Chapter 1

INTRODUCTION

1.1. FULLERENES

1.1.1. INTRODUCTION

It was astro and chemical physicists Richard Smalley, Robert Curl and Harry Kroto who discovered a new form of carbon in 1985 at Rice University in Houston and shared a Nobel Prize in 1996 for their discovery [1]. Their discovery added to the two known allotropic forms of carbon, i.e. diamond and graphite. This form was named Buckminster-fullerene as a tribute to Richard Buckminster Fuller an architect and inventor, who designed the geodesic dome. Fullerenes are moleculs composed entirely of carbon, in form of a hollow sphere (C_{60}), ellipsoid (C_{70}) or other structures depending on the number of carbons in that particular structure. They are similar in structure to graphite, which is composed of a sheet of linked hexagonal rings, but contain pentagonal (or sometimes heptagonal) rings that prevents the sheet from being planar. The typical strutures of these three forms of carbon is shown in Figure 1.1.

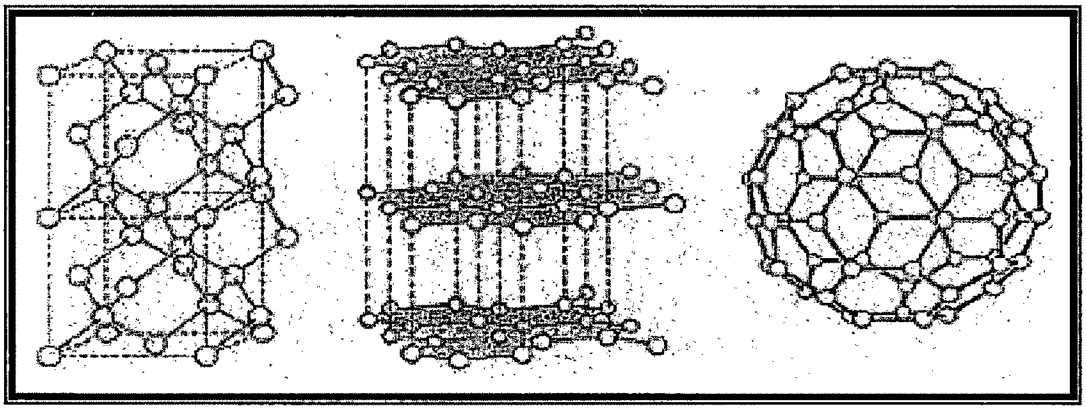


Figure 1.1 The three allotropic form of carbon: left to right diamond, graphite and [60]fullerene

Interestingly, C_{60} and other higher fullerenes were found to be present in normal candle soot as well found to exist naturally in interstellar dust and in geological formations on Earth, in the ppm range. Some of these places are Shunga/Russia[2], New Zealand[3] and

Sudbury/Canada[4]. Formerly, six crystalline forms of the element carbon were known, namely two kinds of graphite, two kinds of diamond, chaoit and carbon (VI). The latter two were discovered in 1968 and 1972.

1.1.2. PRODUCTION

W. Kratschmer, D.R. Huffman and co-workers were the first to report the formation of soot from graphite rods that contained macroscopic quantities of C_{60} and even C_{70}. This was done by causing an arc between two graphite rods to burn in a helium atmosphere and extracting the carbon condensate using an organic solvent [5], [6]. The typical set up used by them is shown in Figure 1.2.

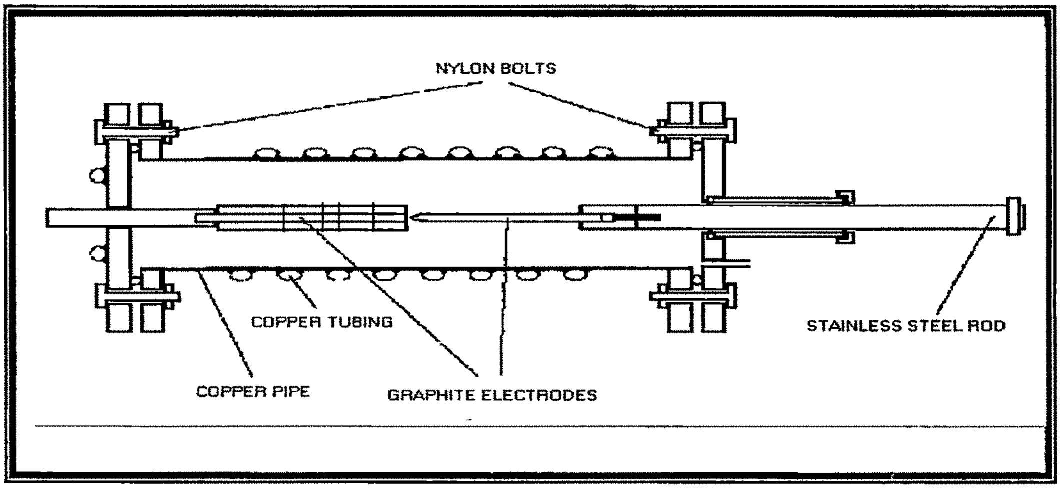


Figure 1.2 The Kratcshmer-Huffmann apparatus as used for the production of C_{60} and C_{70}.

In this method the starting material that was used was pure graphite soot. During the process the system was first purged with helium gas and then the reaction jar filled with about 100 torr of helium gas. Black soot was produced inside this jar by evaporating the graphite electrodes in an atmosphere of 100 torr of helium. The resulting black soot was scraped from the surface of the jar and dispersed in benzene [5]. The black soot could be extracted with the organic solvents like toluene, 1, 2-dichlorobenzene or carbon disulphide [6]. It was reported that the carbon soot samples produced by this method at elevated quenching gas pressures (> 100 torr He) shows absorption features and have been observed in the infrared (the strongest at 1429, 1183, 577, and 528 cm^{-1}). Broader features were also observed in the ultraviolet (the strongest at 340, 270, and 220 nm) [7]. It was in 1992 that A. Walter and his coworkers described a method for the purification of gram quantities of the C_{60} [8].

Now-a-days a simple ac or dc arc method is used for the production of C_{60} and other fullerenes in a good yield is the method used commercially.

1.1.3. STRUCTURE

[60]fullerene has a structure of truncated icosahedron, which resembles a football made of hexagons and pentagons, with a carbon atom at the corners of each hexagon and a bond along each edge. The C_{60} molecule has two bond lengths. The 6:6 ring bonds (between two hexagons) considered "double bonds" are shorter than the 6:5 bonds (between hexagon and a pentagon). The ball and stick models of [60]fullerene and [70]fullerene are shown in Figure 1.3 and Figure 1.4 respectively.

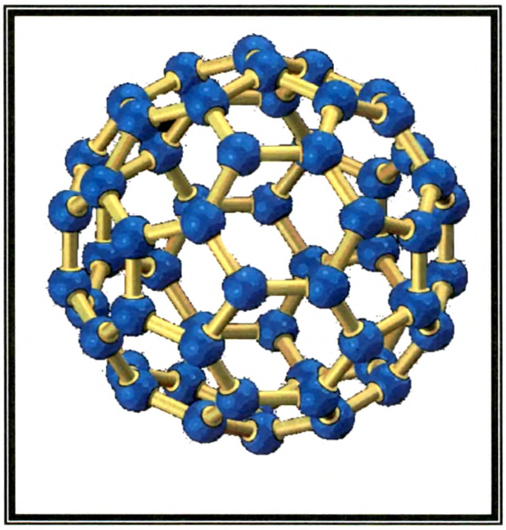


Figure.1.3. The ball and stick model of [60]fullerene

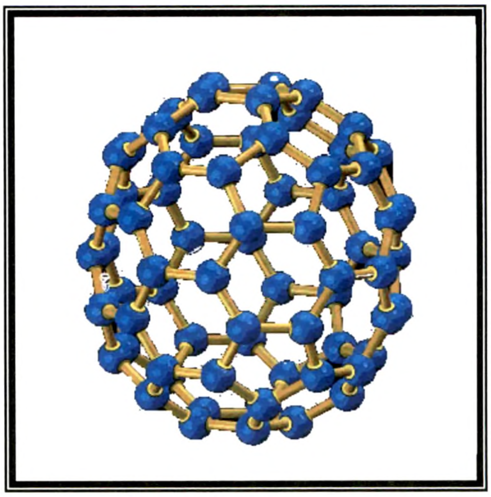


Figure.1.4. The ball and stick model of [70]fullerene

The number of carbon atoms in a fullerene shell can vary, and for this reason, numerous new carbon structures have become known with range from smallest C_{30}-$C_{540.}$

[60]fullerene consists of 20 hexagonal and 12 pentagonal rings as the basis of icosohedral symmetry of closed cage structure. Each carbon atom is bonded to three others and is sp^2 hybridized. C_{60} contains the structural element of corannulene. This bowl shaped corannulene, shown in Figure 1.5(a), was first synthesized by Barth and Lawton in 1970[9] and is the first example of a curved molecular π system. Similar attempt was made to synthesize a curved fragment named Sumanene[10] and is shown in Figure 1.5(c). C_{60} contains six pyracyclene units shown in Figure 1.5(b), which is responsible for the six-fold electrochemical reduction of C_{60}[11].

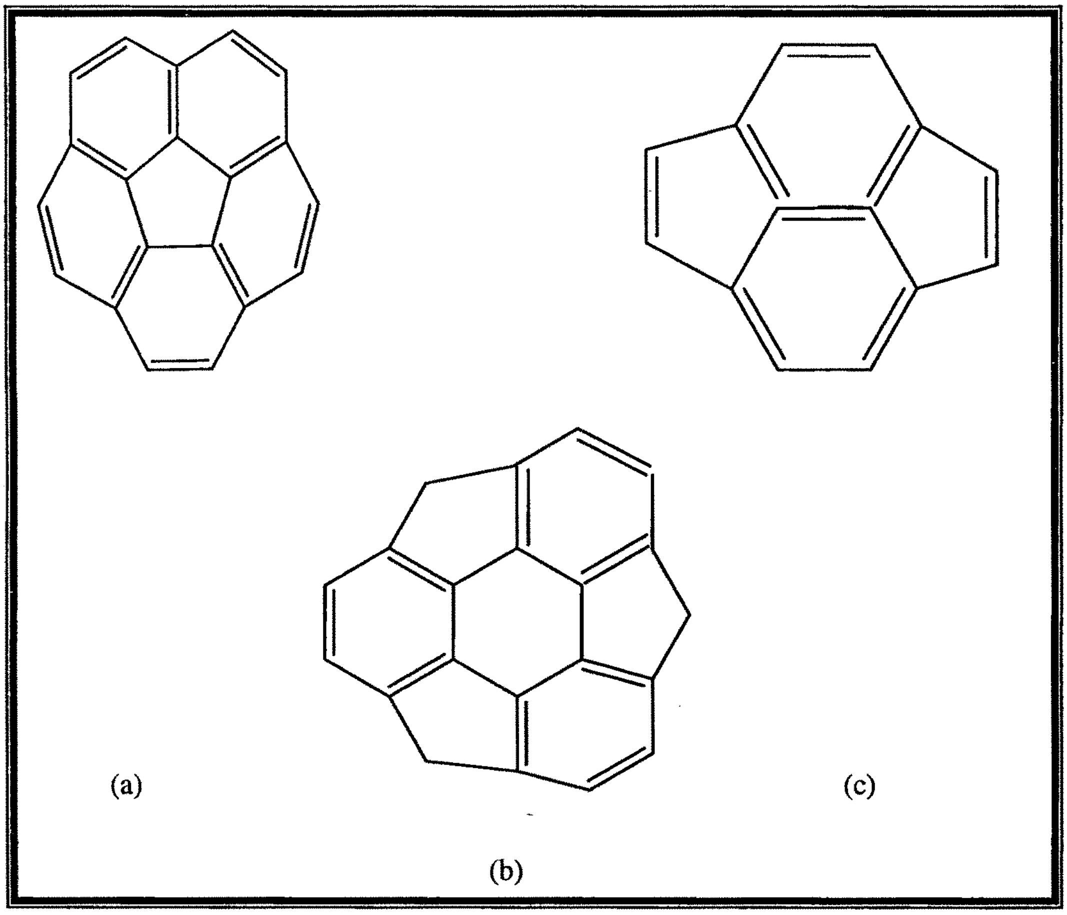


Figure.1.5. Structures of (a) Corannulene, (b) Sumanene and (c) Pyracyclene

The C_{60} molecule has two bond lengths, the C-C double bonds are located between two six membered rings and called 6,6-bonds (1.38Å) and are exocyclic with respect to five membered rings. The C-C single bonds are located between six and five membered ring and called 6,5-bonds (1.45Å). The hexagons and pentagons are arranged into a cage such that each pentagon is surrounded by hexagons. Because of this arrangement each carbon is bound only to other carbons, and imparts a special stability to C_{60}, making it the most stable of the fullerenes.

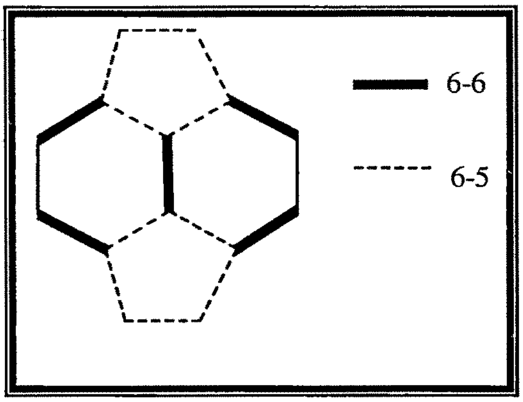


Figure.1.6. The 6-6 and 6-5 bonds of [60]fullerene

Thus C_{60} is not "super aromatic" as is expected due to the number of conjugated ring systems. As there are no double bonds in the pentagonal rings this results in poor electron delocalization [12]. Thus, C_{60} behaves more like an electron deficient alkene, and reacts readily with electron rich species.

1.1.4. PHYSICAL PROPERTIES

Some of the physical properties of [60]fullerene are shown in Table 1.1.

Table 1.1. Physical properties of [60]fullerene

Property	**Value**
Density	1.65 g cm^{-3}
Standard heat of formation	9.08 k cal mol^{-1}
Index of refraction	2.2 (600nm)
Temperature of sublimation	800K
Resistivity	1014 ohms m^{-1}
Vapors pressure	5 x 10^{-6} torr at R.T and 8 x 10^{-4} torr at 800K

1.1.5. Chemistry of [60]fullerene

From the first spectroscopic evidence, it was assumed that C_{60} would be extremely aromatic molecule. This was supported by the calculation that there are 12,500 resonance structures possible for C_{60}[13]. As presence of double bond in pentagonal ring produces Mills-Nixon effect[14], there is only one structure for C_{60}, which avoids any double bonds in the pentagonal ring. Thus there are two important consequences because of this, (i)the delocalization of electrons is poor in C_{60} so it is much more reactive than alkenes and gives more stability to the fullerene derivatives and (ii) fullerene has no hydrogen atom or other groups attached. Therefore, it cannot undergo substitution reactions however substitution reactions can take place on its derivatives.

[60]fullerene contains sp^2-hybridized carbons which shows electrons-withdrawing inductive effect[15] and they are strongly electron attracting and therefore readily react with nucleophiles. But the unique feature of [60]fullerene is that the large number of products that arise are from addition of just one reagent. The first reactions reported for C_{60} were the addition of amine [16] and the formation of the C_{60}-osmium tetraoxide adduct; that gave the first x-ray structure[17].

$$C_{60} \longrightarrow C_{60}(OsO_4)(\text{4-tert-butylpyridine})_2$$

From simple Huckel calculations it has been shown that C_{60} has a filled five-fold degenerate HOMO and a low lying triply degenerate LUMO[18] thus having a low reduction potential of 0.99eV and it can accept up to six electrons[19]. In contrast, oxidation of C_{60} is difficult (I.P +1.76 eV) but C_{60} radical cations can be produced by photo induced electron transfer and by other methods[20].

Since C_{60} behaves like an electron deficient alkenes and not as an aromatic hydrocarbon, it readily reacts with electron rich species. Its characteristic reactions involve cycloaddition as well as additions of nucleophiles and free radicals across the bonds between two six membered rings [21].

A wide range of conditions have been used to make the hydrogenated fullerenes. The simplest hydrogenated derivative of a fullerene synthesized is $C_{60}H_2$ and was first reported in 1993[22]. Other synthetic methods like electrochemical reduction of C_{60}, heterogeneous catalytic hydrogenation [23] have been used to get hydrogenated C_{60} derivatives. The first successful chemical reaction on the surface of C_{60}, (hydrogenation to $C_{60}H_{36}$ and subsequent dehydrogenation back to C_{60}), preparation of a highly hydrogenated fullerene and the first generation of ionic forms of C_{60} in solution have also been reported [24].

$$C_{60} \xrightarrow[\text{Rhodium/ } C_6D_6]{\text{Reduction, Pressure}} C_{60}H_2$$

Halogenation of fullerenes is a radical reaction. C_{60} has been partly chlorinated, fluorinated and brominated [25-27]. However iodination of the C_{60} molecule was not possible because of the weak C-I bond. At higher temperatures (~150 °C) halogens were easily removed. The stability order was found to be fluro-> chloro- >bromofullerenes. The same order has been observed for the solubility of halogenated fullerenes. The fluorinated material is very soluble in polar solvents and reactes rapidly with sodium bicarbonate, whereas the reaction with water is very slow due to the hydrophobic nature of the material. Brominated C_{60} acts as a Fridle-crafts alkylating reagent [28].

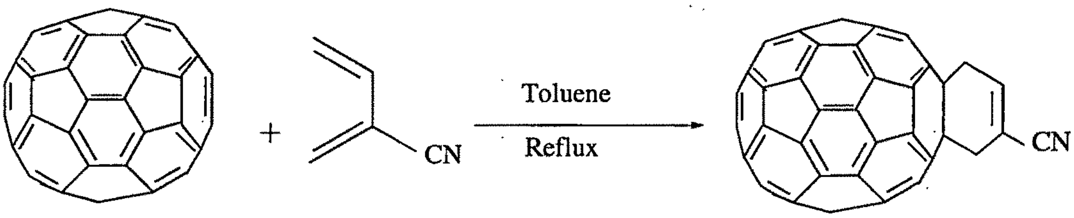
$$C_{60} \xrightarrow[250\ ^{\circ}\text{C}]{Cl_2\ \text{(g)}} C_{60}Cl_{24}$$

$$C_{60} \xrightarrow[\text{R.T/50 }^{\circ}\text{C}]{Br_2\ \text{(neat)}} C_{60}Br_n \qquad n = 2 \text{ or } 4$$

Aromatic substances containing electron supplying group were substituted on C_{60} molecules and this type of reactions were done in the presence of Lewis acid as a catalyst. The type of reaction is SN_1 substitution. The structure of $C_{60}Ph_5H$ was formed via electrophilic aromatic substitution. Reaction of $C_{60}Cl_6$ with benzene/$FeCl_3$ gave $C_{60}Ph_5Cl$, which was readily reduced to $C_{60}Ph_5H$[29].

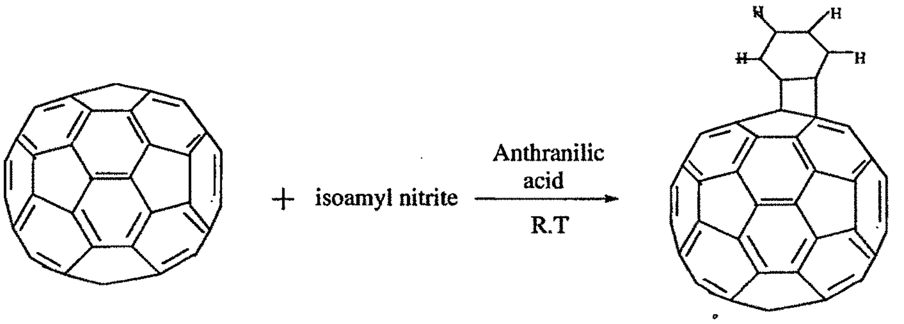
$$C_{60}Cl_6 \xrightarrow{\text{Benzene/FeCl}_3} C_{60}Ph_5Cl \xrightarrow{\text{Reduction}} C_{60}Ph_5H$$

A number of functional derivatives of fullerenes have been synthesized using cycloaddition to the fullerene molecule. Many of these were useful for the biological applications. [60]fullerene has been found to undergo Diels-Alder reactions with anthracene, furan and cyclopentadiene[30]. Rubin and his coworker have reported the X-ray structure of the Diels-Alder adduct of the C_{60} [31] in 1993.



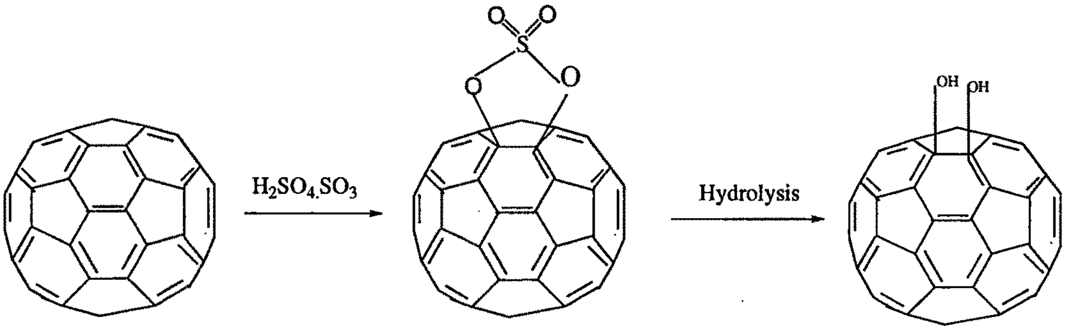
[60]fullerene 3-cyano butadiene

A series of C_{60} derivatives have also been synthesized by reacting C_{60} with benzyne [32]. The substitution of the benzyne to the C_{60} is the regioselctive reaction and depending on the reaction conditions stability of the compound was predicted.



[60]fullerene monobenzyne adduct

Nucleophilic, electrophilic and organoradical addition reactions have been reported as the most efficient methods for the polyfunctionalization of C_{60}. On incorporating polyol functional group on to a C_{60} cage, a polyhydroxylated C_{60} molecule ([60]fullerenols) form. To determine the electrophilic approach Chiang.L.Y and his coworkers have developed an aqueous acid reaction for introducing multiple hydroxyl groups directly on to fullerene[33]. The resulting polyhydroxylated fullerols exhibited high solubility in acidic aqueous medium. This reaction is shown below.



[60]fullerene hydrosulphated fullerene hydroxylated fullerene

A host of electron rich transition metals, lathanides, and actinides were reacted and bound to fullerenes and their derivatives. A large number of compounds that have reactive organic fragments like metalalkyls, carbenes, carbines, amides etc. have also been found to react with [60]fullerene. The anion of C_{60} also shows interesting metal chemistry and it is expected that this will lead to discovery of new catalysts or useful materials[34].

Compounds of fullerenes have been classified into two different categories. These are exohedral (inside the cage) and endohedral (outside the cage) compounds. Examples of the former include metals such as La enclosed in a C_{82} cage, and examples of the latter include transition metal complexes, with Ir. As the C_{60} structure is hollow, with room for one or more other atoms, such compounds are known as the endohedral fullerenes. When a metal atom is inside then they are known as the metallofullerenes[35]. Even though C_{60} is the most common fullerene, few endohedral materials have been synthesized using C_{60} as cage molecule, the reason being small size of the molecule. Most of endohedral materials are made out of C_{82}, C_{84} or even higher fullerenes. The atoms that form stable endohedral compounds include lanthanum, yttrium, scandium, and some of the noble gases[36]. Encapsulation of one or more metal atoms inside fullerene cages is one of the most exciting topics in the fullerene science, since it could give rise to new species or materials with novel properties which are unexpected for hollow fullerenes. The notation for endohedral materials is to use the @ symbol. The material that is encapsulated into the fullerene cage is written first. Thus recently progress has been made in the successful isolation and purification of Sc@C-82, Y@C-82, La@C-82, Gd@C-82, La-2@C-80, and Sc-2@C-84 in large quantities[37].

The most important and most versatile of all species of fullerenes is exohedral fullerenes or fullerene derivatives, which are molecules formed by a chemical reaction between fullerenes and other chemical groups. Exohedral fullerenes are also known as functionalized fullerenes. As fullerenes possess the conjugated π-system of electron, two main types of primary chemical transformations are possible on fullerene surface: addition reactions and redox reactions, which lead to covalent exohedral, adduct and salt respectively[38]. As fullerenes are insoluble in water, numerous derivatives of fullerenes have been synthesized with improved aqueous solubility.

The total of the possible reactions on fullerenes is summarized in Figure 1.7.

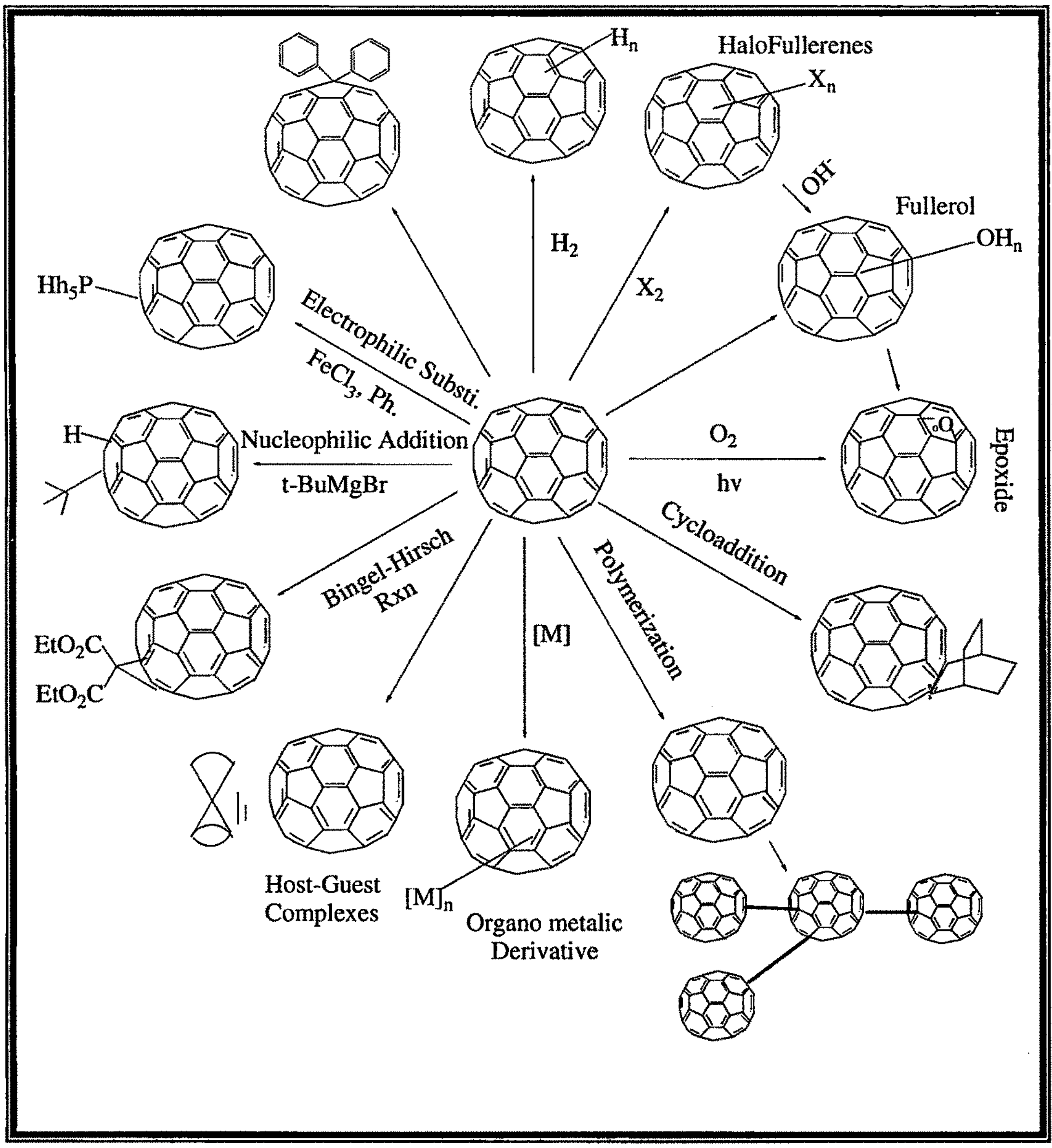


Figure 1.7. Reactions of [60]fullerene

1.1.6. APPLICATIONS

Investigations on fullerene and specifically [60]fullerene have shown a great increase in applications. $C_{60}F_{60}$ has been called a molecular ball of Teflon and can act as a lubricant [39]. As [60]fullerene can not be fully fluorinated it is not used as a lubricant and if partly fluorinated then it reacts with water that is not a good property for the lubricant. Fullerenes can be turned in to diamond with the least pressure and heat thus is a good source for the synthetic diamond [40]. There are many more applications of fullerene and are discussed below.

C_{60} has the reverse ability to reduce by up to 6 electrons. The reduction potential of C_{60} is similar to quinine and leads to application in quenching of oxygen radicals.

[60]Fullerene absorbs strongly in the ultra-violet region and moderately in the visible region of the spectrum [41] and forms a long live triplet state and sensitizes the formation of singlet molecular oxygen with 100% efficiency[42]. Thus it can be used as a photo sensitizer in photodynamic therapy. Recently D. M.Guldi and his coworkers have done the photophysical characterization of the crown linked methanofullerene ligand [43].

C_{60} is also called a "free radical sponge" [44], since it rapidly reacts with all sorts of reactive free radicals. This property of C_{60} is useful for a number of biomedical applications.

For the interaction of fullerene with peptides, proteins and DNA, computer models have been reported. Computer simulations of fullerene compound with DNA and binding of C_{60} to a specific peptide helix show evidence for molecular reorganization. However it has been shown that DNA cleavage occurs at guanine residues in the peptide chain without any significant sequence selectivity[45].. Viruses present in blood get inactivated by C_{60} in the presence of oxygen and light. Singlet oxygen generating agents are known to inactivate enveloped viruses and these are induced by photodynamic reactions. Since C_{60} has the approximately same radius as the cylinder that describes the active site of the HIVP and it is hydrophobic in nature, there is a strong possibility of strong hydrophobic interactions between C-O derivative and active site surface. These interactions can make

C_{60} derivatives inhibitor of the HIVP[46]. A water soluble fullerene derivative was found to be active against HIV protease in cell culture. Diversion of cell depends on chromosome rearrangements, which involve structure of cell microtubules. These microtubules are the target for anti-cancer drugs. Since cancer cells divide rapidly, where normal cells do not, it has been observed that water-soluble fullerene compound affects the microtubule assembly.

Fullerene can be made superconducting at above the temperature of nitrogen liquid but is difficult to maintain the low temperature so, fullerene and their derivatives have been observed to be super conducting at low temperature. There have been reports of superconducting material made of C_{60} doped with metallic potassium or rubidium[47]. The recent discovery of ferromagnetism in rhombohedral C_{60} polymers[48] has opened up the possibility of a whole new family of magnetic fullerenes. Magnetic behavior in fullerenes involves electrons from an unfilled *p* band.

The high chemical reactivity of the C_{60} molecules allows the synthesis of an enormous number of fullerene-derived polymers which are suitable for biological applications. Syntheses of water-soluble polymers and hydrophilic polymers have potential for biological applications. Water soluble derivatives or conjugates of fullerene were found to be effective in the inhibition of HIV protease [46].

Fullerenes have also been found to be promising materials for gas membranes due to the large unit cell parameters of its crystal structure. The crystallographic pores in the crystal form a high ordered sub-system, which can be used as a high density membrane for separation of gas mixtures into individual molecules. New fullerene derivatives like tri-cyanohydrofullerene,$HC_{60}(CN)_3$,andpoly{4[2[2(2methoxyethoxy)ethoxy]ethoxy]benzyl} fullerene, $C_{60}(TEO)_n$, have been used as additives for proton conducting membranes and incorporated into Nafion. The fullerene–Nafion composites are used as a proton exchange membrane fuel cells. These membranes have been found to exhibit an excellent handling with no extensive phase separation [49].

The high pressure, high temperature transformation of fullerites to super-hard carbon is another promising area with respect to their commercial utilization for the development of new generation wear-resistant material. Amorphous Si_xC_{1-x} films is an example of materials presenting low indentation hardness and high wear resistance [50]. A schematic of the potential applications of [60]fullerene is given in Figure 1.8.

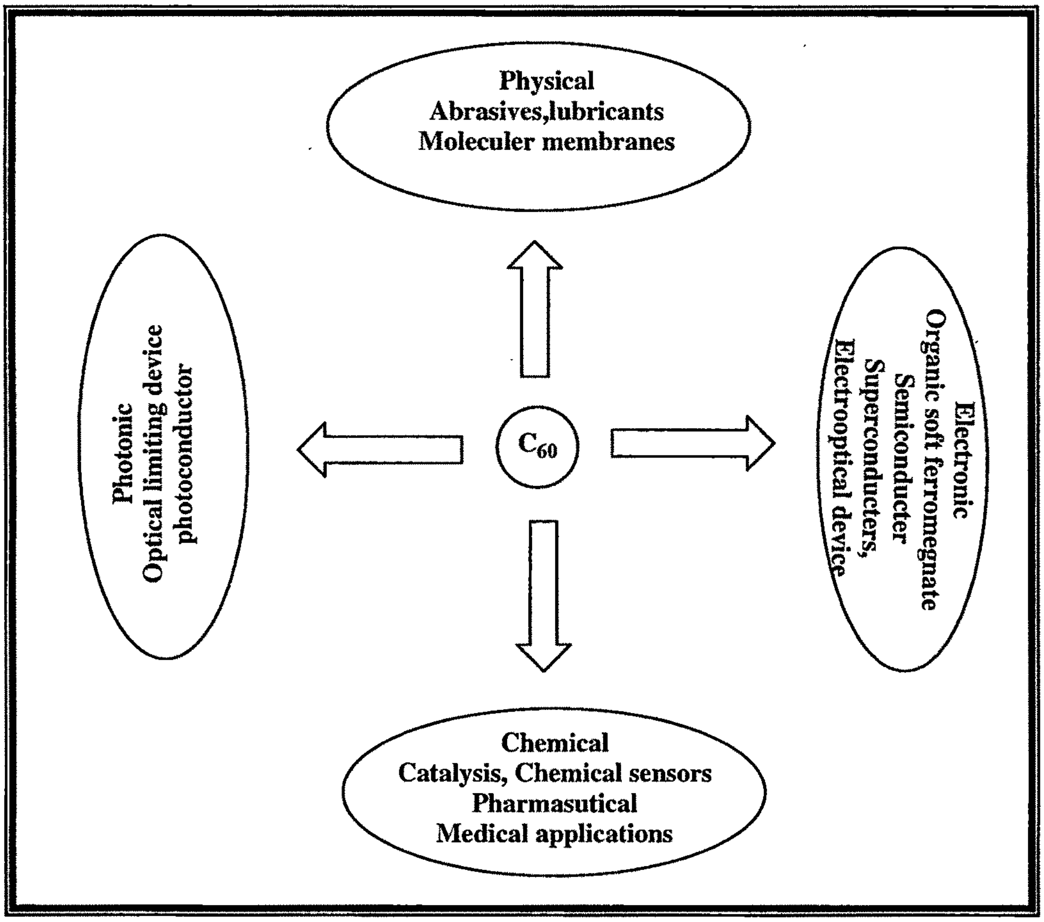


Fig.1.8. Schematic of the potential applications of [60]fullerene

A quantitative study on growth of published literature on fullerenes has shown a peak of about 1500 articles in the single year 1993[51]. The published research in the areas of covalent chemistry, chemical and crystal structure, electrochemistry, photo physical properties and superconductivity has declined subsequently. The main areas of research in fullerene science are summarized in the Figure 1.9 shown below.

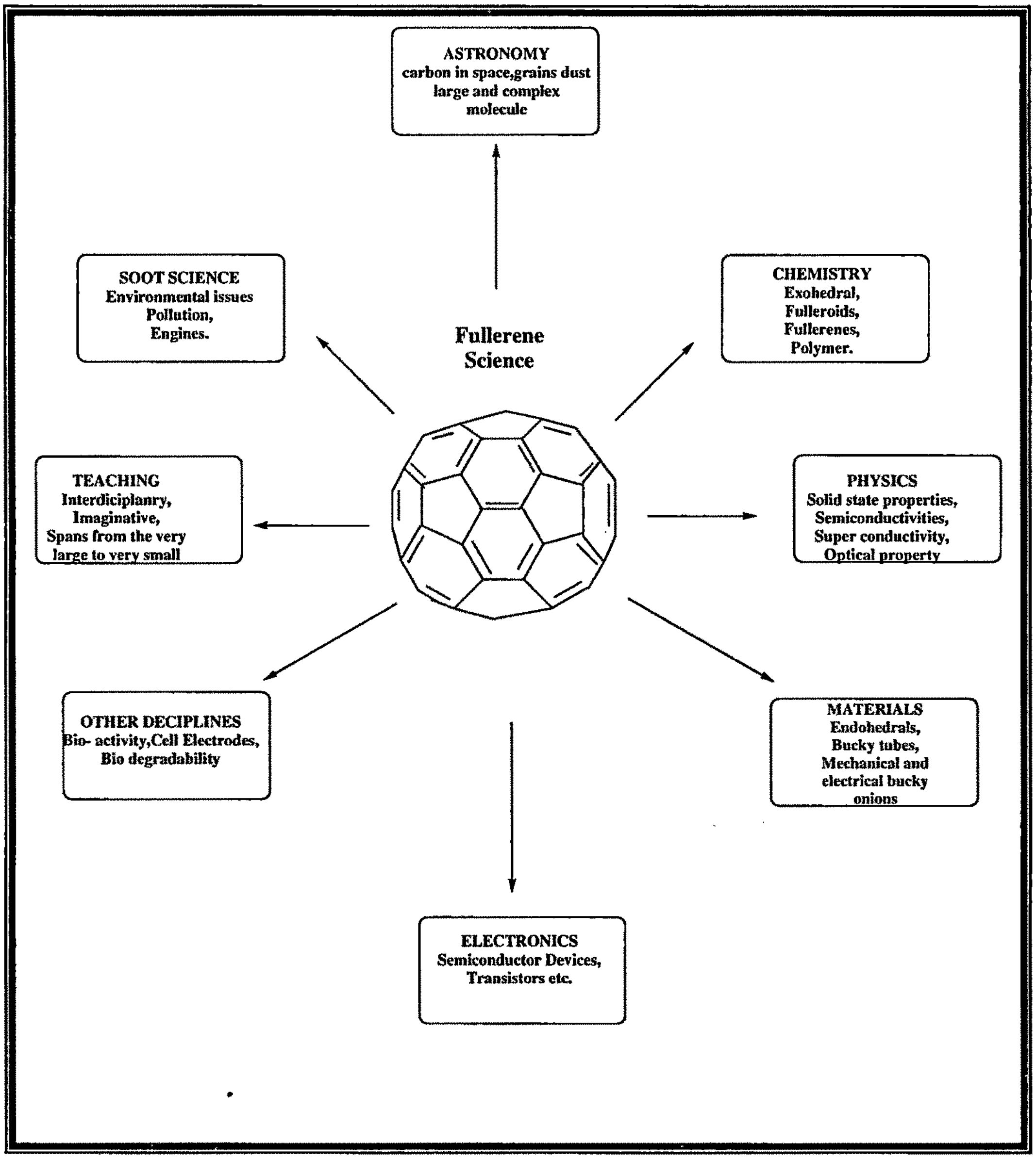


Figure.1.9 Overview of the areas of research on [60]fullerene

1.1.7. SOLUBILITY OF [60]FULLERENE

The solubility of C_{60} and other fullerenes is of fundamental interest and also has potential applications. Fullerenes have rigid, well-defined geometries, in contrast to the other solutes. Fullerene interacts with the solvent in two aspects of geometry: the molecular surface area and the molecular volume. Other factors involving the specific molecular interactions between the fullerene and the solvent molecule[52] are useful to determine its solubility. C_{60} has been described as a van der Waals solid form of inert gases, with little interaction between the molecules. The solubility of C_{60} is expected to be low as the energy required for the breaking of large number of solvent bonds during the dissolution is higher. The higher solubility of C_{60} in aromatic solvents as compared to aliphatic solvents has been explained on the basis of interaction between the solute and the solvent molecule[53]. [60]Fullerene is a hydrophobic molecule and is soluble in almost 150 solvents. Most common aromatic solvents are toluene, 1, 2-dimethyl benzene, 1, 2-dichloro benzene and carbon disulphide. The solubility of C_{60} in aliphatic hydrocarbons increases with the increase in the carbon number. Studies on C_{60} solubility shows that it is not soluble in polar solvents such as methanol or water. Increasing molar volume, solvent polarity diminished the solubility of C_{60} where as electron pair donation ability, and polarizability enhanced its solubility [54]. Solutions of pure [60]fullerene have a deep purple colour. The typical solubility values of [60]fullerene in some few solvents is shown in Table 1.2. The solubility of fullerenes has been found to be dependent on temperature and decreases with increase in temperature.

Table 1.2. Solubility of [60]fullerene in variety of solvents

Solvent	Solubility (mg/ml)
1,2,4-trichlorobenzene	20.0
carbon disulfide	12.0
toluene	3.2
benzene	1.8
chloroform	0.5
carbon tetrachloride	0.4
cyclohexane	0.054
n-hexane	0.046
tetrahydrofuran	0.037
acetonitrile	0.02
methanol	0.0009

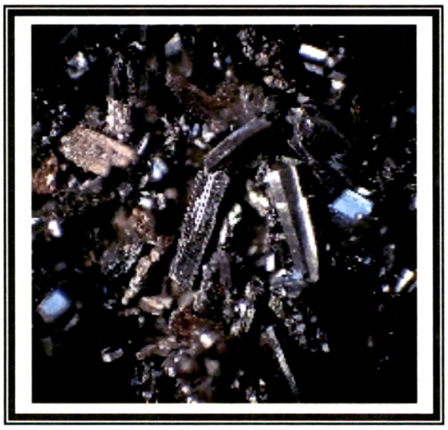


Figure 1.10. [60]Fullerene in the crystalline form.

The biological studies of fullerenes have been hindered because their hydrophobic nature. In an effort to overcome problems associated with their insolubility in aqueous solutions, several strategies have been attempted. One of them is to attach water-soluble moieties like PEG on the fullerene surface. Other methods include inclusion complexes of C_{60} with water-soluble hosts like cyclodextrins, calixarenes, liposomes, polyvinylpyrrolidone etc, or solubilization by surfactants. Other novel method was to make C_{60} fullerene water-soluble was to give ultra sonic treatment to a colloidal solution of C_{60} in toluene and water and the solution was found to be unaffected by boiling and changing of pH [55].

Functional groups with appropriate structure have been used to design water-soluble derivatives or co-polymers of fullerenes to make hydrophilic compounds. Mono-functional derivatives of C_{60} containing polar side chain are quite water-soluble and form poly adduct such as polyhydroxy fullerols. Polyhydroxy fullerols are water-soluble and have been used for biological experiments where the solubility of fullerene in water increases by adding polar groups to the C_{60} core.

1.1.8. SUPRAMOLECULAR HOST-GUEST COMPLEXES OF [60]FULLERENE WITH VARIOUS WATER-SOLUBLE HOSTS

Noble prize winner J. M. Lehn introduced the concept of supramolecular chemistry in 1988 [56] and has been described as the chemistry of the non-covalent bond. In supramolecular chemistry, host-guest chemistry describes complexes that are composed of two or more molecules or ions held together in unique structural relationships by hydrogen bonding or by ion pairing or by van der Waals forces.

Among the supramolecular structures with noncovalent binding, the guest–host chemistry of fullerenes, specifically [60]fullerene has been a topic of interest for more than a decade. The formation of supramolecular structures, assemblies, and arrays held together by weak intermolecular interactions and noncovalent binding has been used for applications being used in both materials science research and biological systems. The interest in [60]fullerene chemistry is due to its unique physical and chemical properties. These include (i) optical limiting property due to electronic absorption in the entire UV-VIS range; (ii) efficient singlet oxygen generating ability; (iii) radical scavenging character; and (iv) superconductivity on doping with alkali metals. The property (i) and (iv) are useful in materials research, the property (ii) and (iii) have applications in the biomedical area.

New methods of incorporating the essentially hydrophobic molecule with low solubility in most organic solvents are being developed. Novel supramolecular structures with enhanced solubility in both polar and nonpolar solvents have been prepared [57, 58]. A number of host molecules have been found to form supramolecular structures with [60]fullerene including calix[3,5,6,8]arenes[59-62], calix[4]naphthalene[63], cyclotriveratrylene[64],triptycenes[65],porphyrine[66],γ-cyclodextrin[67]and β-cyclodextrin[68]. Noncovalent interactions between cyclodextrins and other host molecules have been effectively used. The biomedical applications of fullerene imply its possible reaction with most essential cellular components like nucleic acids and proteins. Earlier studies on DNA-fullerene interactions have shown that the [60]fullerene cleaves DNA and is base specific[69]. It has also been shown that [60]fullerene interaction with HIV virus protease inhibited the activity of the latter[70]. The specific role of the [60]fullerene in these systems is not very clear.

1.1.8.1. Lysozyme

Lysozyme is an enzyme (MW-14,600) found in hen egg-white, tears, and other secretions. This is commonly referred to as the "body's own antibiotic" since it kills bacteria. It breaks the polysaccharide walls of many kinds of bacteria and provides some protection against infection. Alexander Fleming (1881-1955), the discoverer of penicillin, described lysozyme in 1922 [71]. Its structure was described by David Chilton Phillips (1924-1999) in 1965 [72,73]. Phillips provided an explanation for how enzymes speed up a chemical reaction in terms of its physical structures. The mechanism proposed by Phillips was revised in 2001 by Vocadlo, D. J and his co workers [74]. Lysozyme is a best object for X-Ray analysis due to many unique properties. First of all Lysozyme is easy to purify from hen egg white. Secondly, this protein is very easy to crystallize, as compared to other proteins.

The primary structure of hen egg-white lysozyme shown in Fig 1.11, is a single polypeptide chain of 129 amino acids. There are four pairs of cysteines that form disulfide bridges between positions 6 and 127, 30 and 115, 64 and 80, 76 and 94 (counting from the N-terminal lysine). These cross-bridges show that this polypeptide is not a straight chain (like cellulose) but the chain folds and allows the cysteines to be close to each other. The alternative name for lysozyme is 1,4-N-acetylmuramidase, L-7001, N,O-diacetylmuramidase, PR1-Lysozyme, Globulin G1, Globulin G, Lysozyme g, Mucopeptide N-acetylmuramoylhydrolase, Mucopeptide glucohydrolase and Muramidase.

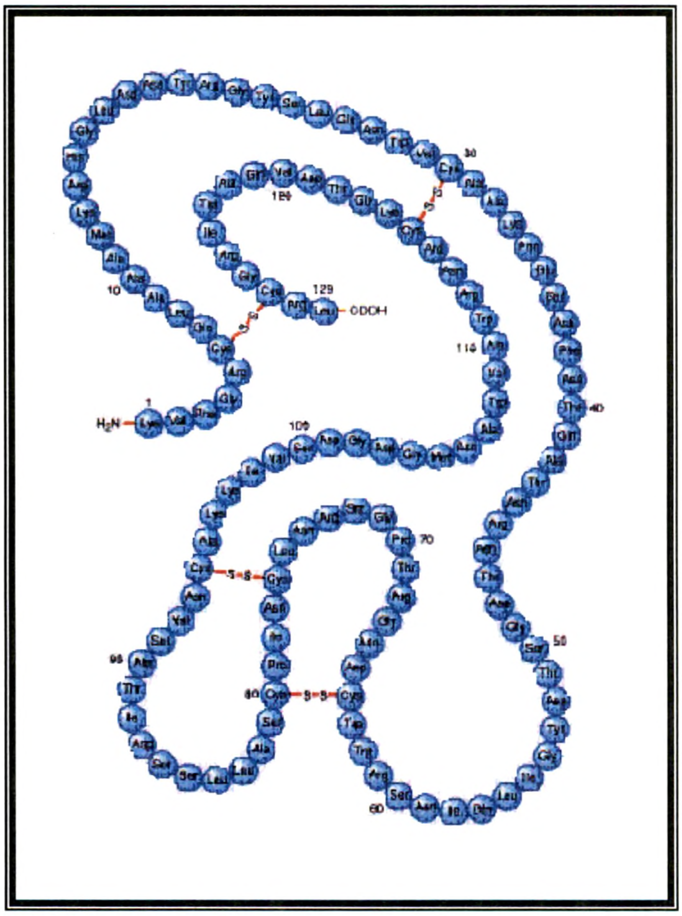


Figure 1.11. The primary structure of hen egg-white lysozyme.

The solid ribbon representation of lysozyme is shown in Figure 1.12 below. The alpha-helices are shown in red colour; beta-strands in blue and the irregular loops are shown in grey.

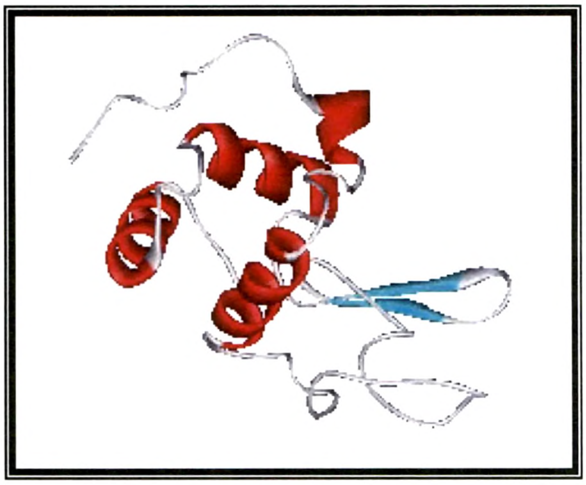


Figure 1.12. Solid ribbon representation of lysozyme

Lysozyme is a single domain protein with beta helical and beta sheet structure[75], with the inner core of the protein being hydrophobic. Lysozyme has been shown to dissolve in solvents like DMSO and glycerol. Lysozyme does not bind hydrophobic molecules like 8-anilino,1-naphthalenesulfonic acid, however, when denatured it interacts with the latter through exposed and hydrated hydrophobic amino acid residues[76]. Three dimensional structure of lysozyme is available in both solution as well solid states.

1.1.8.2. Calixarenes

Adolf Von Bayer pioneered the chemistry of calixarenes although he was unable to determine its structure and did not realise its potential. The first attempt to control the reaction betweent the substituted phenol and formaldehyde was made by Alois Zinke and Erich Ziegler in 1942 [77] where they suggested that p-substituted phenols reacts with formaldehyed to yield a cyclic tetramer.

The space filling model of a cyclic tetramer shows that it is not a flat molecule but is cup- or vase shaped, as shown in the Figure.1.13.

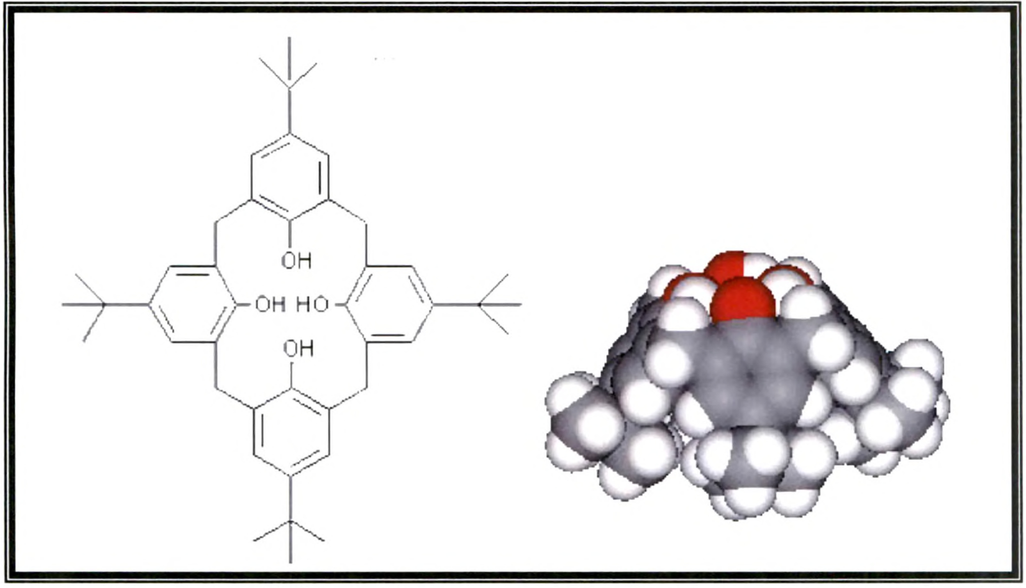


Figure 1.13 (a) Calix[4]arene with para-tert-butyl substituents (b) 3D representation of a cone conformation.

As this molecule resembles the shape of a type of Greek vase called calix crater, it was David Gutsche who gave[78] the name "calixarene" in 1975. 'Calix' defines the shape of molecule and 'arene' indicates the presence of aryl groups. To define a particular calixarene as a cyclic tetramer a bracket [4] is placed between calix and arene –thus calix[4]arene. This nomenclature has been generally accepted and is currently being applied to all the cyclic oligomers obtained by condensing aldehyde with phenols.

These aromatic components are derived from phenol[79], resorcinol [80] or pyrogallol, for phenol and the aldehyde most often used is simply formaldehyde, while large aldehydes are generally required for the resorcinol and pyrogallol. The reaction between the phenol and aldehyde is a electrophilic aromatic substitution reaction followed by an elimination of water and then second aromatic substitution. The reaction is either acid or base catalyzed. The most common of calixarene are the even numbered calix[4]arene, calix[6]arene and calix[8]arene [81-83].

Calixarenes are difficult to synthesize because generally it is easier to get a complex mixture of linear and cyclic oligomers with different number of repeating units. In 2005, pyrogallol[4]arene was synthesized by simply mixing isovaleraldehyde with pyrogallol and catalytic amount of p-toluene sulfonic acid in a mortar and pestle[84].

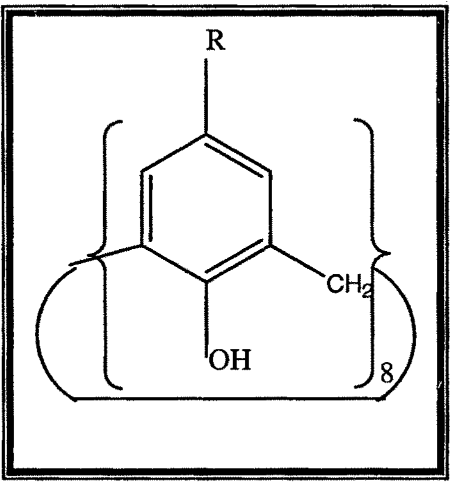


Figure 1.14. The representation of the structure of calix[n]arene

The conformational characteristics of phenol-derived calixarenes have been studied in considerable detail by Kammerer and coworkers[85], Andreetti, Pochini and Ungaro and coworkers[86], Munch[87] and Gutsche and coworkers[88], and those of the resorcinol-derived calixarenes have been studied by Hogberg [89] and Abis and coworkers[90]. Andreeti and his group have shown that the phenol-derived calix[4]arenes and calix[5]arenes exists preferentially in the cone conformation, whereas the larger and more flexible calix[6]arenes assume a somewhat less cone like shape whereas cyclic octamers adopt a flat 'pleated loop' form.

Calixarenes are characterised by a wide upper rim, a narrow lower rim, and a central annulus, with phenol as a starting material, the 4-hydroxyl groups are intra annular on the lower rim. In a resorcin[4]arene the 8 hydroxyl groups are placed extra annular on the upper ring. In calix[4]arenes the internal volume is around 10 cubic nanometers.

Calixarenes exist in different chemical conformations because rotation around the methylene bridge is not difficult. In calix[4]arene four up-down conformations exist: cone, partial cone, 1,2 alternate and 1,3 alternate[88]. The four hydroxyl groups interact by hydrogen bonding and stabilize the cone conformation.This conformation is in dymamic equilibrium with the other conformations. As the number of aryl groups in cyclic array increases, the number of conformations likewise increases. Calix[5]arene can have only four conformers, calix[6]arene has eight and calix[8]arene has sixteen etc. Figure 1.15 below show the various conformations of calix[4]arene.

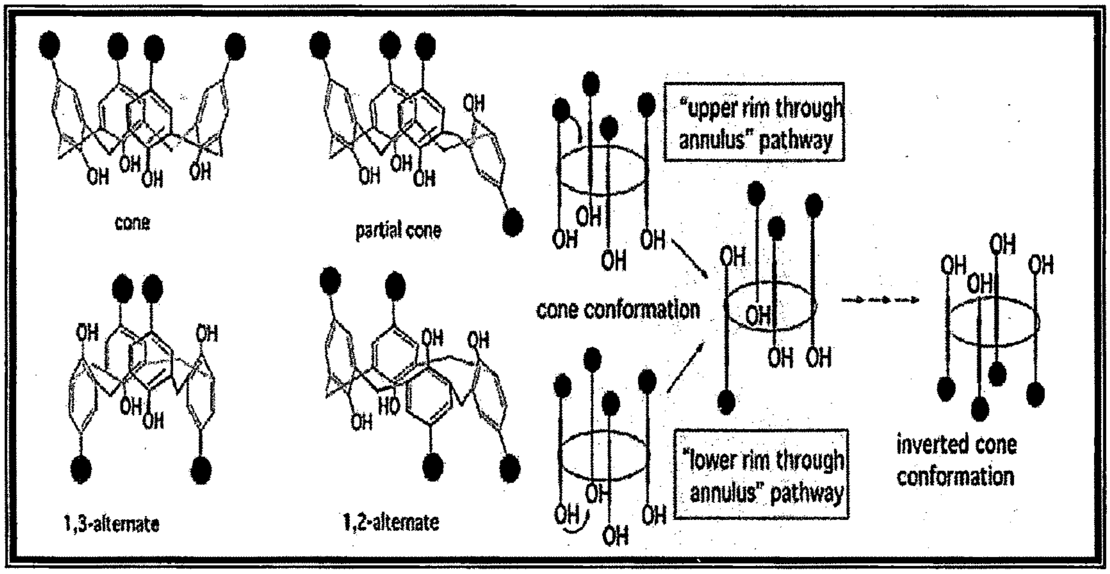


Figure1.15. Conformations of Calix[4]arene and the possible mechanisms for their interconversions.

Calixarenes are sparingly soluble and are high melting crystalline solids[91]. They possess limited solubility in organic solvents and virtually no solubility in water. This hinders their purification and characterization. It is expected that derivatization should alter the solubility of calixarenes and even render them soluble in water.

Ungaro and co-workers [92] prepared the first water-soluble carboxyl containing in 1984. Subsequently Shinkai and his coworkers reported another water-soluble calixarene where a sulfonic acid group was introduced in the para position at the upper rim [93]. The calixarenes are stronger acids than their monomeric phenolic counter parts, but the accurate measurement of their acid value has not been achieved [94]. Numerous studies of the complexation behavior of water-soluble calix[n]arenes have been reported. Calix[n]arenes behave as a third generation of host molecules after Cyclodextrins and Crown ethers. Calixarenes are cyclic oligomers made up of benzene units similar to cyclodextrins which are made up of glucose units. Calixarenes are of potential interest for host-guest complexation studies [79]. Calixarenes have hydrophobic cavities that can hold smaller molecules or ions. Calixarenes are efficient sodium ionophores and are applied as such in chemical sensors and also exhibit great selectivity towards other cations. Calixarenes are used in commercial applications as sodium selective electrodes for the measurements of sodium levels in blood [95].

Calixarenes have been shown to complex fullerenes [96]. This host-guest phenomenon is driven by π-π interactions. Inclusion complexes obtained from various calixarenes have found applications for the direct purification of fullerenes from carbon soot [96]. In addition, such host-guest systems have been used for the incorporation of fullerenes into well-ordered systems such as Langmuir films or self-assembled monolayers. In light of their unique electronic properties, fullerene derivatives are also suitable building blocks for the preparation of molecular devices displaying photo induced energy and electron transfer processes.

1.1.8.3 Cyclodextrins

Cyclodextrins are a family of cyclic oligomers of glucose, which typically contains 6-8 monomers in a ring. These natural products resulted from the degradation of starch by the bacterium Bacillus macerans and were discovered in 1891 by Villiers[97]. There are three naturally occurring cyclodextrins with 6, 7 or 8 D-glucopyranonsyl residues (α-, β-, and γ-cyclodextrin respectively) linked by α-1, 4 glycosidic bonds. All three cyclodextrins have similar structures i.e. bond lengths and orientations. They present a bottomless bowl-shaped (truncated cone) molecule stiffened by hydrogen bonding between the 3-OH and 2-OH groups around the outer rim shown in Figure 1.16.

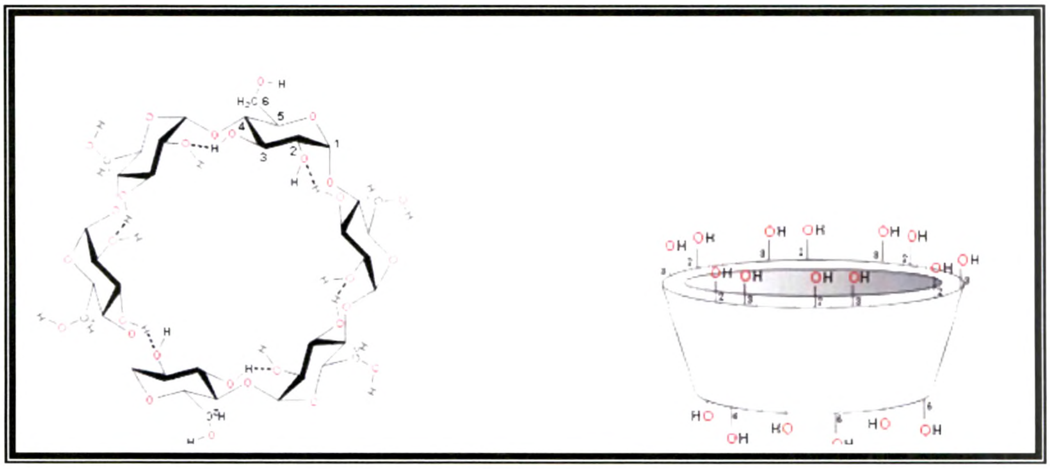


Figure1.16. Bottomless bowl-shaped (truncated cone) general structure of cyclodextrins molecule.

The hydrogen bond strengths are in the order: α-cyclodextrin < β-cyclodextrin < γ-cyclodextrin. All the three cyclodextrin units have cavities with different diameter which depends on the number of glucose units present, while the side rim depth is same for all three (at about 0.8 nm).

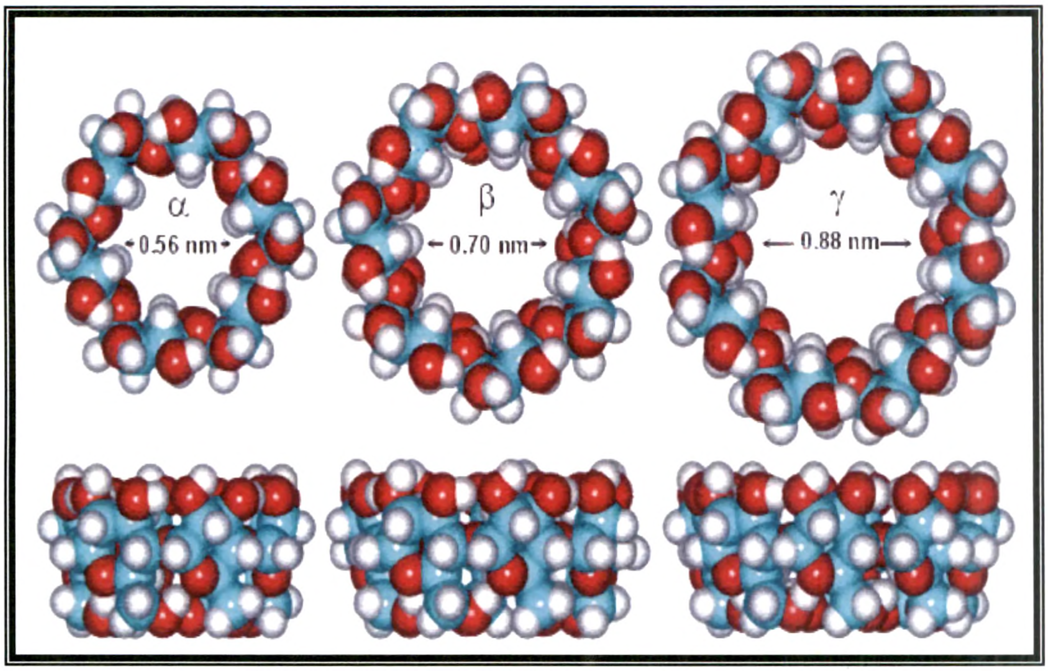


Figure1.17. Different cavity sizes in α, β and γ-cyclodextrin

Cyclodextrins can be represented as toroid in which primary hydroxyl groups are located inside and secondary ones outside. These make the interior of the toroids hydrophobic and are able to host other hydrophobic molecules, while the exterior is sufficiently hydrophilic to allow such host-guest complexes to dissolve in water. Cyclodextrins bind other molecules in 1:1, 2:1 and 1:2 ratios depending on the molecules involved. For example, two molecules of γ-cyclodextrin and β-cyclodextrin bind well to single C_{60}-fullerene molecules [67, 68, 98]. The binding is driven by the enthalpic and entropic gain. Such binding allows the cyclodextrins to be used to increase the water solubility of normally hydrophobic compounds, as well as to minimize the undesirable properties such as odor, or taste in certain food additives. Cyclodextrin complexes have been widely used in the pharmaceutical, food and cosmetic and toiletry fields [99].

1.2. MICROEMULSIONS

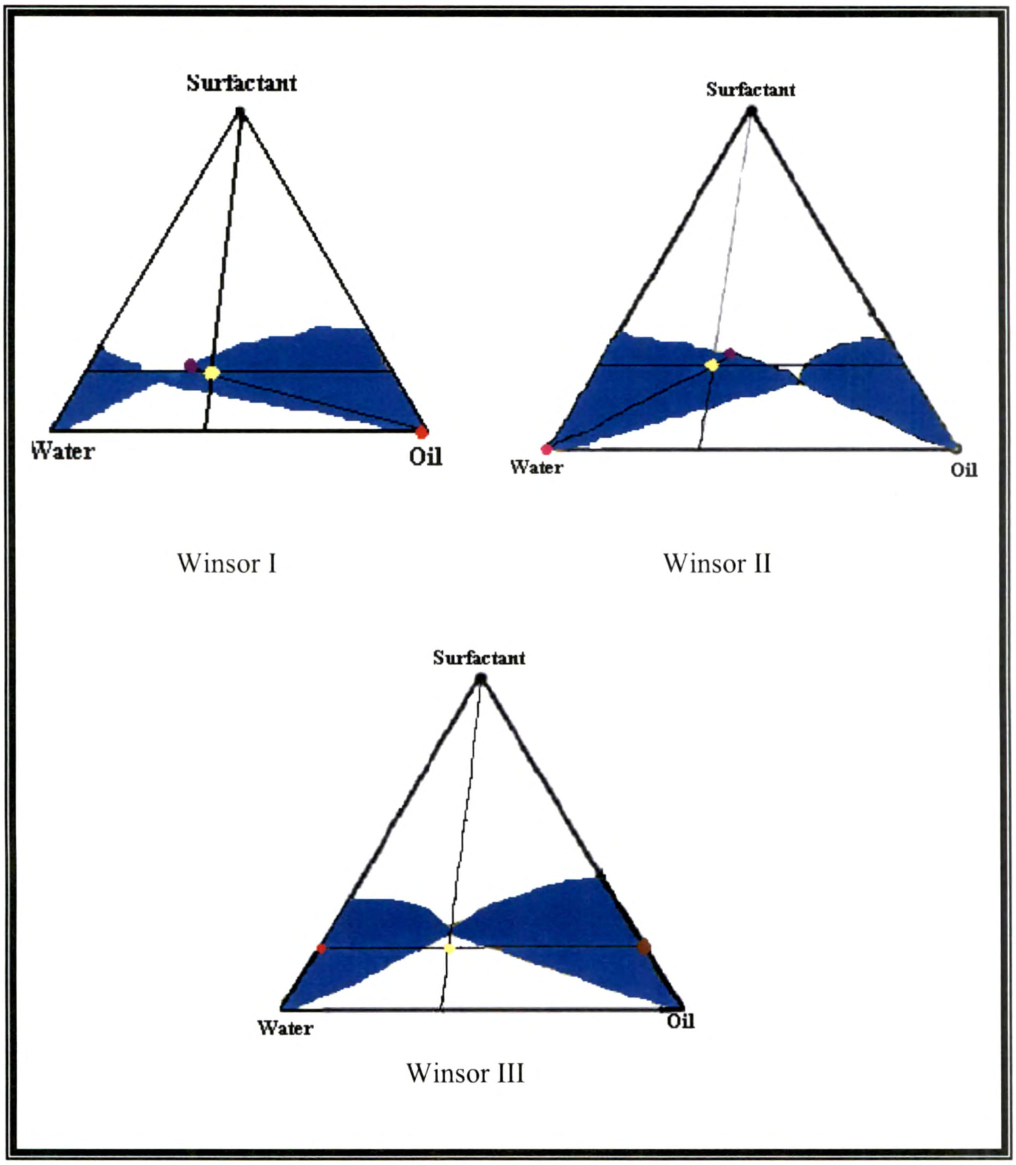
Microemulsions (μE) are thermodynamically stable fluids composed of water and oil, which are separated by a layer of surfactant molecules. Surfactants molecules with a hydrophilic head and a hydrophobic tail, serve to stabilize the fluid and prevent it from macroscopic phase separation into water and oil. Microemulsion or swollen miceller systems represent an intermediate state between miceller solutions and true emulsions, resulting from the mutual solubility of water and oil. Such mutual solubility requires the addition of surface-active agent so that water in oil or oil in water microemulsion may be obtained according to the components nature and their concentration ratio. Many systems require the presence of alcohol and /or salt for their formation and stability [100].

Microemulsion is not really a "special case" of a regular emulsion or macroemulsion. Regular emulsions and microemulsions are fundamentally different. The most fundamental difference between these two is that microemulsions exist in a state of thermodynamic equilibrium whereas macroemulsions are meta stable two phase systems.

Microemulsions are typically classified into three main categories, or Winsor-type systems [101]. Winsor's Type I microemulsions consist of oil-swollen micelles in a water, where as a Type II system consists of water-swollen reverse micelle in an oil. Type III microemulsion systems can be understood as an accumulation of swollen micelles that touches one another, forming either some kind of percolated dispersion or a perfectly bicontinuous structure[102,103] in which all water domains are connected and all oil domains are likewise connected. The boundary between the oil and water domains does not exhibit a strong curvature means the Winsor type III diagram contains a region exhibiting a three-phase behavior, the occurrence of which is not limited to surfactant systems.

Winsor's approach is actually the conceptual framework to understand the formulation and microemulsions. In Figure 1.18, the phase behavior of a SOW (surfactant-oil-water) ternary system is shown. Ternary-phase diagrams are very helpful in understanding the role of the surfactant in microemulsion systems. In a ternary-phase diagram, each of the vertices corresponds to a pure component (surfactant, oil, and water), and the composition of a particular mixture of the three components is represented as a point inside the triangle. In the case of the Type I and Type II microemulsion systems shown in

Figure 1.18, the dome in the lower part of the triangle represents a two-phase (polyphasic) region. A SOW mixture inside the dome will separate into two phases, as shown in Figure 1.18.



Winsor I

Winsor II

Winsor III

Figure1.18 The three types of phase behavior for surfactant-oil-water system according to Winsor.

Microemulsions are generally differentiated from ordinary emulsions by their spontaneous formation, transparency, thermodynamic stability, small particle size, and the limited range of concentrations over which they exist. These thermodynamically stable and optically isotropic systems have a wide range of applications in various industrial processes such as oil recovery, cosmetics, pharmaceuticals, food, and medicine [104]. Because of many interesting properties involved in the microemulsion systems, considerable efforts (both experimentally and theoretically) have been devoted to their studies [105].

The structure of microemulsions has been extensively studied using NMR, fast freeze-fracture, cryo-electronmicroscopy, and electrical birefringence [106]. A microemulsion can form a variety of phases that depend on its composition and on the values of the state variables [105]. Recent work has shown that by carefully changing the nature of surfactant, micro emulsions can also display storage stability over a temperatures range of -10°C to +55°C and exhibit excellent long term dilution properties in water of varying degrees of hardness[106].

As solublization is concerned, the best microemulsions are found to have a zero net-curvature condition for the surfactant layer between the oil and water domains. Solublization can be measured in two ways. First, it can be measured through the solublization parameter, which is defined as the maximum amount of oil (respectively, water) solubilized in the microemulsion per unit amount of surfactant. The word "maximum" implies an excess of this phase, and is hence a multiphase system. Another way to measure solublization is to measure the minimum amount of surfactant necessary to produce a single phase in an SOW system (i.e., the surfactant, oil and water coexist as one phase). This concept can easily be observed in a ternary diagram representing the phase behavior since it corresponds to the height of the polyphasic zone located near the OW side.

Solublization is related to the presence of surfactants that are able to produce micelles. Since micelles are aggregates with 100 or so molecules, a small amount of surfactant less than 10% is sufficient to solubilize large amounts of both oil and water, one outside the micelles (or inverse micelles) and the other one inside them [107].

1.2.1. Surfactant Aggregates or Micelles

Surfactants or surface active agents are amphiphilic ie. They contain both hydrophilic and hydrophilic groups. Surfactants are classified into four groups namely, anionic, cationic, non-ionic and zwitterionic. The monomer surfactant molecules self aggregate to form large aggregates called micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their ionic/polar heads form an outer shell that maintains favorable contact with water.

When surfactants assemble in oil, the aggregate is refered to as a "Reverse micelle". In reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. The schematic structures of the micelles and reverse micelles are shown in Figure 1.19.

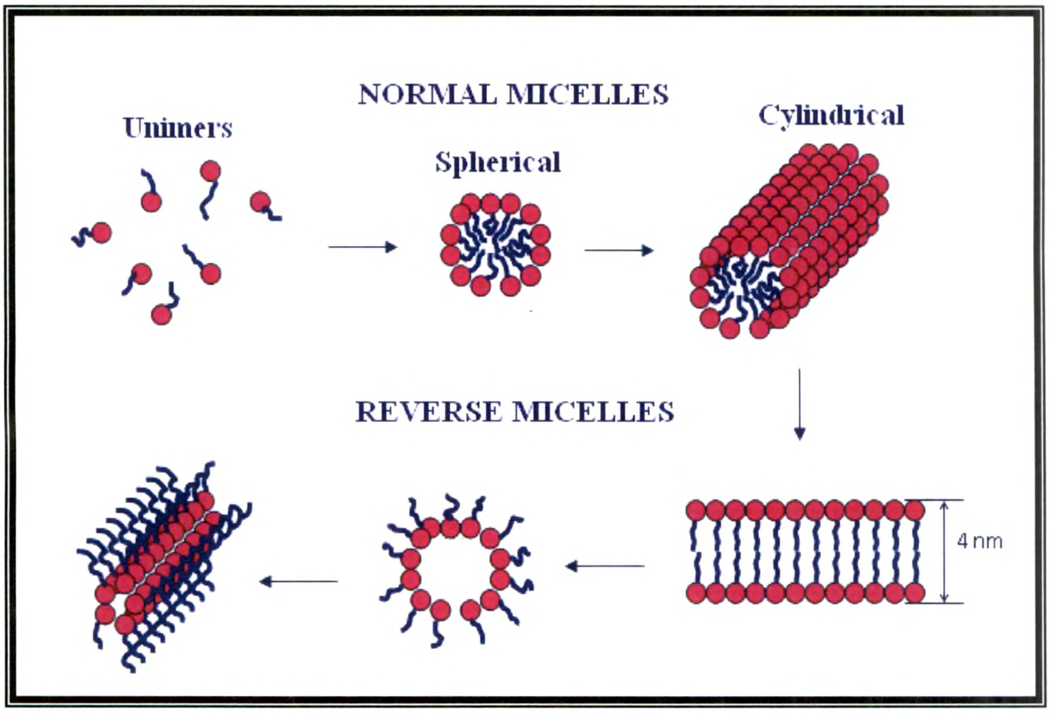


Figure 1.19 Surfactant aggregates with hydrophilic head (●) and hydrophobic tails (~).

Micelles only form when the concentration of surfactant is equal or greater than the critical micelle concentration(CMC), and the temperature of the system is greater than the critical micelle temperature, or Krafft temperature. The formation of micelles can be understood using thermodynamics i.e micelles form sponteneously because of a balance between entropy and enthalpy. The properties of micelles depends on the parameters such as temperature, pressure concentration and the chemical nature of the surfactant. Solublization is the phenomenon of increased solubility of a substance, normally insoluble or slightly soluble in a continuous solvent phase, in the presence of supramolecular aggregates in that phase. Solublization word was initially used for aqueous miceller solutions but has been used to other solutions containing organized surfactant assemblies (e.g., reversed micelles, vesicles, bilayers) and to block-copolymer micelles.

Solublization of small guest molecules in reversed micelles is less studied, although it has attracted increased attention over the past 20 years. Much of the initial work on solublization in reversed micelles focused on the solublization of water[108], which is of primary importance in enhanced oil-recovery applications. More recently, the focus has turned to the solublization of proteins and the other biological macromolecules, because of its importance in membrane studies and in biotechnology.

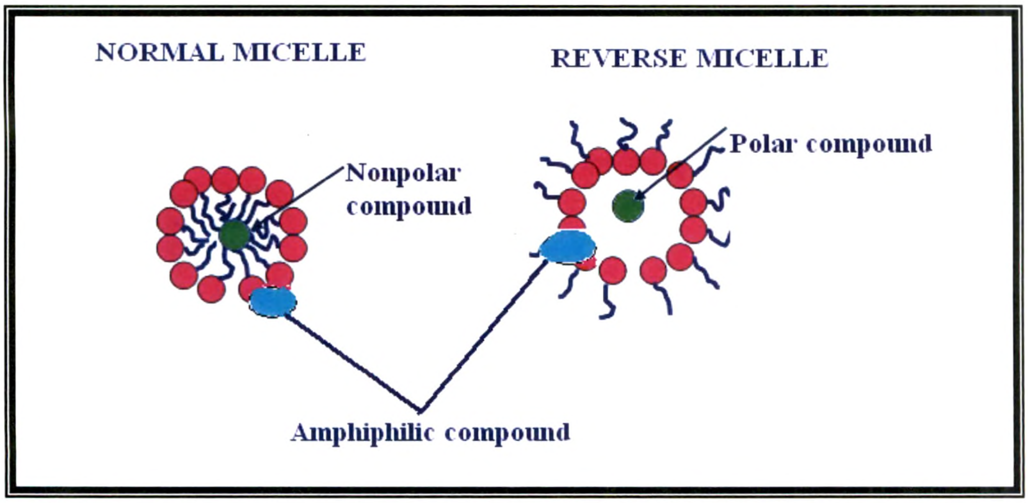


Figure 1.20 Solublization of compound insoluble in bulk solvent in to the surfactant micelle.

Nonionic surfactants are known to form reverse micelles in organic media. In this case, the hydrophilic groups are sequestered in the micelle core and the hydrophobic groups remain solvent-exposed on the surface of the micelle. These reverse micelles are extremely difficult to form from surfactants with charged head groups, since hydrophilic sequestration would create highly unfavorable electrostatic interactions. In a recent report C_{60} has been shown to be soluble in pure nonionic surfactant (hexaethyleneglycolmono-dodecyl ether $C_{12}EO_6$ [109]. It has been reported that stable dispersion using Triton X-100 C_{60} exists in miceller form with mean diameter of ~10 nm [110] depending on the molar ratio of C_{60}/Triton X.

1.3. AIM OF THE THESIS

Although C_{60}, buckminsterfullerene, was discovered in 1985, it was not until Kratschmer discovered in 1990 that C_{60} was soluble in benzene. The practical problem of fullerene solubility has partially been solved just by collecting experimental data. The solubility of C_{60} is known in almost 150 solvent, probably the highest number among all chemical substances but because of the hydrophobic nature of the C_{60} it is very difficult to dissolve fullerene in water and other polar solvents.

The hydrophobic nature of C_{60} and its unique shape render this molecule very interesting for its potential use in superconductors, ferromagnets, lubricants, photoconductors, catalysts, medical materials etc. However, a wider application of [60]fullerene is heavily limited by their poor solubility in aqueous media, especially for biological applications/activities such as enzymatic inhibition, anti-HIV activity, neuroprotection, antibacterial activity, DNA-cleavage and photodynamic therapy.

To overcome the difficulty of strong hydrophobicity of this carbon allotrope, [60]fullerene was made water soluble using various water-soluble host molecules which form supramolecular structures with [60]fullerene. This thesis contains a study of synthesis of various water-soluble supramolecular complexes of [60]fullerene. With this in view the following synthetic water-soluble host-guest supramolecular systems of [60]fullerene have been studied.

(A) Lysozyme-[60]fullerene adduct

(B) Reverse micelles of [60]fullerene and its microemulsion study

(C) [p-(N, N-Diallylaminomethyl)] Calix[8]arene-[60]fullerene complex

(D) α, β-Cyclodextrin-[60]fullerene Complex

The first approach to select lysozyme as a water-soluble host is because lysozyme is an enzyme (MW-14600) found in egg white, tears, and other secretions. Commonly referred to as the "body's own antibiotic". since it kills bacteria. [60]fullerene has a binding capacity to a specific peptide helix, it was proposed to prepare the adduct of [60]fullerene with lysozyme.

It has been reported that [60]fullerene shows ferromagnetism in pristine state when exposed to ultraviolet radiation over a period of time. One of the major drawbacks of the above mentioned organic ferromagnets is (1) their aqueous solubility and (2)

Biocompatibility. Pristine fullerene is water-insoluble and so has to be made water-soluble for further applications. Polymerized fullerenes would be much more difficult to be solubilized in water. In view of these observations it was thought to induce magnetism by first making a water-soluble fullerene and irradiating this complex with various energies of ultraviolet radiation.

Another approach is to incorporate C60 in to water-soluble superstructures such as α, β-cyclodextrin, [p-(N, N-Diallylaminomethyl)]calix[8]arene and amphiphilic surfactant structure (Reverse Micelle).