregation behavior of AAIlS in aqueous solution [527]. Further, Rao et al. [528] reported for the first time formation of unilamellar vesicles and reverse vesicles of a single chain amino acid based IL, ProC$_3$LS in a hydrophilic ethanolammonium formate (EOAF) and hydrophobic 1-butyl-3-methylimidazolium bis-(trifluoromethylsulfonylimide) ([Bmim][Tf$_2$N]) IL medium, respectively. Such vesicular structures, being non-aqueous, nonvolatile, and able to dissolve both hydrophilic and hydrophobic solutes can provide unique benefits in applications of drug delivery, biotransformations, catalysis and material synthesis.

In addition, choline, another green natural product, is an essential micronutrient for normal functioning of all cells. Recently, several cholinium-based ILs had been synthesized and characterized [529-531]. The majority of cholinium based ILs were reported to be readily biodegradable and low toxic, owing to its cholinium cation containing a hydroxyl group [532]. Weaver et al. [531] synthesized five choline phosphates ILs and reported that these cholinium ILs exhibited excellent biocompatibility and biodegradability. Recently, Calvino-Casilda and co-
workers reported on the synthesis of biocompatible cholinium-based AAILs ([Ch][AA]), which were successfully applied as catalysts for the Knoevenagel condensation reaction between benzaldehyde and different active methylene compounds with high conversion and selectivity [533]. A series of ILs were prepared by Fukaya et al. [510] using mainly naturally-derived and inexpensive materials. Hydrogen maleate, propionate, tiglate, and hydrogen succinate anions gave RTILs upon coupling with the choline cation. In particular, choline combined with the hydrogen maleate anion gave an RTIL of moderate viscosity. These ionic liquids (ILs) exhibited higher polarities, especially greater hydrogen bonding abilities, than conventional ILs. In a recent work, five AAILs, cholinium glycinate ([Ch][Gly]), cholinium L-alaninate ([Ch][L-Ala]), cholinium β-alaninate ([Ch][β-Ala]), cholinium prolinate ([Ch][Pro]), and cholinium serinate ([Ch][Ser]), were synthesized from choline hydroxide and amino acids via simple neutralization reactions by Tao et al. [534]. These ILs were characterized by $^1$H NMR, Fourier transform infrared (FT-IR), elemental analysis, thermogravimetry, and differential scanning calorimetry (DSC) analysis. Very recently, synthesis and characterization of two new choline esters, namely hydrogen choline chloride succinate ([CholSucc][Cl]) and hydrogen choline chloride glutarate ([CholGlut][Cl]), were reported by Lopes et al. [535]. Recently, interest in morpholinium-based ILs has increased because of their properties. In fact, they are less toxic than the commonly used imidazolium, pyridinium or tetraalkylammonium-based ILs [536]. In particular, ILs based on the morpholinium cation are preferred because of their electrochemical stability [537]. Mukherjee et al. [538] studied the microbiological activities of ILs comprising alkyltriphenylphosphonium cation with long alkyl chain as surfactants [e.g., triisobutyl(methyl)phosphonium tosylate and trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl)phosphinate] against two bacterial strains Bacillus subtilis (gram positive) and Escherichia coli (gram negative) by measuring the diameter of the inhibition zone (‘diz’). They found that both the ILs exhibited greater antimicrobial activity than that of conventional nonionic surfactant (TX-100).

In order to facilitate the application of choline ionic liquids as environmentally benign solvents, Kunz and his coworkers initiated systematic studies. They synthesized biocompatible choline carboxylates (ChCm, with alkyl chain lengths of m = 12-18) [539, 540] and investigated the biodegradability of ChCm (alkyl chain lengths of m = 12-18) according to the OCDE 301F standard (process of testing of degradation of organic chemicals). Further, the cytotoxicity of ChCm surfactants was determined, both for odd- and even-numbered fatty acids [541]. Studies
were carried out using two different human cell lines, namely cervix carcinoma cells (HeLa) and keratinocytes (SK-Mel-28). For a better comparability to common soaps and to shed light on the influence of the cation, sodium and potassium homologues were also investigated [541]. Further, they reported the aqueous binary phase diagrams and a detailed investigation of the lyotropic liquid crystalline phases formed by choline carboxylate surfactants (ChCm) with chain lengths ranging from \( m = 12–18 \) and at varied surfactant concentrations. They concluded that Choline carboxylate surfactants are powerful alternatives to the well-known classical alkali soaps, since they exhibit substantially increased water solubility while maintaining biocompatibility, in contrast to simple quaternary ammonium ions [542]. Further, Kunz et al. [543] also synthesized the charged room temperature liquid surfactant by combination of choline and other natural cations with and other natural cations and discussed the potential benefits of ionic liquids in microemulsions. Further, they also synthesized a new class of ILs based on combination of simple alkali metal ions (Na\(^+\) or K\(^+\) or Li\(^+\)) and oligoether carboxylates (2,5,8,11-tetraoxatridecane-13-oate, TOTO) as anion [544]. In this report, they also showed that the cytotoxicity of such simple alkali metal carboxylate ILs was very low. In continuation of such study, they also replaced the alkali cations by tetraalkylammonium (TAA) ions and studied the TOTO salts of tetraethyl- (TEA), tetrapropyl- (TPA), and tetrabutylammonium (TBA). In addition, the environmentally benign quaternary ammonium ion choline (Ch) was included in the series [544]. Very recently, Klien et al. [545] synthesized a green surfactant, choline alkylsulfates (ChDS) by the combination of a most classical surfactant anion, dodecylsulfate (DS), with choline (Ch), a natural green cation. Further, cytotoxicity tests on HeLa and SK-Mel 28 cells were presented and compared to other surfactants, showing that ChDS was no more harmful than its sodium counterpart SDS.

6.4. Application of ILs

Recent ionic liquid applications include use in sensors [546] and solar cells [547], as well as thermal fluids [548], analytical techniques [549], lubricants [550], ionogels [551], chemical engineering (separation, extraction and membranes) [552, 553], chemistry (organic synthesis, catalytic reactions, nanomaterial synthesis and polymerization reactions) [489, 554], energy conversion (batteries, fuel cells and heat storage) [555] and biotechnology (biocatalysis, biomolecules purification and biofuels production) [556-560]. The room temperature ionic liquids (RTILs) have now become familiar names among the scientific community for their
various applications ranging from reaction media to pharmaceutical drugs and electrolytes in energy storage cell [561].

Recently, researchers have employed ILs as a component in enzyme immobilization. In these systems, enzymes or proteins are incorporated in ILs that are supported on host matrices such as cellulose [562], chitosan [563], gelatin [564], or silica-gel [565]. ILs themselves can be functionalized by chemical modification of, mainly, their cationic components to obtain “task-specific ILs” [566]. ILs bearing a vinyl group in the cationic component can be directly polymerized by free radical polymerization [567]. Recently, Park et al. [568] fabricated cellulose/charcoal and cellulose/heparin/charcoal composites using ionic liquids to enhance the biocompatibility of charcoal and decrease the size of their active pores. Surface morphological studies of these biocompatible charcoal composites showed that their uniformly coated surfaces could inhibit the adsorption of proteins while allowing the removal of small drug molecules. The activated partial thromboplastin time demonstrated that heparinized surfaces on the cellulose/heparin/charcoal composite had excellent blood compatibility. These charcoal composites can be useful for the rapid and safe removal of small and hydrophobic drugs from the digestive system of overdose patients by circulating blood in an extracorporeal circuit. Further, Sekar et al. [569] used choline-based biocompatible salts as “nutrients” for the growth of *Staphylococcus lentus* bacteria. The N-alkyl-N-methylmorpholinium ionic liquids [i.e., N-alkyl-N-methylmorpholinium (alkyl = ethyl, butyl, hexyl, and octyl) bis(trifluoromethansulfonyl)-imide (Tf$_2$N)] were found to be more cost-effective compared to the commonly used imidazolium and pyrrolidium salts [570], reported to be more structured than the imidazolium ILs [571] and used as electrolytes for fuel cell devices, thermal transfer fluids, and acid-catalyzed reaction media as replacements of conventional solvents [572].

Goto and coworkers [573] demonstrated that enzymes are dramatically activated and stabilized in ILs by chemical modification with comb-shaped poly(ethylene glycol). Further, the review of Moniruzzaman et al. [559] brings together a number of techniques that could be used for activation and stabilization of enzymes in ILs, which are receiving increasing attention as “green” solvents for organic synthesis and catalytic processes. Many enzyme formulations showed exciting potential for carrying out biocatalytic reactions in ILs, suggesting a promising future for modified enzymes in these new non-aqueous solvent systems. The enzyme formulation is particularly important when hydrophilic ILs are used as the reaction media. However, for most
of the formulations, additional studies are required to obtain quantitative and physically robust data to design optimal enzyme formulations for use in IL media. The Diels-Alder reaction between cyclopentadiene and methyl acrylate in ionic liquids ([bmim][BF$_4$], [bmim][BF$_6$], [bmim][PF$_6$], [bmim][SbF$_6$], [bmim][TF$_2$N], ) was studied in detail by Vidisˇ et al. [574]. The effect of contamination of the ionic liquids by common impurities, viz. sodium and chloride ions, and water, on the selectivity has been investigated. The presence of high concentrations of chloride was found to decrease the selectivity. Biodegradable ionic liquids have also been investigated as solvents in synthetic organic reactions [575, 576]. Very recently, Isik et al. [518] proposed the preparation of biocompatible ion gels by photopolymerization of the cholinium lactate methacrylic monomer inside the cholinium lactate IL, which can be used as drug delivery agents, electrochemical transistors for biosensors, and mediums for biocatalysis. On the other hand, the same bio-ionic liquid monomer 2-cholinium lactate methacrylate was used to obtain poly(ionic liquid)-cellulose composites by a fast UV curing process, which are the major component of plant cell walls, the most abundant biopolymer on earth and one of the most exploited natural resources.

The use of ionic liquids to replace organic solvents in biocatalytic processes has gained much attention. Cull et al. [577] used the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim][PF$_6$], for the two-phase biotransformation of 1,3-dicyanobenzene to 3-cyanobenzamide and 3-cyanobenzoic acid using the nitrile hydratase from Rhodococcus R312. This work established ILs as potential alternative to organic solvents for multiphase biotransformations. The first reported enzyme-catalyzed reaction in an IL, performed by Erbeldinger et al. [578] They involved the thermolysin-catalyzed synthesis of Z-aspartame in [bmim][PF$_6$] and showed enzyme activity progressed as seen in conventional organic solvents and the enzyme maintained very high stability in IL.

Very recently, a book covering fundamentals, biotransformations and organocatalysis of ionic liquids has been published [579]. Another book of Kokorin summarized recent applications of ILs in polymer sciences, material chemistry, catalysis, nanotechnology, biotechnology, and electrochemical applications [580].

6.5. Room temperature ionic liquids (RTILs) in microemulsions or reverse micelles

In this short introduction, recent developments in the formation, characterization and application of IL-based self-assembled systems with special emphasize on microemulsions/RMs has been
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dealt briefly. The chemistry of room temperature ionic liquid based microemulsions (RTIL-mEs) is at an incredibly exciting stage of development. No longer are mere curiosities, RTIL-mEs are beginning used as solvents for a wide range of synthetic procedures. The advent of systems that are easy to handle allows those without specialist knowledge in the field to use them for the first time. Ionic liquids have also been used as additives in microemulsion formulation. A number of reactions (e.g., Diels-Alder Reaction, aminolysis, Matsuda-Heck reaction etc.) have been investigated so far to show the potential of the ionic liquid based microemulsions. The retention of catalytic activity in IL-based microemulsions is due to the entrapment of enzyme molecules into aqueous microdroplets formed in w/IL microemulsions, indicating that these IL-based reaction systems provide a protective environment for the enzymes. In recent years, great attention has been paid to IL-based microemulsions due to their potential application prospects in biology, pharmaceutical, catalysis and material synthesis, which have been discussed latter. In addition, ILs are not only promising candidates for the formulation of high temperature stable colloidal systems by an appropriate choice of ingredients, and also low temperature stable microemulsions can be gained, which has been discussed in subsequent section [581]. This is one of the advantages of IL based microemulsions over conventional aqueous microemulsions.

Recent developments in the formulation, characterization and possible applications of IL-based self-assembled systems in general and microemulsion/reverse micelle in particular, have been summarized by different research groups, giving an excellent overview of the relevant literature [505, 582-589]. Recently, studies on the formation and characterization of self-assembled systems (viz. micelles, microemulsions etc.) in presence of different types of ILs (as replacement of surfactant or polar component or oil or additive) have been reported from the viewpoints of academic and applied aspects [590-592].

Amphiphilic ionic liquids that are comprised of a hydrophilic imidazolium head group and a hydrophobic tail are good surfactant candidates for forming micelles and microemulsions. In recent studies, Galgano and El Seoud [593] evaluated the micellar properties of the surface active ionic liquid (SAIL) 1-hexadecyl-3-methylimidazolium chloride, C\textsubscript{16}MeImCl and compared to those of two series of cationic surfactants that carry a pertinent structural moiety; 1-hexadecylpyrindinium chloride, C\textsubscript{16}PyCl (a heterocyclic ring), and benzyl (3-hexadecanoylaminoethyl)dimethylammonium chloride, C\textsubscript{15}AEtBzMe\textsubscript{2}Cl (benzyl moiety and a hydrogen-bond forming amide group). They concluded that the structural versatility of SAILs,
with practically unlimited combinations of counter-ions and substituted imidazolium rings, offers exciting possibilities for applications. The interfacial region composed of SAILs can be “fine-tuned” according to ones needs by a judicious combination of the head-cation (nature of the substituent introduced) and the counter-ion (charge density, hydration) and also involved in various interactions, e.g., electrostatic and hydrophobic.

In comparison with traditional surfactant-based microemulsions, IL-based microemulsions show several advantages. First, the strong attraction between the imidazolium group and counter ion of the ILs at fluid/fluid interface prevents its leakage into the conjugated water solution. Second, the broad imidazolium head group shows a higher capacity for solutes than the tert-ammonium cationic system. Third, the imidazolium head is strongly attractive, via the aromatic ring of penicillin, strong $\pi-\pi$ interaction. Finally, the versatile structures of ionic liquids provide an amplitude platform for exploring the hydrophilic-lipophilic equilibrium in IL-based microemulsions/reverse micelles, which is a key for optimizing the partitioning behaviors in microemulsions. Therefore, long chain ionic liquids (e.g., 1,3-dialkylimidazolium chlorides or bromides or tetrafluoroborates) are reported to be used as surfactants for formulation of the microemulsion systems [505]. Some of these reports are summarized here. The phase behaviors of three long-chained imidazolium ionic liquids, $C_{12}$mimBr (1-dodecyl-3-methylimidazolium bromide), $C_{14}$mimBr (1-tetradecyl-3-methylimidazolium bromide) and $C_{16}$mimBr (1-hexadecyl-3-methylimidazolium bromide), with p-xylene and water were investigated by Li et al. [594]. The formation of non-aqueous microemulsion by using EMIM-EtOSO$_3$ and EMIM-HexOSO$_3$ as polar phase(s) in the presence of toluene as oil component and CTAB as surfactant is possible on heating the mixture up to 80°C. The temperature treatment is necessary due to the better solubility of the surfactant and the ionic liquid in the oil phase. In addition, the isotropic phase range can be enlarged by heating up the mixture to 80°C. Pentanol as co-surfactant was used for tuning the range of microemulsion area. It has a stabilizing effect on the interfacial layer, which results in a widening of the microemulsion area, i.e., the solubilization capacity is increased by the addition of cosurfactant [595]. Safavi et al. [596] formulated a microemulsion comprising imidazolium ILs, 1-octyl-3-methylimidazolium chloride [omim][Cl], as a surfactant, a hydrophobic IL as a substitute for traditional organic solvent, 1-bmimPF$_6$ and water at 25°C. Zech et al. [597] also formulated microemulsions with 1-hexadecyl-3-methyl-imidazolium chloride ([C$_{16}$mim][Cl]) as surfactant, decanol as cosurfactant, dodecane as continuous oil phase
and RTILs (ethylammonium nitrate (EAN) and 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF$_4$]) as polar microenvironment and characterized the systems by employing electrical conductivity, DLS and SAXS studies.

Recently, Lü et al. [598] attributed to the unique role of the ionic liquid (bmimBF$_4$), which is capable of modifying the physicochemical properties of water/AOT/decane (Dc) microemulsions by measuring the critical concentrations and the critical temperatures. Liu et al. [599] showed that the concentration of a model IL, bmimBF$_4$ can act as an effective interfacial control parameter for tuning the formation of microemulsions. Wei et al. [600] also studied water solubilization, volume-induced and temperature-induced percolation of conductance behavior of AOT-based microemulsions in presence of ILs. In another study, they investigated the effects of ionic liquid, [bmim][BF$_4$] on the properties (e.g., polarity, conductivity, viscosity, and droplet size) of nonionic surfactant based water-TX-100-hexanol-cyclohexane microemulsion [601]. Gao and his coworkers studied the effect of water on the microstructure of [bmim][BF$_4$]/TX-100/benzene or triethylamine microemulsion by phase behavior, DLS, UV-Vis, FTIR and $^1$H NMR studies [602, 603]. Rojas et al. [267] investigated the influence of the ionic liquid (ethylmethylimidazolium hexylsulfate) on structure formation in the optically clear phase region in water/toluene/pentanol mixtures in presence of the cationic surfactant CTAB by conductivity, DLS, $^1$H NMR and cryo-SEM techniques. Gao et al. [604] studied the effect of water on [bmim][BF$_4$]/TX-100/benzene microemulsion and it was shown that the addition of small amount of water to the IL microemulsion contributed to the stability of microemulsion and thus increased the amount of solubilized [bmim][BF$_4$] in the microemulsion. In another contribution, they showed that the addition of water decreased the droplet size of [bmim][BF$_4$]-in-cyclohexane microemulsion [202]. The role of small amounts of water in the 1-butyl-3-methylimidazolium bis(2-ethylhexyl) sulfo succinate ([bmim][AOT]) derived [bmim][BF$_4$]-in-benzene reverse microemulsions were also emphasized by Bai et al. [605]. Very recently, Paul and Panda [606] reported the understanding of the physicochemical properties of pseudo ternary microemulsion system comprising aqueous solution of 1-butyl-3-methyl imidazolium methanesulfonate, [bmim][MS], polyoxyethylene (20) sorbitan monolaurate (Tween-20), 1-pentanol (Pn) and n-heptane (Hp) by employing phase study, method of dilution, viscosity, DLS, conductivity and spectroscopic probing techniques. Seth et al. [607, 608] also reported the interaction of water with room temperature ionic liquid (RTIL) [bmim][PF$_6$] in [bmim][PF$_6$]/TX-100/water ternary
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microemulsions by solvent and rotational relaxation of coumarin 153 (C-153), coumarin 151 (C-151) and coumarin 490 (C-490). A review, in the form of a book chapter, on solvent dynamics and rotational relaxation processes in IL-based microemulsions is available in literature [589]. While the studies mentioned above involve water as one of the component, water-free IL-based microemulsions are also studied by many groups [263, 264, 505, 602, 604, 609-617]. In the first report on formation of IL-in-oil microemulsions, Gao et al. [263] prepared [C₄mim][BF₄]/TX-100/cyclohexane microemulsions and characterized them by phase behavior, conductivity, DLS, freeze-fracturing electron microscopy and UV-Vis techniques. Eastoe et al. [610] further investigated size and shape of the same microemulsions by small-angle neutron scattering (SANS) measurement. They observed regular swelling behavior of microemulsions with addition of the IL, which indicates that the volume of dispersed nanodomains is proportional to the amount of IL added [610]. This study was followed by several other reports on similar systems. In these investigations [C₄mim][BF₄], TX-100 and toluene [611], p-xylene [612], or benzene [604] were used as the polar phase, surfactant and oil phase respectively. The water-free IL-based microemulsions with charged surfactants are rather scarcely reported [593, 597, 614, 618, 619]. Falcone et al. [613] used cationic surfactant, benzyl-n-hexadecyldimethylammonium chloride (BHDC) for the formulations of [C₄mim][BF₄]/BHDC/benzene and [C₄mim][TF₂N]/BHDC/benzene microemulsions and compared their behavior with [C₄mim][BF₄]/TX-100/benzene and [C₄mim][TF₂N]/TX-100/benzene microemulsions. With their recent multinuclear NMR study on the behavior of [C₄mim][BF₄] in [C₄mim][BF₄]/BHDC/benzene and [C₄mim][BF₄]/TX-100/benzene microemulsions [618], they concluded that the structure and behavior of entrapped ILs in the microemulsions depend strongly on the surfactants used. Rabe and Koetz [595] investigated the characteristic behavior 1-ethyl-3-methylimidazolium-ethylsulfate (EMIM-EtOSO₃) or 1-ethyl-3-methylimidazolium-hexylsulfate (EMIM-HexOSO₃)/CTAB/pentanol/toluene IL/o microemulsions by conductometric titrations, DLS, rheological measurements, and Cryo-Scanning Electron Microscopy. Recently, Blach et al. [620] investigated the behavior of two ionic liquids (ILs) with high electron donor ability such as 1-butyl-3-methylimidazolium trifluoromethanesulfonate (bmimTfO) and 1-butyl-3-methylimidazolium trifluoroacetate (bmimTfA) entrapped in anionic, AOT/chlorobenzene and cationic, BHDC/chlorobenzene reverse micelles (RMs) using DLS and FT-IR spectroscopy. In their recent study, Kunz and his coworkers [621] reported that use of ionic surfactants in
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combination with ILs is better for preparation of the microemulsions with high temperature stability and temperature insensitivity. They explored thermal stability of EAN/[C<sub>16</sub>mim][Cl]/dodecane microemulsions at ambient pressure, which exhibited stability over the temperature range 30°C to 150°C. Harrar et al. [622] reported a detailed investigation of nonaqueous 1-ethyl-3-methyl-imidazolium-ethylsulfate ([emim][etSO<sub>4</sub>])/limonene/polyethylene glycol tert-octylphenyl ether (Triton X-114 or TX-114) microemulsions as a function of ionic liquid (IL) content and temperature. These systems were characterized by phase diagrams, conductivity measurements, and small angle X-ray scattering (SAXS) techniques. Rojas et al. [623] also formulated N,N'-Alkylimidazolium Alkylsulfate ([Emim][EtSO<sub>4</sub>])/1-butyl-3-methylimidazolium dodecylsulfate ([Bmim][DodSO<sub>4</sub>])/toluene microemulsion and characterized the systems by conductivity measurements, and DLS and SAXS techniques. Sarkar and his coworkers [607] reported the formulation of a novel ionic liquid-in-oil (IL/O) mixed microemulsion where the polar core of the ionic liquid, 1-ethyl-3-methylimidazolium n-butylsulfate ([C<sub>2</sub>mim][C<sub>4</sub>SO<sub>4</sub>]), is stabilized by a mixture of two nontoxic nonionic surfactants, polyoxyethylene sorbitan monoooleate (Tween-80) and sorbitan laurate (Span-20), in a biocompatible oil, isopropyl myristate (IPM). The formation of the microemulsion droplets was confirmed from the dynamic light scattering (DLS) and phase behaviour study. Very recently, Rao et al. [590] presented facile methods to adjust the structural parameters of microemulsions using different hydrophobic ILs ([C<sub>2</sub>mim][Tf<sub>2</sub>N], [C<sub>4</sub>mim][Tf<sub>2</sub>N], [C<sub>6</sub>mim][Tf<sub>2</sub>N], [C<sub>4</sub>mim][PF<sub>6</sub>], and [C<sub>4</sub>mim][BF<sub>4</sub>]) as additives (polar phase). They characterized ILs/[C<sub>4</sub>mim][AOT]/benzene ternary systems by performing phase behavior study, DLS and <sup>1</sup>H NMR measurements. Apart from these reports, Sarkar and his coworkers have published a good number of publications on characterization of nonaqueous ionic liquid containing microemulsions [624].

Microemulsions with magnetic properties were formed by employing a magnetic room temperature ionic liquid, MRTIL, (1-butyl-3-methylimidazolium tetrachloroferrate, [bmim][FeCl<sub>4</sub>]) as polar phase, cyclohexane as oil, and an appropriate mixture of ionic surfactant (SDS, AOT, various C<sub>10</sub>E<sub>j</sub> and C<sub>16</sub>mimCl) and decanol as a cosurfactant, and microstructures of these systems were characterized by SANS and electric conductivity. This system showed structurally the same as that of normal microemulsions but with the magnetic properties added to it by the incorporation of [bmim][FeCl<sub>4</sub>] into the systems formulation [625]. Zech et al. [626]
demonstrated that biodiesel, which gained more and more attention in recent years because of its synthesis from renewable resources, can replace conventional oils as nonpolar phase in nonaqueous microemulsions containing the RTIL, ethylammonium nitrate (EAN) as polar phase. They studied the thermal stability and microstructure of these systems by conductivity, SAXS measurements, in addition to the phase diagram and the viscosity of the microemulsions. They concluded that these high temperature stable, nonaqueous, free of crude oil based organic solvent microemulsions highlight an efficient way towards the formulation of environmentally compatible microemulsions and open a wide field of potential applications.

Recently, two studies have demonstrated the microemulsification of RTIL in compressed or supercritical CO\(_2\) [616, 627]. These new CO\(_2\)-ionic liquid systems might have certain advantages, especially when water sensitive compounds are involved. Recently, Wang et al. [628] reported the successful formulation of microemulsions with vegetable oil (castor oil) as the continuous phase, RTIL 1-butyl-3-methyl-imidazolium tetrafluoroborate as the polar phase, Triton X-100 as the surfactant, and 1-butanol as the cosurfactant. A pseudo-ternary phase diagram, DLS data, UV-vis spectra, and kinematic viscosity measurements were employed to characterize and analyze the phase behavior and microstructure of the vegetable oil-based IL microemulsions. Further, they proposed that this vegetable oil-based IL microemulsion has tremendous potential as renewable biolubricant base-stocks. Li et al. [603] also formulated a novel IL microemulsion, consisting of 1-butyl-3-methylimidazolium tetrafluoroborate (bmimBF\(_4\)) and nonionic surfactant TX-100 in triethylamine which was used either as an organic solvent or a Lewis base. The unique solubilization behavior of water in this system revealed the possibility of using the triethylamine microemulsions as a template to prepare metal hydroxides as well as metal oxides in the microemulsions, which is not possible in case of traditional microemulsions.

Recently, Kunz and his coworkers formulated high temperature stable microemulsions by using the room-temperature ionic liquid ethylammonium nitrate (EAN) as polar phase, dodecane as continuous phase and 1-hexadecyl-3-methyl imidazolium chloride ([C\(_{16}\)mim][Cl]), an IL that exhibits surfactant properties, and decanol as cosurfactant at ambient temperature. They were able to obtain thermal stability ranging from 30\(^{\circ}\)C up to 150\(^{\circ}\)C [621, 629]. According to them, these high temperature microemulsions are predestined for high temperature applications, such as reaction media, lubricant formulations and size controlled nanoparticle synthesis. Apart from
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these, Rao et al. [630] demonstrated the wide range of thermal stability of [C_{6}mim][TF_{2}N]/N,N-
dimethylethanolammonium 1,4-bis(2-ethylhexyl) sulfo succinate (DAAOT)/IPM microemulsions
by performing phase behavior study, DLS measurements, and \textsuperscript{1}H NMR measurements and also
by using coumarin-480 (C-480) as a fluorescent probe molecule. Mukherjee et al. [149] formulated
microemulsions with two alkyltriphenylophosphonium cation based ILs, in which
triisobutyl(methyl)phosphonium tosylate (IL 1) as polar phase, IPM as oil phase, trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate (IL 2) as surfactant and isopropanol as cosurfactant and characterized by phase diagram, dilution method and DLS
technique. These ILs studied against Sarcoma-180 cell lines and evidenced proficient anti-cancer
activity of IL 1 and moderate activity of IL 2. Good temperature stability up to 353 K was
obtained for this microemulsion system.

Harrar et al. [581, 631] presented a detailed investigation of nonaqueous TX-100 or TX-
114/limonene/1-ethyl-3-methylimidazolium ethylsulfate ([emim][etSO_{4}]) microemulsions and
identified the different microstructures. However, the systems containing high amounts of ionic
liquid showed remarkably large temperature stability down to - 10\textdegree C or - 35\textdegree C, respectively.
These newly introduced low-temperature stable microemulsions have prospect of several
applications, for example in extraction processes, formulation of lubricants, or as reaction media
for water-sensible reactions.

First report, on IL-in-IL microemulsions (IL/IL) was reported by Cheng et al. [632] composed of
hydrophobic IL [bmim][PF_{6}] as apolar phase and hydrophilic protic IL, propyl ammonium
formate (PAF) as polar phase stabilized by anionic surfactant, AOT.

6.6. Application of IL-based microemulsions

IL-based microemulsions have been extensively investigated in various fields, for example, drug
delivery, material synthesis, enzymatic reaction, polymerization, biocatalysis, organic synthesis,
protein extraction and capillary electrophoresis. The hot applications of IL-based
microemulsions in the synthesis of nano-materials, biocatalysis, and organic reactions are
summarized [633].

One of the important applications of ionic liquid (IL)-based non-aqueous microemulsions is drug
dissolution, and has been discussed earlier. Drug delivery using such self-assembled systems is
yet to fully achieve its potential for a large number of drugs, which are insoluble or sparingly
soluble in water. These limitations demand the need for alternative solvents that can be used as a
polar core in microemulsions for the solubilization of sparingly soluble drug molecules. Considering these aspects, ILs could be a good candidate because they are capable of dissolution of various poorly soluble compounds including drug molecules [487]. Pharmaceutical industries have posed challenges in the topical and transdermal administration of drugs which are poorly soluble or insoluble in water and most of organic solvents. In an approach to overcome these limitations, ionic liquid-in-oil (IL/o) microemulsions (mEs) were synthesized and characterized by Moniruzzaman et al. [634] to increase the solubility of a sparingly soluble drug and to enhance its topical and transdermal delivery. The formulation of microemulsions was composed of a blend of nonionic surfactants, Tween-80 and Span-20, IPM as an oil phase, and IL [C_{1}mim] [(CH_{3}O)_{2}PO_{2}] (dimethylimidazolium dimethylphosphate) as a pseudo phase. Among various weight ratios of Tween-80 to Span-20 investigated in the microemulsion systems, the ratio 3:2 showed excellent solubility and skin permeation enhancing effect for acyclovir (ACV), which was used as a model sparingly soluble drug. Taken together these results, they assumed that IL-assisted nonaqueous microemulsion could serve as a versatile and efficient nano-delivery system for insoluble or sparingly soluble drug molecules that require solubilizing agents for delivery. Microemulsions with their high solubility of ACV, excellent formulation stability and enhanced permeation to the Yucatan micropig (YMP) porcine skin were obtained from a blend of surfactant composed of 3:2 weight ratio of Tween-80 to Span-20. Finally, it was concluded that IL/o microemulsions create a new opportunity for the controlled delivery of sparingly soluble drugs that have problems of administration by other routes. In continuation, Moniruzzaman et al. [635] reported a novel IL-in-oil (IL/o) microemulsion which was able to dissolve pharmaceuticals that are insoluble or sparingly soluble in water as well as most of pharmaceutical grade organic liquids. Towards this approach, the nanometer-sized ionic liquid droplets in IPM were formed with a blend of nonionic surfactants, Tween-80, and Span-20. In order to explore the use of newly developed microemulsion as a potential drug carrier, they investigated the solubility of some drug molecules (e.g., acyclovir, methotrexate and 1-[(5-(p-nitrophenyl) furfurylidene) amino] hydantoin sodium) that are insoluble or sparingly soluble in most of the conventional solvents. In another study, Moniruzzaman et al. [636] reported solubility studies indicating a high degree of solubilization of such drugs in IL based mixed Tween-80/Span-20 microemulsions. They concluded that this microemulsion formed with ILs
having unique physical, chemical and biological properties, which may offer novel opportunities to develop a potential drug delivery carrier for poorly soluble drugs molecules, acyclovir.

In another study, Moniruzzaman et al. [560] explored the use of water-in-ionic liquid microemulsions composed of anionic surfactant, AOT/hydrophobic IL [C₈mim][Tf₂N] (1-octyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)amide)/water/1-hexanol as the reaction medium for the enzymatic oxidation of pyrogallol catalyzed by horseradish peroxidase (HRP). The results demonstrated that the rate of HRP-catalyzed reactions in IL microemulsions increased significantly compared with that obtained in conventional oil microemulsions. Therefore, they concluded that a water-in-ionic liquid microemulsion may be a very promising system for performing enzymatic reactions with HRP in ILs media. According to them, the findings will be of value for the development of ILs as a medium for the HRP-catalyzed oxidation of a variety of organic compounds such as phenols, biphenols, anilines, benzidines, and related heteroaromatic compounds. Further, they formulated aqueous microemulsion droplets in a hydrophobic ionic liquid 1-octyl-3-methyl imidazolium bis (trifluoromethyl sulfonyl) amide), [C₈mim][Tf₂N] stabilized by a layer of anionic surfactant; AOT in the presence of 1-hexanol as a cosurfactant to overcome the insolubility of enzymes in most of the ILs. Further, they reported that the catalytic activity of one of the enzymes (Pseudomonas cepacia lipase or lipase PS) became higher in this medium compared to AOT derived microemulsions in isooctane [637]. Das et al. [638] reported the significant enhancement in performance of interfacially active enzymes, Chromobacterium viscosum (CV) lipase and horseradish peroxidase (HRP) in mixed RMs of CTAB and imidazolium-based amphiphiles having varying alkyl chains. Zhou et al. [639] studied the catalytic activities of two fungal oxidases, lignin peroxidase and laccase for the oxidation of o-phenylenediamine, in the TX-100 stabilized water-in-[bmim][PF₆] microemulsion. Both of these fungal oxidases were catalytically active in this medium. The laccase-catalyzed oxidation of 2,6-dimethoxyphenol in the water-in-[bmim][PF₆] microemulsion exhibited a catalytic activity which could be regulated by the composition of the interfacial membrane [640].

Zheng and coworkers [641] synthesized the hollow silica spheres in benzene/TX-100/[bmim][BF₄] microemulsion. For comparison, a traditional aqueous microemulsion system was used to replace the nonaqueous IL template, and no hollow silica spheres were formed. This indicates that [bmim][BF₄] plays an important role in the formation of the hollow structure of
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silica spheres. Furthermore, Zhao and coworkers [642] synthesized silica products with two different morphologies using nonaqueous TX-100/benzene/[bmim][BF$_4$] microemulsion droplets as templates. By adjusting the reaction conditions, ellipsoidal nanoparticles were formed under acidic conditions, while hollow silica spheres were obtained under alkaline conditions. Li et al. [643] used the water-in-IL microemulsion to prepare the tetragonal ZrO$_2$ nanoparticles. For comparison, ZrO$_2$ particles synthesized by water-in-oil microemulsion were assembled after calcinations. The results indicated that the IL, [bmim][PF$_6$] plays an important role in preventing the congregation of the product. Very recently, Zhang and coworkers [644] synthesized the bimetallic palladium/gold nanoparticles in the IL-based microemulsion. The palladium and gold precursors were dissolved in dispersive and continuous phase of microemulsion (H$_2$O/TX-100/[bmim][PF$_6$]), respectively. Harada and his coworkers [554, 645] also synthesized metal particles of silver (Ag) by the photoreduction of silver nitrate (AgNO$_3$) or silver perchlorate (AgClO$_4$) in water-in-ionic liquid (IL) microemulsions consisting of nonionic surfactant Tween-20 or Triton X-100, water and ionic liquid, 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIm][PF$_6$]) or -butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF$_4$]) or 1-octyl-3-methylimidazolium tetrafluoroborate ([OMIm][BF$_4$]). In another study, they synthesized Ag particles in water/Tween-20/[BMIm][BF$_4$] or [OMIm][BF$_4$] microemulsions mixed with a high-pressure (25 MPa) CO$_2$. They concluded that the water droplet size under high-pressure of CO$_2$ was more effectively regulated in the process of Ag particle formation than that under ambient air, and thereby preventing Ag particles from aggregation and precipitation [646].

There are some limits on conducting polymerization reaction in conventional microemulsions, for example, the solubility limitation for apolar solutes remains. This limitation can be overcome by IL-based microemulsion due to the combined advantages of both ILs and microemulsions. Surfactants based on imidazolium ILs, including polymerizable surfactant ILs, were synthesized by Yan and Texter [494]. These ILs were used to stabilize polymerizable microemulsions, which are useful for producing polymer nanoparticles, gels, and open-cell porous materials. Wang and coworkers [647] conducted the copolymerization of styrene and acrylonitrile in IL microemulsion under reverse atom transfer radical polymerization using 2,2'-azobis(isobutyronitrile) (AIBN) as initiator, FeCl$_3$·6H$_2$O/succinic acid (SA) as complex catalyst, cetytrimethylammonium bromide (CTAB) as surfactant and [bmim][PF$_6$] as IL. The copolymers of styrene and acrylonitrile (SAN) with predetermined molecular weights and narrow molecular
weight distribution were obtained. Chen et al. [648] used methyl methacrylate (MMA)/1-dodeyl-3-methylimidazolium bromide/[bmim][BF_4] microemulsions for free radical and atom transfer radical polymerizations. Polymers with reproducible size, well controlled molecular weight and low polydispersity were produced. After the polymerization process, the remaining components were recycled and reused. Zhang and coworkers [649] studied the electro-polymerization behavior of pyrrole in water-in-[bmim][PF_6], bicontinuous, [bmim][PF_6]-in-water microemulsions. They reported that the water-in-[bmim][PF_6] microemulsion represents as an optimal medium with fastest-polymerization rate. Guo and coworkers [650] synthesized the PANI core decorated with TiO_2 (PANI/TiO_2) nanocomposite particles in a microemulsion consisting of deionized water as the continuous aqueous phase and an oil solution (aniline) and hydrophobic IL, [bmim][PF_6] as the dispersed phase in the presence of anatase TiO_2 nanoparticles. Further, Yan et al. [651] reported the formation of proton conducting membranes by surfactant stabilized protic IL (PIL) nanodomains dispersed in polymerizable oil, a mixture of styrene and acrylonitrile.

In a series of studies, Huang and his coworkers [639, 640, 652] formulated and characterized single TX-100 and mixed AOT/TX-100 water-in-IL microemulsions stabilized in 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]) and subsequently, investigated lipase catalyzed hydrolysis of lignin peroxidase (LiP), biomacromolecule laccase, and 4-nitrophenyl butyrate (p-NPB) in these systems. Cheng and coworkers [653] used IL-in-water microemulsion as a pseudostationary phase (PSP) for the detection of a large number of acidic compounds in capillary electrophoresis (CE). A fast and an efficient separation of eight phenolic acids was achieved by using [bmim][PF_6] as oil drops, Tween-20 as the surfactant, and borate as the background electrolyte (BGE). The detection efficiency of the analytes by using IL-in-water microemulsion is much higher than that by using oil-in-water microemulsion eletrokinetic chromatography (MEEKC).

The Diels-Alder Reaction (DAR) between N-ethylmaleimide and 2,3-dimethyl-1,3-butadiene was studied in water/AOT/isooctane microemulsion in presence of 1-butyl-3-methylimidazolium tetrafluoroborate (IL) [654]. The apparent second-order rate constants were determined from spectrophotometric measurements. The effect of solvent on the rate constants was investigated and interpreted. The experimental results showed that the reaction rate in the microemulsion with IL was enhanced and it was faster compared to pure isooctane and generic AOT microemulsion.
as well. Selective extraction of hemoglobin was achieved by using water/AOT/[bmim][PF₆] microemulsion system from complex biological sample matrices [655]. García-Ríos and his coworkers analyzed the properties of the interface of IL-in-oil microemulsions by absorption solvatochromic shifts, $^1$H NMR and kinetic measurements in order to investigate the properties of the IL within the restricted geometry provided by microemulsions and the interactions of the IL with the interface. They performed a quantitative kinetic study in IL based microemulsions and found that the rate constant for aminolysis of nitrophenyl laurate at the interface of [bmim][BF$_4$]/TX100/cyclohexane IL-in-oil microemulsions is between two and four times higher than in water/TX100/cyclohexane w/o microemulsions [656]. Gayet and coworkers [657] established a IL-in-oil microemulsion system consisting of benzylpyridinium bis(trifluoromethanesulfonyl)imide ([BnPyr]NTf$_2$), TX-100 and toluene, in which the Matsuda-Heck reaction between metoxybenzene diazotate and 2,3-dihydrofuran took place. The study showed that the reaction yield in IL-in-oil microemulsion was higher (almost doubled) than that in neat ILs. Consequently, after the formation of IL microemulsion, not only the reaction efficiency was significantly improved, but also the consumption of IL was reduced. The results provided a basis for designing nonaqueous IL microemulsion microreactors, and also showed that this reaction medium might have a good prospect in the applications relating to biocatalysis and nanomaterials synthesis. Zhang et al. [658] also prepared Pd nanoparticles in situ in the H$_2$O/TX-100/[bmim][PF$_6$] microemulsion. Surfactant TX-100 served as reductant as well as stabilizer of the nanoparticles. It was reported that H$_2$O/TX-100/[bmim][PF$_6$] microemulsion containing Pd nanoparticles represents a very efficient catalyst system for the ligand-free Heck reaction. Xia et al. [659] studied first time an environmentally-friendly, enzyme-promoted procedure for the Henry (nitroaldol) reaction, which is considered one of the most powerful and atom economical C–C bond-formation reactions and is widely employed in organic chemistry using water/TX-100/[Bmim][PF$_6$] microemulsions as reaction medium. The report revealed that amino acylase from Aspergillus oryzae showed better catalytic activity for the addition reaction of nitromethane with a series of aromatic aldehydes, and a highest yield of 90% was obtained.
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