Chapter 2


2.1 Organized Surfactant Assemblies:

2.1.1 Surfactants:

2.1.1.1 What are surfactants?

Surfactants are those compounds that lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. The term surfactant is a blend of surface active agent. Surfactants are usually organic compounds that are amphiphilic, meaning that they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Surfactants will migrate to the water surface, where the insoluble hydrophobic group may extend out of the bulk water phase, either into the air or (if water is mixed with oil) into the oil phase, while the water soluble head group remains in the water phase. This alignment and aggregation of surfactant molecules at the surface serves to alter the surface properties of water at the water/air or water/oil interface. Upon introduction of surfactants (or any surface active material) into the system, they will initially partition into the interface, reducing the system free energy by:

1. Lowering the energy of the interface (calculated as area times surface tension) and

2. Removing the hydrophobic parts of the surfactant from contact with water.

Subsequently, when the surface coverage by the surfactants increases and the surface free energy (surface tension) decreases, the surfactants start aggregating into micelles, thus again decreasing the system's free energy by decreasing the contact area of hydrophobic parts of the surfactant with water. In the ideal case upon reaching CMC, any further addition of surfactants will just increase the number of micelles. A balance between hydrophilicity and hydrophobicity exists in surfactant molecules. This depends on the molecular structure and type. This is called the hydrophilic–lipophilic balance (HLB). Surfactants having greater hydrophobicity are more surface-active and vice versa. With increasing hydrophobicity in a homologous series, micelle formation becomes easier. Thus, HLB is one of the fundamental properties of surfactants, especially of nonionic surfactants, in relation to their self-association. HLB can be estimated from the chemical formula of surfactants. Hydrophilic surfactants which have high water solubility are good stabilizers for oil-in-water (o/w) emulsions and also have higher HLB values. Those having low water solubility have lower HLB and they are good stabilizers for water-in-oil (w/o) emulsions.
2.1.1.2 Classification of surfactants:

Surfactants can be classified in three different ways.

[A] According to the composition of the hydrophobic tail:

The tail of surfactants can be:

- **A hydrocarbon chain:** aromatic hydrocarbons, alkanes, alkenes, cycloalkanes, etc.
- **An alkyl ether chain:** Ethoxylated surfactants such as polyethylene oxides; ethoxy groups are inserted to increase the hydrophilic character of a surfactant. Another example of this category are propoxylated surfactants. Polypropylene oxides are inserted to increase the lipophilic character of a surfactant.
- **Fluorosurfactants:** these surfactants have a fluorocarbon chain.

[B] According to the composition of the head group:

Surfactants are classified according to the composition of their head group as nonionic, anionic, cationic, and zwitterionic. (Fig. 2.2)
Non-ionic surfactants:

These surfactants do not have any electrical charge. They are excellent grease removers that are used in laundry products, household cleaners and hand dishwashing liquids. Ethers of fatty alcohols are the most commonly used non-ionic surfactants. Non-ionic surfactants have either a polyether or a polyhydroxyl unit as the polar head group (Fig. 2.3). The characteristic feature of this group is a polyoxyethylene chain. The polyoxyethylene moiety is made by polymerization of ethylene oxide. Non-ionic surfactants can be derived from different sources like

(a) Alcohols- Fatty alcohols, Cetyl alcohol, Stearyl alcohol, Oleyl alcohol; (b) Ethers- Polyoxyethylene glycol alkyl ethers (Brij), Polyoxypolypropylene glycol alkyl ethers; (c) Glucoside ester- Glucoside alkyl ethers, Decyl glucoside, Lauryl glucoside, Octyl glucoside, polyoxyethylene glycol octylphenol ethers, (Triton X-100); (d) Esters- Glycerol alkyl esters, Polyoxyethylene glycol sorbitan alkyl esters, Polysorbates (Tweens), Sorbitan alkyl esters (Spans).

Fig. 2.3 Structure of a Non-ionic surfactant

Anionic surfactants:

These are the most widely used type of surfactants having negatively charged head groups. They are used for laundering, dishwashing liquids and in shampoos due to their excellent cleaning properties. The most commonly used anionic surfactants are based on permanent anions (sulfate, sulfonate, phosphate) or pH-dependent anions (carboxylate). For example:

(a) Sulfates- alkyl sulphate, ammonium lauryl sulfate, sodium lauryl sulfate (SLS) or sodium dodecyl sulphate (SDS);
(b) Sulfonates- dioctyl sodium sulfosuccinate (AOT) (Fig. 2.4), alkyl benzene sulfonates;
(c) Phosphates- alkyl aryl ether phosphates, alkyl ether phosphates,
(d) Carboxylates- alkyl carboxylates, fatty acid salts (soaps), sodium stearate, carboxylate fluorosurfactants, perfluorooctanoate (PFOA or PFO).
Cationic surfactants:

The cationic surfactants (having positively charged head groups in solution) adsorb strongly to surfaces as majority of surfaces carry a net negative charge. There are different types of cationic surfactants based on pH-dependent primary, secondary or tertiary amines. Primary amines become positively charged at pH < 10, secondary amines become charged at pH < 4. Few examples are alkyltrimethylammonium salts like cetyltrimethylammonium bromide (CTAB) (Fig. 2.5), cetyl trimethyl ammonium chloride (CTAC), cetlypyridinium chloride (CPC), dioctadecyldimethylammonium bromide (DODAB).

Amphoteric surfactants:

Amphoteric surfactants may contain two charged groups of different sign. Whereas the positive charge is almost always ammonium, the source of the negative charge may vary (carboxylate, sulphate, sulphonate). Examples of Zwitterionic surfactants are CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), Sultaines, polyalkyl glucosides (Fig. 2.6), carboxylates, amino acids, Imino acids, Betaines, Phosphates and lecithin. Phospholipids, the most abundant of all the amphiphiles in our body, are examples of zwitterionics.
According to the composition of the surfactant counter-ion:

Counterions of surfactants can be monoatomic (inorganic) e.g. alkali metal, alkaline earth metal, transition metal, chloride (Cl\(^-\)), bromide (Br\(^-\)), iodide (I\(^-\)) or polyatomic (organic) e.g. pyridinium, triethanolamine (TEA).

2.1.1.3 Micellization of surfactants:

Surfactants associate to form micelles above a certain critical concentration known as the critical micellar concentration (CMC).\(^5\)\(^\text{–}^7\) Micellization has been treated either as a stepwise association phenomenon or as a phase transition process.\(^8\)\(^,\)\(^9\) The critical micellar concentration (CMC) is the concentration above which any added surfactant molecules appear with high probability as micellar aggregates (Fig. 2.7).

The Krafft temperature is known as Krafft point or critical micelle temperature.\(^5\) This is the minimum temperature at which surfactants form micelles. Below the Krafft temperature, there is no value for the critical micellar concentration (CMC) i.e. micelles cannot form. Various methods for determination of CMC are discussed below.

- **Determination of CMC by Conductometric method:**

Below the CMC, the addition of surfactant to an aqueous solution causes an increase in the number of charge carriers and consequently, an increase in the conductivity. Above the CMC, further addition of surfactant increases the micelle concentration while the monomer
concentration remains approximately constant (at the CMC level). Since a micelle is much larger than a surfactant monomer it diffuses more slowly through solution and so is a less efficient charge carrier. A plot (Fig. 2.8) of conductivity against surfactant concentration is thus expected to show a break at the CMC.¹⁰

![Conductivity plot for CMC determination](image)

**Fig. 2.8** Conductivity plot for CMC determination

- **Determination of CMC by Tensiometric method:**

  Before the CMC, the surface tension changes strongly with the concentration of the surfactant. After the CMC, the surface tension remains relatively constant or changes with a lower slope. Amphiphile adsorption at the air/water interface lowers the surface tension (γ) of the solution. After a critical surfactant concentration (C), γ becomes constant (with a break in the γ versus log C plot) (Fig. 2.9), this is the CMC. At very low amphiphile concentration, γ decreases sharply and often nonlinearly by a co-operative adsorption process till a plateau is reached at CMC.

![Tensiometric plot for CMC determination](image)

**Fig. 2.9** Tensiometric plot for CMC determination

**2.1.2 Surfactant-based Organized Assemblies:**

An organized medium refers to a microscopic region within a micro heterogeneous system in which some properties such as polarity and viscosity differ from bulk media due to
restricted motion of the molecules in such media. Self-organization of molecules usually happens through hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions and electrostatic effects. Hence it is evident that in the formation of such self-assemblies, instead of covalent bonding, weaker and reversible non-covalent interactions are involved. Self-organization or formation of molecular self-assemblies leads to an increase in internal organization of a system. A few examples of such self-organized media are—(i) micelles, (ii) reverse micelles, (iii) self-assembled monolayers, (iv) bilayers, (iv) liposomes, (v) lipids, (vi) folded protein structures, (vii) protein-bile salt aggregates, (viii) bile salt gels, (ix) liquid crystals, (x) cyclodextrins, (x) nanoparticles etc.\textsuperscript{11}

These media are useful for performing chemical reactions since they exhibit high solubilization capacities and usually give rise to reactivity and selectivity enhancement. Vast applications of organized media can be found in modern science. An amphiphile (surfactant) spread on water can lead to the formation of different aggregates—vesicles, micelles, emulsions or microemulsions, depending on its concentration, its molecular structure and the experimental conditions. Such aggregates—(a) may concentrate products, reactants or analytes and so improve the analytical sensitivity\textsuperscript{12} and (b) may solubilize substances and so favorably change the analytical selectivity. Bilayer membrane vesicles for instance, apart from their wide applications in cosmetic and pharmaceutical industries, have a great analytical potential due to their ability to reversibly sequester metal ions avoiding matrix interference.\textsuperscript{13} Micellar solutions have also found wide applications in different areas of analytical chemistry, showing their capacity to concentrate and separate a significant variety of analytes. Organized media, such as surfactant micelles and cyclodextrins, provide increased selectivity and lower detection limits in the spectrofluorimetric analyses of many compounds. Among the numerous micelle-based separation techniques, cloud point extraction offers an excellent enrichment factor for metal ions, allowing their quantification at the microgram/litre level.\textsuperscript{14}

Surfactant-based organized systems like microemulsions are single-phase systems, although they contain both polar and non-polar domains in which reactants of different solubility can dissolve. Such systems are able to dissolve both organic and inorganic components, which will meet at the interface where the reaction can take place. The large internal interface of these systems significantly increases the probability of contact between incompatible reagents as compared to the situation in macroscopic two phase systems. The improved contact leads to increased reactivity.

Organized media such as micelles and reverse micelles are the focus of study in contemporary research for performing highly selective and very fast reactions. Organic reactivity in micro-organized media is illustrated by results on catalysis in micelles and on the properties of water in reverse micelles. With cationic micelles, interesting catalytic effects on reactions between hydrophobic and hydrophilic reagents are obtained when the hydrophilic partner alone goes into the Stern layer by shifting the counter-ions of the surfactant.\textsuperscript{15} Self-organized surfactant systems such as microemulsions, vesicular solutions, dispersions or lyotropic mesophases can serve as templates for the structure-directed synthesis of organic polymers.\textsuperscript{16}
2.1.2.1 Micelles and mixed micelles:

A micelle\(^{17}\) is an aggregate of surfactant molecules dispersed in a liquid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic single-tail regions in the micellar centre (Fig. 2.10). This type of micelle is known as a normal micelle. Micelles are approximately spherical in shape. Other shapes such as ellipsoids and cylinders are also possible. The shape and size of a micelle is a function of the molecular geometry of the surfactant molecules and solution conditions such as surfactant concentration, temperature, pH and ionic strength. The process of forming micelles is known as micellization which has been discussed in section 2.1.1.3. Micelles composed of ionic surfactants have an electrostatic attraction to the ions that surround them in solution, the latter are known as counterions.\(^{18}\) Micelles only form when the concentration of surfactant is greater than the critical micelle concentration (CMC) and the temperature of the system is greater than the critical temperature or Krafft temperature.

![Micelle Diagram](image)

**Fig. 2.10** Schematic representation of micelle formation

When surfactants are present above the CMC they can act as emulsifiers that will allow a compound that is normally insoluble (in the solvent being used) to dissolve. This occurs because the insoluble species can be incorporated into the micelle core, which is itself solubilized in the bulk solvent by virtue of the favorable interactions of the surfactant head group with the solvent species. The most common example of this phenomenon are detergents, which clean poorly soluble lipophilic material (such as oils and waxes) that cannot be removed by water alone. Detergents also clean by lowering the surface tension of water making it easier to remove material from a surface. The emulsifying property of surfactants is also the basis for emulsion polymerization. Micelle formation is essential for the absorption of fat-soluble vitamins and complicated lipids within the human body. Bile salts formed in the liver and secreted by the gall bladder form micelles. This allows the absorption of complicated lipids (lecithin) and lipid soluble vitamins (A, D, E and K) within the micelle by the small intestine.
In higher animals lipid molecules are largely water-insoluble, but must be digested, absorbed, transported and secreted in an aqueous medium. One of the means by which these phenomena may occur is by the association of insoluble lipids with soluble lipids in small molecular aggregates called “mixed micelles (Fig. 2.11). Mixed micelles are divided into two categories depending on whether the micelle-forming component (soluble lipid) is an aliphatic detergent (soaps etc.) or a complex aromatic detergent (bile salts etc.). The amount of insoluble lipid solubilized by either of the two types of detergents varies markedly with the type of insoluble lipid.

Fig. 2.11 Structure of mixed micelles

The very marked ability of the natural detergent bile salt to disperse and solubilize lipids such as phospholipids, monoglycerides and “acid soaps” is physiologically important as these lipids are the main insoluble lipids found in bile and in intestinal contents during fat digestion.

2.1.2.2 Reverse Micelles and Microemulsions:

In a non-polar solvent, it is the exposure of the hydrophilic head groups to the surrounding solvent that is energetically unfavourable, giving rise to a water-in-oil system. In this case the hydrophilic groups are sequestered in the micelle core and the hydrophobic groups extend away from the centre (Fig. 2.12). These “inverse micelles” are less likely to form on increasing headgroup charge, since hydrophilic sequestration would create highly unfavourable electrostatic interactions.

Certain surfactants, such as the widely used anionic surfactant sodium bis (2-ethylhexyl) sulfosuccinate (commonly known as AOT) (Fig. 2.4), dissolved in organic solvents can form spherical aggregates called “inverse” or “reverse” micelles. AOT reverse micelles are thermodynamically stable aggregates of the amphiphilic surfactant, resulting in a hydrophilic headgroup region with hydrophobic tails that extend into a nonpolar continuous phase. These reverse micelles have nanometer-sized water cores stabilized by a curved monolayer of the surfactant (Fig. 2.12b). This arrangement allows a variety of polar and ionic species to be dissolved into the water cores. The core is described by the molar ratio of water to surfactant molecules in the solution, \( w_0 = ([H_2O]/[AOT]) \). Besides AOT, TX-100 and the non-ionic surfactant poly (oxy ethylene) nonyl phenyl ether are also known to form reverse
micelles in organic solvent. Some ionic liquids such as 1-butyl, 3-methyl-imidazolium hexafluoro phosphate have also been used to prepare microemulsions. The chemistry of the reverse micellar water pool has drawn the interest of several scientists for its very interesting properties like micropolarity, microproticity, microviscosity and especially its heterogeneous structure which differs widely from that of the bulk water. Several techniques such as NMR and FTIR, as well as molecular dynamics simulations support the existence of discrete water environments based on their distance from the surfactant interface. In fact, three different types of water have been proposed to exist within these systems- (i) water strongly bound to the surfactant, (ii) free water and (iii) water with intermediate characteristics near the interface.

Reverse micelles have also been used as micro reactors to produce well-defined nanosized crystallites or chemically modified enzymes. Discrete nanoparticles with controlled chemical composition and size distribution are readily synthesized using reverse micelles as confined reaction media. The interfacial activity of reverse micelles can be exploited to couple nanoparticle synthesis and self-assembly over a range of length scales to produce materials with complex organisation arising from the interdigitation of surfactant molecules attached to specific nanoparticle crystal faces. Reverse micelles are often used in homogeneous catalysis, paint drying, adhesion enhancement and also as catalysts for various organic reactions.

![Fig. 2.12 Schematic view of a reverse micelle, A: Inner polar core; B: Interfacial region; C: outer nonpolar solvent](image)

When water, oil and a surfactant are mixed, the surfactant rests at the water-oil interface. These systems depending on their stability are called emulsions or microemulsions. Although the properties of emulsions and a microemulsions are different, both obey the same principle. They try to form enough interface for preventing the polar/non-polar solvent contact. Micro-emulsions are microstructured, thermodynamically stable isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant (Fig. 2.13). The sizes of these structures are in the range of a few hundreds of nanometers. The aqueous phase may contain salt and/or other ingredients and the oil may actually be a complex mixture of different hydrocarbons and olefins. Micro-emulsions may be in two
types: (a) oil in water (o/w) micro-emulsion and (b) water in oil (w/o) micro-emulsion (Fig. 2.13).

![Image: Water-in-Oil and Oil-in-Water microemulsion](image)

Fig. 2.13 Water-in-Oil and Oil-in-Water microemulsion

2.1.2.3 Vesicles:

Most molecules that form bilayer vesicles in water are amphiphilic: they have a hydrophobic and a hydrophilic part. The hydrophilic part (‘‘head group’’) of the molecule interacts favourably with the surrounding water while the hydrophobic part (‘‘tail’’) minimizes its exposure to water. Hence, the formation of bilayer vesicles is driven mainly by hydrophobic interaction. Typically, the head group is polar and/or charged and may contain phosphate, sulfate, ammonium, amino acid, peptide and carbohydrate groups. The ‘‘tail’’ is nonpolar and uncharged. The tail is usually composed of long hydrocarbon chains, which may be saturated or unsaturated, linear, cyclic or branched, aromatic or aliphatic. Overall, the molecule must have a cylindrical shape, so that the molecules arrange into a bilayer, which may be slightly curved so that it can close into a spherical vesicle.

If the ‘‘head’’ is substantially bigger than the ‘‘tail’’, the amphiphiles will tend to form micelles not vesicles.\(^{32}\) If on the other hand the ‘‘tail’’ is larger than the ‘‘head’’, the amphiphiles will assemble into ‘‘inverted’’ phases. With a few notable exceptions (such as vesicles composed of polymeric amphiphiles or equimolar mixtures of cationic and anionic amphiphiles), vesicles are metastable in aqueous solution and energy is required to dissolve the amphiphiles in water and induce the formation of vesicles. The energy required to obtain vesicles is usually provided by heat, rapid stirring, sonication, extrusion or a combination of these. Vesicles can be differentiated according to their size. Small unilamellar vesicles (SUVs, size upto100 nm) contain about ten thousand molecules. Large unilamellar vesicles (LUVs, 100–1000 nm) contain about a hundred thousand molecules. Giant unilamellar vesicles (GUVs) and multilamellar vesicles (MLVs) contain millions of molecules. MLVs have an onionlike structure \(^{32}\) (Fig. 2.14) and consist of many bilayer membranes which can be converted to a unilamellar structure by sonication or extrusion. Vesicles are flexible and dynamic colloidal structures. Vesicles display Brownian motion in solution and the diffusion rate of a vesicle correlates inversely with its size. The membrane of a vesicle is often flexible so that the vesicle can easily change shape.

In cell biology, a vesicle is a small bubble within a cell and thus a type of organelle. Enclosed by lipid bilayers, vesicles can form naturally for example during endocytosis. If
there is only one phospholipid bilayer, they are called unilamellar vesicles otherwise they are called multilamellar. The membrane enclosing the vesicle is similar to that of the plasma membrane and vesicles can fuse with the plasma membrane to release their contents outside the cell. Vesicles can also fuse with other organelles within the cell.

In basic research, vesicles serve as models for cell membranes. They also serve as delivery vehicles for drugs, genetic material, enzymes and other molecules into living cells and through other hydrophobic barriers. They are also used as a support for semiconductor particles in applications such as in the photoconversion of solar energy.

![Fig. 2.14 Schematic diagram of small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV) and multilamellar vesicles (MLV)](image)

### 2.1.2.4 Liposomes:

Liposomes are nano size artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol. The name liposome is derived from two Greek words 'Lipid' meaning fat and ‘Soma’ meaning body. Bangham discovered that phospholipids combined with water, immediately form a bi-layered sphere because one end of each molecule is water soluble, while the opposite end is water insoluble. Liposomes usually contain an aqueous core or in other words an inner aqueous compartment enclosed by a lipid bilayer (Fig. 2.15). Liposomes are formed when thin lipid films or lipid cakes are hydrated by sonication and stacks of liquid crystalline bilayers become fluid and swell. During agitation, hydrated lipid sheets detach and self associate to form vesicles. Sonication is generally considered a "gross" method of preparation and newer methods such as extrusion are employed to produce materials for human use. The physicochemical characteristics of the liposomes like particle size, lamellarity, surface charge, sensitivity to pH changes and bilayer rigidity can be manipulated. Depending on the choice of lipid and preparation techniques, liposomes can vary widely in size. An important parameter in the formation of liposomes is the rigidity of bilayer. The hydrated phospholipid bilayers can be in a “fluid” (liquid crystalline) state or in a gel state. The gel bilayers melt at the transition temperature (T<sub>c</sub>) and get converted to the “fluid” state. For the formation of liposomes, the “fluid” state is preferred. Liposomes can be characterized by electron microscopy, dynamic light scattering and fluorescence polarization techniques. Liposomes show promising results in drug delivery but their applicability is limited primarily because of their short half-life in blood circulation. The circulation time of liposomes in the blood stream is dramatically increased by attaching polyethylene glycol (PEG) units to the bilayer, known as long
circulating (Stealth) liposomes. Liposomes are potential carriers for controlled drug release in tumours, therapeutic agents and antibiotics, for gene therapy and other uses. Liposomes are also used in some non-medical areas like bioreactors, catalysts, cosmetics and ecology. Liposomes are biocompatible, completely biodegradable and non-toxic. They can be formulated as a suspension, as an aerosol, or in a semisolid form such as gel, cream and lotion and often as a dry vesicular powder (proliposome) for reconstitution. They can be administered through ocular, pulmonary, nasal, oral, intramuscular, subcutaneous, topical and intravenous routes. Liposomes supply both a lipophilic environment and aqueous environment in one system and are therefore suitable for delivery of hydrophobic, amphiphilic and hydrophilic drugs. Liposomes can encapsulate not only small molecules but also macromolecules like superoxide dismutase, haemoglobin and interferon-9. Liposomes reduce toxicity and increase stability of entrapped drugs via encapsulation. Liposomes have the flexibility to couple with site-specific ligands to achieve active targeting (Anticancer and Antimicrobial drugs).

![Fig. 2.15 Structure of a Liposome](image)

In cosmetic industry, liposomes have a major application. The first liposomal cosmetic product to appear on the market was the anti-ageing cream ‘Capture’ launched by Dior in 1986. One of the reasons for the widespread use of liposomes in the cosmetic industry is their ease of preparation and the ability to improve the absorption of active ingredients by skin. Liposomes facilitate the continuous supply of agents into the cells over a sustained period of time, making them ideal candidates for the delivery of vitamins and other molecules to regenerate the epidermis. Several active ingredients, biomolecules (e.g. vitamins A and E) and antioxidants (e.g. lycopene and carotenoids) have often been incorporated into liposomal membranes. Phosphatidylcholine, one of the main ingredients of liposomes, has been widely used in skin care products and shampoos due to its softening and conditioning properties. Liposomes have also been used for treatment of hair loss.
2.1.2.5 Niosomes:

Liposomes have certain limitations. They have high production cost and low solubility. Liposomal vesicles are very much prone to oxidation and in turn are susceptible to destabilization and degradation. Thus, liposomes require special handling and storage. Any change in surface charge of liposomes results in altered physical properties which in turn may render them toxic.\[^{51}\]

Niosomes are a better alternative to liposomes - these are vesicles containing non-phospholipid constituents.\[^{52}\] Niosomes are lamellar structures that are microscopic in size. They comprise non-ionic surfactants of the alkyl or dialkyl polyglycerol ether class along with cholesterol.\[^{53}\] The surfactant molecules tend to orient themselves in such a way that the hydrophilic ends of the non-ionic surfactant point outwards, while the hydrophobic ends face each other to form the bilayer (Fig. 2.16). The bilayers contain nonionic surfactants and cholesterol. Studies have shown that niosomes behave like liposomes \textit{in-vivo} while simultaneously increasing the circulation of entrapped drugs. They also show higher metabolic stability.\[^{54}\]

Niosomes are chemically stable, biodegradable, biocompatible and can encapsulate large amount of active drugs in approximately less volume\[^{53}\] and are also cost efficient. This makes them an appropriate choice as a drug carrier over liposomes. Niosomes prepared by thin layer evaporation method are less toxic and provide precise control over the availability of active drug at the stratum corneum as compared to other classical formulations for stratum corneum.\[^{55}\] Niosomes are useful because of their sustained release profile, less toxicity and specific drug targeting.\[^{56}\] The size of niosomes increases on the incorporation of entrapped drug which is a result of interaction of solute with surfactant head groups, increasing charge and mutual repulsion of the surfactant bilayer and thus increasing the size of vesicles.\[^{57}\]

![Fig. 2.16 Structure of a niosome](image)

Niosomes can be prepared by various methods.

(a) Ether injection method

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C. The surfactant mixture in ether is injected through a gauge needle into an aqueous solution of the material.\[^{58}\] Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions, the diameter of the vesicle ranges from 50 to 1000 nm.
(b) Hand shaking method (Thin film hydration technique)

The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

(c) Sonication

In this method, an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for a few minutes using a sonicator with a titanium probe to yield niosomes.

(d) Micro fluidization

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities in precisely defined micro channels within the interaction chamber. The result is greater uniformity, smaller size and better reproducibility of niosomes formed.

(e) Multiple membrane extrusion method

It is a good method for controlling niosome size. Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into a thin film by evaporation. The film is hydrated with aqueous drug solution and the resultant suspension extruded through.

(f) Reverse phase evaporation technique (REV)

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

(g) The “bubble” method

It is a novel technique (Fig. 2.17) for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of a round-bottomed flask with three necks positioned in a water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards “bubbled” at 70°C using nitrogen gas.
There are some therapeutic applications of niosomes which are discussed below. The cells of reticuloendothelial system (RES) preferentially take up the vesicles. Such localized drug accumulation has however been exploited in treatment of animal tumors known in the liver and spleen and in parasitic infection of liver. Niosome carrier systems can be directed to specific sites in the body by use of antibodies. Immunoglobulins seem to bind quite readily to the lipid surface, thus offering a convenient means for targeting of drug carriers. Doxorubicin, the anthracyclic antibiotic with broad spectrum anti tumor activity, shows a dose dependant irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing tumors increased their life span and decreased the rate of proliferation of sarcoma. Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism. Intravenous administration of methotrexate entrapped in niosomes to tumor bearing mice resulted in total regression of tumor. Baillie et. al reported increased sodium stibogluconate efficacy of niosomal formulations and showed that the effect of two doses given on successive days was additive. Niosomes can be used as a carrier for hemoglobin. Niosomal suspension shows a visible spectrum superimposable onto that of free hemoglobin. Vesicles are permeable to oxygen and hemoglobin dissociation curve can be modified similarly to non-encapsulated hemoglobin.

In the cosmetic industry, niosomes have been used as delivery vehicles. Many encapsulation techniques have been proposed for carrying cosmetic actives. They can be used to encapsulate aqueous solutes and act as drug and cosmetic carriers. Cosmetics based on niosomes were developed and patented by L’Oréal in the 1970s and 80s. The first product ‘Niosome’ was introduced in 1987 by Lancôme. The advantages of using niosomes in cosmetic and skin care applications include their ability to increase the stability of entrapped drugs, improved bioavailability of poorly absorbed ingredients and enhanced skin penetration. In spite of having a lot of advantages, niosomes also have some limitations. Like liposomes, aqueous suspensions of niosomes may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion. The traditional method for producing niosomes or liposomes involves drying the lipid to a thin film from organic solvent, and then hydrating this film with the aqueous solvent of choice. The resulting multilamellar vesicles can be further processed by sonication, extrusion, or other treatments to optimize drug entrapment. All of these methods are time consuming and may involve specialized equipment. The thin film approach allows only for a
predetermined lot size so material is often wasted if smaller quantities are required for a particular application or dose.

2.2 Nanoparticles:

Nanoscale matter is that which has dimensions ranging from 1-100 nm.\textsuperscript{69, 70} Thus study of nanomaterials lies at the interface between chemistry (which deals with atoms and molecules of dimensions < 1 nm) and condensed matter physics (which deals with solid made up of an infinite array of atoms or molecules of dimensions > 100 nm). Nanoparticles can be classified according to their size. Matter in the nanometer regime has properties distinct from bulk matter. The reduction of bulk materials to the nano region induces size-dependent effects arising due to:

(a) Significant increase of the surface–to-volume ratio leading to a large interfacial area.

(b) Change in the physico-chemical properties of the interfacial species.

(c) Changes in the electronic structure and arrangement of the species within the nanoparticles.

(d) Confinement and quantum size effects.

2.2.1 Special properties of Nanomaterials:

2.2.1.1 High surface to volume ratio:

Nanoparticles have a relative larger surface area when compared to the same volume of the material. For example, let us consider a cube of length $l$, the surface area of the cube will be $6l^2$, the volume of the cube = $l^3$. Therefore the surface-area-to-volume ratio will be $6l^2/l^3 = 6/l$. It means that the surface-area-to-volume ratio increases with the decrease in length of the cube and vice versa. The high surface-area-to-volume ratio (Fig. 2.18) of nanoparticles provides a tremendous driving force for diffusion, especially at elevated temperatures.

![Fig. 2.18](image.png)

**Fig. 2.18** Figure illustrating the high surface-to-volume ratio in nanomaterials.
2.2.1.2 Surface plasmon resonance:

Surface Plasmon Resonance (SPR) is the collective oscillation of valence electrons in a solid stimulated by incident light (Fig. 2.19). The resonance condition is established when the frequency of light photons matches the natural frequency of surface electrons oscillating against the restoring force of the positive nuclei. SPR in nanometer-sized structures is called localized surface plasmon resonance (LSPRs). LSPRs are collective electron charge oscillations in metallic nanoparticles that are excited by light. Light intensity enhancement is a very important aspect of LSPRs and localization means the LSPR has very high spatial resolution limited only by the size of nanoparticles.

![Fig. 2.19 The collective oscillation of valence electrons in a solid stimulated by incident light](image)

2.2.1.3 Quantum confinement:

A quantum dot is a semiconductor nanostructure that confines the motion of conduction band electrons and valence band holes, or excitons (bound pairs of conduction band electrons and valence band holes) in all three spatial directions (Fig. 2.20). The confinement can be due to electrostatic potentials (generated by external electrodes, doping, strain or impurities), the presence of an interface between different semiconductor materials (e.g. in core-shell nanocrystal systems) or the presence of the semiconductor surface (e.g. semiconductor nanocrystal) or a combination of these. A quantum dot has a discrete and quantized energy spectrum.

![Fig. 2.20 Quantum confinement in Quantum dots](image)
2.2.2 Synthesis of nanoparticles:

There are two basic protocols for preparing nanoparticles- Bottom-up approach and Top-down approach (Fig. 2.21)

![Fig. 2.21 Schematic representation of TOP-DOWN and BOTTOM-UP methods, MNPs-metal nanoparticles](image)

2.2.2.1 Bottom-up approach:

From atomic and molecular precursors, through spontaneous chemical reaction and by self-assembly, bottom-up approaches involve those based on the preparation of colloidal solutions and use of mesoporous materials. This involves the build-up of a material atom-by-atom, molecule-by-molecule. Examples are preparation methods involving sol-gel fabrication, chemical reduction of precursors, preparation by ultra sonication, UV-irradiation, γ irradiation and solvothermal methods.

The sol-gel process is a wet-chemical technique\(^{71,72}\) widely used in the fields of materials science and ceramic engineering. The ultimate microstructure of the final component will be strongly influenced by changes implemented during processing. The precursor sol can be either deposited on a substrate to form a film (e.g. by dip-coating or spin-coating), cast into a suitable container with the desired shape (e.g. to obtain monolithic ceramics, glasses, fibers, membranes, aerogels) or used to synthesize powders (e.g. microspheres, nanospheres). Advantages of the sol-gel process are that it is a cheap and low-temperature technique that allows for the fine control of the product size and composition. Even small quantities of dopants such as organic dyes and rare earth metals can be introduced in the sol and end up being uniformly dispersed in the final product.

γ-irradiation technique\(^{73}\) is one in which the precursor solution is sealed and irradiated with γ-rays from a Co\(^{60}\) source at various dose rates to produce nanoparticles. Solvothermal synthesis is a method for preparing a variety of materials such as metals, semiconductors, ceramics and polymers. The process involves the use of a solvent under moderate to high pressure (typically between 1 atm and 10,000 atm) and temperature (typically between 100 °C and 1000 °C) that facilitates the interaction of precursors during synthesis. If water is
used as the solvent, the method is called “hydrothermal synthesis.” It is very similar to the hydrothermal route (where the synthesis is conducted in a stainless steel autoclave), the only difference being that the precursor solution is usually not aqueous. Using the solvothermal route one gets the benefits of both the sol-gel and hydrothermal routes. Thus solvothermal synthesis allows for precise control over the size, shape distribution, and crystallinity of nanoparticles or nanostructures. These characteristics can be altered by changing certain experimental parameters including reaction temperature, reaction time, solvent type, surfactant type and precursor type. Solvothermal synthesis has been used in the laboratory to make nanostructured titanium dioxide, graphene, carbon and other materials. The high photocatalytic activity of TiO\textsubscript{2} leads to the degradation of organic and biological molecules into smaller and less harmful compounds.

In the sonochemical process, powerful ultrasound radiation (20 kHz to 10 MHz) is applied to molecules to enhance the rate of chemical reaction. Acoustic cavitation is a physical phenomenon which is responsible for sonochemical reaction. This method, initially proposed for the synthesis of iron nanoparticles is nowadays used to synthesize different metals and metal oxides. The main advantages of the sonochemical method are its simplicity, operating conditions (ambient conditions) and easy control of the size of nanoparticles by using precursors with different concentrations in the solution. Ultrasound is effective due to the cavitation phenomena involving the formation, growth and collapse of bubbles in a liquid.

### 2.2.2.2 Top-down approach:

This involves suitable subdivision of larger structures into finer particles. Examples are attrition, ball milling, grinding of solids and vapour deposition. Attrition method involves the grinding of particles in a ball mill.

Chemical Vapour Deposition (CVD) of films and coatings involve the chemical reactions of gaseous reactants on or near the vicinity of a heated substrate surface. This atomistic deposition method can provide highly pure materials with structural control in the atomic scale. Moreover, it can produce single layer, multilayer, composite, nanostructured and functionally graded coating materials with well-controlled dimension and unique structure at low processing temperatures. The unique features of the CVD technique has allowed the coating of complex shape engineering components and the fabrication of nanodevices, carbon–carbon (C–C) composites, and ceramic matrix composites (CMCs). The versatility of CVD has led to its rapid growth and it has become one of the main processing methods for the deposition of thin films and coatings for a wide range of applications including semiconductors (e.g. Si, Ge) optoelectronics and energy conversion devices, dielectrics, metallic films for microelectronics and for protective coatings, fibre production and fibre coating.
2.2.3 Stability of the nanoparticles:

Use of metal nanoparticles is gradually increasing due to their function as chemical catalysts, adsorbents, biological stains and elements of optical, electronic, and magnetic devices. As the size of the particle decreases to the 1-100 nm range, the electronic, optical, catalytic and thermodynamic properties of the metal particles deviate from bulk properties. Due to the very high surface energy, nanoparticles tend to agglomerate and often undergo oxidation. To avoid agglomeration and oxidation, their stabilization is an important issue. Metal particles in the ultrafine state have been prepared using various methods such as chemical reduction, chemical deposition, thermal decomposition, photochemical reduction and radiolysis.

Nanomaterials thus prepared, need a protective agent for stabilization. Until now noble metal colloids have been prepared and stabilized by particle stabilizers such as polymers, surfactants, ligands and dendrimers. Many of these agents play a crucial role in controlling the size and shape of the nanoparticles. The organic layer formed around the nanoparticles (adlayer) has to be sufficiently thick to provide a steric barrier that counterbalances the attractive van der Waals forces responsible for particle agglomeration. The formation of thick adlayers around nanoparticles is an efficient approach to prevent agglomeration in the diluted systems typically used in biomedical applications.

The nanoparticles synthesized in solution have very high surface energy. Various organized media have been reported to serve as stabilizing agents. Few examples are microemulsions, block copolymer micelles and other amphiphilic systems. Polymers such as polyethylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone and surfactants like sodium dodecyl sulfate, Triton-X and carbohydrates like chitosan have been used for this purpose.

Polymers have been widely used to stabilize nanoparticles. Although there are a variety of ways to achieve nanoparticle-polymer composites, two different approaches dominate. The first one consists of the in situ synthesis of the nanoparticles in the polymer matrix either by reduction of the metal salts dissolved in that matrix or by evaporation of the metals on the heated polymer surface. The second one less frequently used, involves polymerization of the matrix around the nanoparticles. Li. et al. synthesized gold and platinum nanoparticles by the reduction of their salts with potassium bitartrate as the reductant and poly(N-vinyl-2-pyrrolidone) (PVP) and polyethylene glycol (PEG) as the protective agent. Synthesis of gold and silver hydrosols was carried out in a one-step process by reduction of aqueous solutions of metal salts using poly(N-vinyl-2-pyrrolidone) (PVP). In most of the works related to the synthesis of nanoparticles in the presence of PVP, this polymer is only considered to play the role of stabilizer or protecting agent. Shape, size and optical properties of the particles can be tuned by changing the employed PVP/metal salt ratio. The CdS/Poly styrene nanocomposites films were prepared through ex situ methodology and deposited by spin-coating technique. A simple method for synthesis of well dispersed cadmium sulphide (CdS) nanoparticles embedded in a polyethylene glycol matrix (PEG 300) in thin film form has been reported by Bhattacharjee et. al.
Nanoparticles in presence of surfactants in solution can be stabilized by micelle formation (Fig. 2.22). The nonionic surfactants in general and polysorbate 80 in particular, have been used in the development of a strategy to obtain stable gold nanoparticles in an aqueous medium at room temperature.

**Fig. 2.22** Schematic illustration of the synthesis of well-dispersed gold nanoparticles by using Polysorbate 80 at room temperature

Colloidal dispersions of bimetallic nanoparticles composed of gold and palladium were prepared by a sonochemical method in which Au(III) and Pd(II) ions in an aqueous solution of sodium tetrachloroauroate-(III)dihydrate and sodium tetrachloropalladate(II) were reduced by ultrasound irradiation in the presence of sodium dodecyl sulfate (SDS). Nanoparticles using a block copolymer micellar template were first made by Sohn et al.

Polymerizable surfactants (called surfmers) and their polymerized products have been reported in micelles, vesicles, liposomes and bilayers. The polymerizable surfactant forms a bilayer on the surface of the nanoparticles with the quaternary positive head groups facing the bulk water, and the polymerizable groups reside in the hydrophobic region. Murphy et. al. showed that polymerization of the bilayer around the gold nanoparticles decreases surfactant desorption and results in gold nanoparticles with remarkable stability against aggregation after extraction with chloroform, dialysis at elevated temperature and centrifugation by using 11-(acryloyloxy)undecyltrimethylammonium bromide. The formation of gold nanoparticles and the crystal growth at the surface of mixed phosphatidylcholine (PC)–ionic surfactant vesicles was investigated by Robertson et. al. The PC-bilayer surface was negatively charged by incorporating sodium dodecyl sulfate (SDS) and positively charged by adding hexadecyltrimethylammonium chloride (CTAB).

Reverse micelles are often used as microreactors because they exchange the content of their water pools by a collision process. Such a micellar core can be considered as a nanoreactor where nucleation and growth of metal particles upon reduction are restricted. Reverse micelles have been used in the past to produce a wide range of nanocrystals. With silver and copper nanocrystals, no traces of oxide are detected. This is mainly attributed to the fact that a functionalized surfactant (i.e. the head polar group of the surfactant is one of the reactants) is used to produce nanocrystals. The reverse micelle-based synthesis technique is a well-documented chemical reaction system for the synthesis of spherical nanoparticles. Many nanosized particles have been prepared in reverse micelles, including metals, metal sulfides and selenides and metal oxides and hydroxides. The growth of metal nanoparticles formed in a reverse micellar water pool is restricted by the pool size and the nanoparticle is stabilized by the shell composed of surfactant molecules. The size of a growing particle can be controlled.
by varying the pool diameter or initial concentration of the reagents. For example, gold nanoparticles were synthesized in reverse micelles of cetyltrimethylammonium bromide (CTAB)\textsuperscript{139} or sodium bis(2-ethylhexyl)sulfosuccinate (AOT)\textsuperscript{140} via the chemical reduction of HAuCl\(_4\) with NaBH\(_4\) and N\(_2\)H\(_4\). Gold nanoparticles were prepared by the reduction of HAuCl\(_4\) in CTAB/octane + 1-butanol/H\(_2\)O reverse micelle using NaBH\(_4\) as reducing agent.\textsuperscript{141} Wilcoxon and co-workers\textsuperscript{142} have studied the synthesis of gold nanoparticles formed in aqueous media and in reverse micelles using chemical and photolytic reduction methods. Steigerwald et al. prepared capped CdSe, ZnS, ZnS/ CdSe and CdSe/ZnS nanocrystallites from reverse micellar solutions.\textsuperscript{143}

2.2.4 Copper Nanoparticles:

Among metal nanoparticles, noble metal nanoparticles are popular due to their brilliant colours and strong SPR bands in the visible part of the electromagnetic spectrum.\textsuperscript{144-148} Silver and gold nanoparticles have been widely studied. Studies with copper nanoparticles (CuNPs) are comparatively less popular. This is because the copper surface is highly prone to aerial oxidation and aggregation.\textsuperscript{149-154}

The production of copper nanoparticles is much more challenging in comparison to other noble metals. When copper nanoparticles are placed in the open air, aggregation immediately occurs due to surface oxidation.\textsuperscript{154} To avoid this problem, an inert environment, such as argon or nitrogen\textsuperscript{155} is used. Protective polymers\textsuperscript{156, 157} and surfactants\textsuperscript{158, 159} have been employed for the synthesis of stable copper nanoparticles. Since, copper is significantly less expensive than silver and gold, therefore it is economically attractive.

Copper nanoparticles can be produced using many different techniques, typically classified as bottom-up or chemical methods and top-down or physical methods. Chemical reduction,\textsuperscript{160-162} microemulsion based techniques,\textsuperscript{163-165} sonochemical reduction,\textsuperscript{166} electrochemical,\textsuperscript{167} microwave-assisted\textsuperscript{168} and hydrothermal\textsuperscript{169} syntheses are the main techniques for the synthesis of nanoparticles through the chemical approach. Biological or biosynthesis\textsuperscript{170} techniques are also considered as bottom-up methods. Physical methods for nanoparticle synthesis are laser (pulse) ablation,\textsuperscript{171} vacuum vapour deposition,\textsuperscript{172} pulsed wire discharge (PWD)\textsuperscript{173} and mechanical milling.\textsuperscript{174} During the chemical synthesis the growth and morphology can be controlled by optimizing reaction conditions, such as temperature, concentration, precursor, capping/stabilizing agent and the type of solvent.\textsuperscript{175}

The chemical reduction of copper salts is the easiest, simplest and the most commonly used synthetic method for copper nanoparticles.\textsuperscript{160-162} In fact, the production of nanosized metal copper particles with good control of morphology and size using chemical reduction of copper salts can be achieved. In the chemical reduction techniques, a copper salt is reduced by a reducing agent such as sodium borohydride,\textsuperscript{160-162} hydrazine (N\(_2\)H\(_4\)),\textsuperscript{168, 176, 177} ascorbate,\textsuperscript{178} polyol\textsuperscript{179} and isopropyl alcohol.\textsuperscript{180}

The pioneering work on microemulsion-mediated synthesis of CuNPs was initiated by Pileni et. al.\textsuperscript{181-183} A reaction mechanism is shown in Fig. 2.23. The precursor metal ion is introduced into the system either as a metal salt dissolved in the aqueous micelle core or by functionalization of the AOT surfactant head group where the sodium counter ion is
exchanged with the desired metal ion. The second method is more popular. The purpose of
the second method is to eliminate the presence of the salt anion from the core of the reverse
micelle. The use of salts and the presence of anions within reverse micelles have been shown
to alter the physical properties of the water environment and surfactant layer resulting in
variations in the size and shape of the reverse micelles and metallic particles
synthesized. Bagwe and Khilar\textsuperscript{188} and Cason et. al\textsuperscript{189} studied the effect of different
solvents on the microemulsion method. They used different solvents and reported their
effects on the growth rate, final size and polydispersity of the nanoparticles. The shape of the
micellar aggregates depends on the nature of the counterion.\textsuperscript{190} Many workers reported that
the concentration of water (w\textsubscript{0}) strongly affects the final size of the particle.\textsuperscript{191-194} Pileni et. al
summarized the effects of various parameters on the shape and size of the nanoparticles
synthesized by the microemulsion method.\textsuperscript{194-197}

\begin{center}
\includegraphics[width=\textwidth]{fig223.png}
\end{center}

\textbf{Fig. 2.23} Mechanism of synthesis of AOT-capped Cu NPs

In contemporary research, the microwave-assisted synthesis of copper nanoparticles
has become popular due to its simplicity, ease of operation, short reaction period and
increasing yield of products compared to the conventional heating methods.\textsuperscript{198, 199} The
commonly used microwave frequency is 2.45 GHz. The main reasons for using the
microwave are the fast and homogeneous reaction conditions during the microwave
synthesis. Bloisi et. al\textsuperscript{200} reported the synthesis parameters for copper nanoparticles.
Zhu et. al\textsuperscript{201} found a fast method for the production of copper nanoparticles by using copper
sulphate as a precursor and sodium hypophosphite as the reducing agent in ethylene glycol
under microwave irradiation. They also studied the effect of parameters like concentration of
reducing agent and microwave irradiation time. The size of copper nanoparticles prepared by
this method was 10 nm.

In the solvothermal process, the chemical reaction takes place in a sealed vessel such
as bomb or autoclave where solvents are brought to temperatures well above their boiling
points.\textsuperscript{202} When water is used as solvent, it is called a hydrothermal process.

Laser ablation method is a commonly used technique for the preparation of copper
nanoparticles in colloidal form in a variety of solvents.\textsuperscript{203, 204} Copper nanoparticles are
prepared in colloidal form to avoid oxidation. The pulse laser ablation process takes place in
a vacuum chamber and in the presence of some background/inert gas. Marine et. al\textsuperscript{203}
reviewed the recent development and reported an analysis of this method. Mechanical/ball milling method and pulsed wire discharge method are the other two physical methods by which copper nanoparticles have been synthesized by different researchers.

Biosynthesis of nanoparticles is also considered to be a bottom-up technique, where the oxidation/reduction is the main reaction that occurs during the process. Metal compounds usually get reduced to their respective nanoparticles because of microbial enzymes or the plant phytochemicals with antioxidant or reducing properties. In the biosynthesis of nanoparticles, the three important parameters that should be evaluated are (a) the choice of the solvent medium (b) the reducing agent and (c) the choice of a nontoxic material for the stabilization of the nanoparticles. Nanoparticles of copper have also been synthesized inside live plants like Indian mustard, Alfa alfa and Sunflower.

2.2.5 Copper oxide nanoparticles:

In recent years, synthesis of inorganic nanostructures with well-defined morphologies has received considerable attention due to their outstanding properties and potential applications. Metal oxide nanoparticles have been one of the key areas of inorganic materials research because of their numerous applications in catalysis, data storage, energy technology and coating fields. Among these materials, CuO is an important p-type transition-metal-oxide semiconductor, having a narrow band gap of \( E_g = 1.2 \text{ eV} \). Copper oxide (CuO) nanoparticles have been of great interest due to their potential applications in many important fields of science and technology such as gas sensors, magnetic phase transitions, catalysts and superconductors. In the past decades, great efforts have been made to study the preparation of nanosized CuO. Conventional methods for the preparation of CuO powders include onestep solid state reaction at room temperature, thermal decomposition of copper salts, mechanical milling of commercial powders and so on. However, none of these methods seems to be suitable for the preparation of highly dispersed CuO nanoparticles. Preparation of highly dispersed CuO nanoparticles has been successfully developed by Zhu et. al. About 6 nm sized CuO nanoparticles with narrow size distribution can be easily prepared by their method. Spherical, ellipsoidal and needle-shaped CuO nanocrystals can be obtained by varying the reaction conditions. Different copper oxide (CuO) (rectangle-like, seedlike, beltlike, and sheetlike) nanostructures have been synthesized by addition of different molar concentration of NaOH solution (0.1, 0.25, 0.50, and 1 M, respectively) to the copper nitrate solution (0.2 M). Monodisperse, stable CuO nanocrystals were synthesized by using a novel, yet simple wet chemistry route reported by M. Yin et. al. Flower-shaped CuO nanostructures have been prepared by a simple solution-based process at 100 °C using copper nitrate, NaOH, and hexamethylenetetramine (HMTA) for 3 h without the use of any complex reagents by Vaseem et. al.

2.2.6 Applications of nanoparticles in Biology and Chemistry:

Nanotechnology is technology that deals with nano meter sized objects. Thus it is expected that nanotechnology will be developed at several levels - materials, devices and
systems. The nanomaterial level is the most advanced at present, both in scientific knowledge and in commercial applications. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties. Now they have entered a commercial exploration period. The unique physical properties (e.g. plasmon resonance, fluorescence enhancement) and chemical properties (e.g. catalytic activity enhancement) of nanometals are a result of the high area/volume ratio. Metal nanoparticles have also found their application in engineering, chemistry, biology and medicine.

Nanomaterials participate in chemical reactions more readily than larger objects of similar chemical nature. Therefore, nanomaterials show greater biological activity. This fact set scientists thinking about the possibility of use of nanoparticles as antibacterial and biocidal agents. Some of the applications of nanomaterials in biology or medicine are: as fluorescent biological labels, drug and gene delivery, bio detection of pathogens, detection of proteins, probing DNA structure, tissue engineering, tumour destruction via heating, separation and purification of biological molecules and cells, MRI contrast enhancement and phagokinetic studies. Nanoparticles exist in the same size domain as proteins, this makes nanomaterials suitable for bio tagging or labelling. In order to interact with biological targets, a biological or molecular coating or layer acting as a bioinorganic interface should be attached to the nanoparticle. Examples of biological coatings include antibodies, biopolymers like collagen or monolayers of small molecules that make the nanoparticles biocompatible.

Copper nanoparticles have wide applications as antimicrobial materials, super strong materials, sensors and catalysts. Among other metals, copper (Cu) is a promising candidate for the development of new generation nanomaterials. On the one hand, copper participates in many major metabolic processes. It shows significant bactericidal activity due to cell membrane, nucleic acid and protein damage. The mechanism of the antibacterial action of copper is predominantly based on DNA structure damage. Nowadays it is known that copper shows biocidal activity not only against bacteria, but also against viruses, such as herpes simplex virus, human immunodeficiency virus, bronchitis and influenza viruses.

2.3: Effect of Nanoparticles on Dyes in Solution:

2.3.1 Dyes:

A dye is a colored substance that has an affinity for the substrate to which it is being applied. The majority of natural dyes are from plant sources – roots, berries, bark, leaves, wood, fungi and lichens. The discovery of man-made synthetic dyes late in the 19th century ended the large-scale market for natural dyes. Many synthetic dyes have since been prepared. Dyes are now classified according to how they are used in the dyeing process e.g. acid dyes, basic dyes, Mordant dyes, Vat dyes, Reactive dyes, disperse dyes, Azo dyes and Sulfur dyes. Other important classes of dyes are food dyes, laser dyes like Rhodamine 6G, Fluorescein and coumarin, fluorescent brighteners for textile fibres and paper, solvent
dyes for wood staining and producing colored lacquers, solvent inks, coloring oils and contrast dyes used for magnetic resonance imaging. Depending on the nature of their chromophore, dyes are divided into the following classes: Acridine dyes have acridine chromophore; Triarylmethane dyes are derivatives of triphenyl methane; azo dyes are based on -N=N- azo structure; nitro dyes are based on a -NO₂ nitro functional group. Phthalocyanine dyes are derivatives of phthalocyanine; Indophenol dyes are derivatives of indophenol etc. Other important classes of dyes are the Xanthene dyes and Fluorene dyes which are derived from xanthene.

2.3.1.1 Xanthene dyes:

Xanthene (9H-xanthene,10H-9-oxaanthracene) is a yellow organic heterocyclic compound. Its chemical formula is C₁₃H₁₈O. Xanthene is used as a fungicide and it is also a useful intermediate in organic synthesis. Derivatives of xanthene are commonly referred to collectively as xanthenes, and among other uses are the basis of a class of dyes which includes fluorescein, eosin, and rhodamines. Xanthene dyes tend to be fluorescent and are colored in solution. Many xanthene dyes can be prepared by condensation of derivatives of phthalic anhydride with derivates of resorcinol or 3-aminophenol. The general skeleton of xanthene dyes and structures of few common xanthene dyes are shown in Fig. 2.24

![Fig. 2.24 Structure of the basic skeleton of xanthene dyes and few examples of such dyes](image-url)
The xanthene dyes are probably the most intensely studied class of luminescent dyes. Xanthene dyes are widely used in research due to the special spectral characteristics of the different ionic forms and their ability to form dimers. The equilibria among the various ionic forms of the dyes are sensitive to temperature, pH, solvent, concentration and other factors.\textsuperscript{237-240} Several workers have proposed that the photophysics and lifetime of xanthene dyes are very much dependent on the hydrogen bonding ability of the solvent.\textsuperscript{241}

Another important aspect of Xanthene dyes is their aggregation. The formation of aggregated dye molecules in concentrated aqueous solution was first suggested to explain the rather substantial deviations from Beer's law evidenced by highly colored organic ions such as methylene blue, certain cyanines and crystal violet. Aggregation of the xanthenes has fundamental consequences in applications as diverse as photographic technology,\textsuperscript{241} tunable lasers,\textsuperscript{242} fluorescence depolarization diagnostic devices\textsuperscript{243} and photomedicines.\textsuperscript{244} Since the formation of aggregates modifies the absorption spectrum and photophysical properties of a dye, it affects its ability to emit at a certain wavelength or to act as a photosensitizer. The applications mentioned are solely dependent on one or both of these phenomena. Thus, the aggregation phenomena of xanthene dyes have been an important and interesting field of research since the last few decades.\textsuperscript{245, 246} Various mechanisms have been suggested to explain the forces holding dye ions together in solution. These include additive forces of van der Waals type, intermolecular hydrogen bonding, hydrogen bonding with the solvents, dispersive forces for halogen substituted xanthene dyes and coordination with the metal ions.\textsuperscript{245-247} Hydrogen bonding appears to be responsible for the dimerization of fluorescein in protic solvents.\textsuperscript{248}

The phenomenon of aggregation depends on solvents, presence of electrolytes, surfactants, pH and also on temperature. The dye aggregates are usually characterized as H-aggregates (card-like stacking) and J-aggregates (head-to-tail) on the basis of the observed spectral shift of the absorption maximum relative to the respective monomer absorption band (hypsochromic for H-type and bathochromic for J-type).\textsuperscript{249, 250} Many J-aggregates exhibit fluorescence and their fluorescence quantum yield quite often surpasses that of the monomeric dyes.\textsuperscript{250}

One of the most interesting behavior exhibited by xanthenes dyes is their pH dependent equilibrium. Xanthene dyes in aqueous solution occur in cationic, neutral, anionic and dianionic forms\textsuperscript{240, 251} making their absorption and fluorescence properties highly pH-dependent. For the fluorescein system, it has been observed that the absorption and fluorescence spectra vary significantly with pH and for this reason this dye can be effectively used as a pH dependent probe.\textsuperscript{240, 251} So the characterization of the protolytic equilibria of fluorescein and spectroscopic properties of its protolytic forms is very important. The protolytic constants relating the chemical activities of cation, neutral form, anion and dianion are $pK_1 = 2.08$, $pK_2 = 4.31$, and $pK_3 = 6.43$. The dianion has the most intense fluorescence with a quantum yield of 0.93 but the anion also shows considerable fluorescence with a quantum yield of 0.37. The neutral and cationic species upon excitation are converted into the anion and fluoresce with quantum yield of about 0.30 and 0.18 respectively.\textsuperscript{251} Margulies et. al\textsuperscript{240} reported the pH dependent equilibrium of fluorescein dye and the absorption spectra of different dye forms (shown in Fig. 2.25).
Xanthene dyes, which are well known for their high quantum yield are used to probe several organized media since they are required in minute quantities. Moreover these dyes are highly sensitive to the local pH of the microenvironment and so these have been successfully used to monitor any change in pH and hydrogen bonding properties of the environment. Benzo[c]xanthene dyes have been successfully used to monitor the pH changes in various medical diagnosis. Xanthene dyes are also well known for the formation of aggregates at high concentrations. Aggregation is favoured in polar solvents with greater hydrogen bonding capacity. Thus the aggregation phenomenon of the xanthene dyes can also be used to monitor the change in polarity and the hydrogen bonding ability of the environment. Sulfonated xanthene dyes are used as light screening dyes in photographic products and processes. New diagnostic agents for positron emission tomography (PET) and methods for use of such agents for imaging of human or animal tissue are described, wherein a primary active component of such agents is a radiolabeled halogenated xanthene or halogenated xanthene derivative. Preferably, the radiolabeled halogenated xanthene is radiolabeled Rose Bengal or a functional derivative of Rose Bengal. The well known xanthene dye, Fluorescein is efficiently used in Fluorescein angiography. Fluorescein angiography is an eye test that uses an orange-colored fluorescent dye (fluorescein) and a special camera, to take pictures and analyze the blood circulation in the retina and choroid. Ring-fluorinated fluoresceins acts as organic photosensitizers for dye-sensitized solar cells. Fluorescein -27 and other xanthene dyes have been used to design dye sensitized solar cells with TiO₂ nanoparticles. Xanthene dyes can bind to proteins without disturbing their secondary structure, hence providing a scope to label proteins non-covalently with this fluorescent probe.
Fluorescein is the most popular Xanthene dye was first synthesized by Adolf von Baeyer in 1871. It can be prepared from phthalic anhydride and resorcinol in the presence of zinc chloride via the Friedel-Crafts reaction (Fig. 2.26).

Fig. 2.26 Scheme showing the synthesis of Fluorescein

The fluorescence\textsuperscript{261} of this molecule is very intense. Its absorbance peak ($\lambda_{\text{max}}^{\text{abs}}$) is at 480 nm and peak emission ($\lambda_{\text{max}}^{\text{em}}$) at 520 in alcohol\textsuperscript{240,251} (Fig. 2.27). Fluorescein has a $\text{pK}_1$ of 6.4 and its ionization equilibrium leads to pH-dependent absorption and emission over the range 5 to 9.

Fig. 2.27 Absorption and emission spectra of Fluorescein in ethanol

Rhodamine B is a Xanthene dye which is often used as a tracer dye within water to determine the rate and direction of flow and transport. Rhodamine dyes fluoresce and can thus be detected easily and inexpensively with instruments called fluorometers. Rhodamine dyes are used extensively in biotechnology applications such as fluorescence microscopy, flow cytometry and fluorescence correlation spectroscopy.\textsuperscript{262} Rhodamine B can be synthesized by the following method (Fig. 2.28)
Methylene blue is a heterocyclic aromatic chemical compound with the molecular formula C\textsubscript{16}H\textsubscript{18}N\textsubscript{3}SCl. It has many uses in a range of different fields, such as biology and chemistry. At room temperature it appears as an odorless, dark green solid powder that yields a blue solution when dissolved in water. The hydrated form has three molecules of water per molecule of methylene blue. This compound may be prepared by treating 4-aminodimethylaniline with hydrogen sulfide dissolved in hydrochloric acid, followed by oxidation with ferric chloride.\textsuperscript{263} (Fig. 2.29).

Fig. 2.28 Scheme showing the synthesis of Rhodamine B

Fig. 2.29 Scheme showing the synthesis of Methylene Blue

Methylene blue is a potent cationic dye with absorption maximum around 670 nm. (Fig.2.30). The absorption characteristics depend on a number of factors including protonation, adsorption to other materials and metachromasy - the formation of dimers and higher-order aggregates depending on concentration and other interactions.\textsuperscript{264}
Methylene blue is capable of autooxidizing \textit{in-vivo} at low concentrations, to the reduced colorless form (leukomethylene blue). At these concentrations, methylene blue and leukomethylene blue are at equilibrium and form a reversible reduction-oxidation system. This autooxidizing property provides a mechanism for electron transfer to oxygen. Thus Methylene Blue is widely used as a redox indicator in analytical chemistry. Solutions of this substance are blue when in an oxidizing environment, but will turn colorless if exposed to a reducing agent.

\section*{2.3.2 Effect of Nanoparticles on Dyes in solution:}

Metallic nanoparticles (NPs) are exploited for their ability to interact with organic compounds and to increase significantly the fluorescence intensity and the photostability of many fluorescent dye molecules. Energy transfer can occur from the metal NPs to the fluorescent dye molecules or by a modified local electromagnetic field experienced by the fluorophores in the vicinity of metal surfaces.

The radiative and nonradiative decay rates of dye molecules, chemically attached to differently-sized gold nanoparticles, have been investigated by means of time-resolved fluorescence experiments.\textsuperscript{265} A pronounced fluorescence quenching is observed for the smallest nanoparticles of 1 \textmu m radius. The quenching is caused not only by an increased nonradiative rate but also due to a drastic decrease in the dye’s radiative rate. Silver particles in aqueous surfactant media and their catalytic properties toward the reduction of a number of dyes as test reactions were performed by Jana et. al.\textsuperscript{266} The dyes used were methylene blue (MB), phenosafranin (PS), fluorescein (F), 2,7- dichlorofluorescein (DCF), eosin (E) and rose bengal (RB). A simple screening method for the analysis of the interaction between several AgNPs (bare-, citrate- and PVP-coated) and dye-containing DMPG vesicles acting as a biomimetic cell-membrane was formulated by Shin et. al.\textsuperscript{267} According to Yusif et. al,\textsuperscript{268} with increase in the concentration of Ag nanoparticles, the laser dyes under investigation were
adsorbed effectively and the fluorescence of the laser dyes is quenched. Dye-doped silica NPs have been demonstrated to be sensitive labeling reagents for biosensing and imaging. Their flexible conjugation, excellent photostability and ultrasensitivity make them a powerful tool in bioanalysis.
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