Fullerenes: From Carbon to Nanomedicine

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Abstract: Fullerenes, the third carbon allotrope, have emerged as agents which could revolutionize the treatment of many diseases. Fullerenes possess different biological applications like neuroprotective agents, antioxidants, anti-HIV activity, enzyme inhibition, antiapoptotic activity and the list is ever increasing. Moreover, they are being utilized as drug carrier systems and also for many non-biological applications like superconductors, catalysis and so on. Their size has made them promising agents for nanotechnology. This article aims at outlining the chemistry, properties and non-biological applications of fullerenes and their evolution to biological applications, thereby traversing their evolution from simple carbon allotropes to present day nano-medicinal agents.

Keywords: Anti HIV, antioxidant, antiapoptotic, enzyme inhibition, nanomedicines, neuroprotective.

INTRODUCTION

Fullerenes are a family of carbon allotrope molecules, in the form of a hollow sphere, ellipsoid, tube or plane. The fullerene revolution began with the discovery of the buckyball (C_{60}), composed of 60 carbon atoms arranged in a hollow soccer ball shape. Buckminster Fullerene (Fig. (**1**)) was named after Richard Buckminster Fuller, the architect who created the dome in 1967 with the same shape as that of the carbon cluster [1].

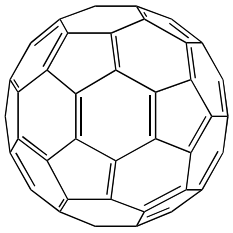

Fig. (1). Buckminsterfullerene.

Fullerenes containing 60-70 carbon atoms with diameter of about 1 nanometer (nm), were discovered in 1985 by Kroto *et al*. The structure was confirmed by mass spectrometry [2] and they were awarded nobel prize for their discovery of fullerenes in 1996.

Chemistry and Properties of Fullerenes

Fullerenes were synthesized by using laser vaporization of graphite as a target to produce clusters of carbon chains. Time of flight mass spectrometry was used to probe the carbon plasma produced by laser vaporization. A strong peak at 720 amu indicated that a carbon molecule with 60 carbon atoms was formed [2]. Fullerenes are separated by sublimation HPLC, or liquid chromatography on alumina [3].

Pure fullerenes have closer packing than impure fullerenes and therefore dissolve slowly [4]. C_{60} and C_{70} are decomposed by light, with oxygen and ozone playing a role [5, 6]. This photolytic degradation is responsible for cage opening reaction of fullerenes. Light catalyzed degradation occurs on alumina chromatography columns especially for $C_{70,}$ and it is observed that carbon/silica gel columns produce C_{60} of higher purity and crystallinity [7]. Films of C_{60} degrade on storage, possibly because of the ability of C_{60} to chemisorb oxygen, which increases its density. On heating in oxygen, C_{60} is gradually oxidized: C-O adducts are formed at 200ºC and decomposition is substantial at 400-500ºC [8, 9].

Fullerenes tend to react as electrophiles. Saturation of double bonds leads to relief of strain, which in turn acts as a driving force for electrophilic reactions. The extent of functionalization is instrumental for the progress of reactions i.e. monoaddition or multiple additions and in case of multiple additions their topological relationships (new substituents huddled together or evenly spaced).

As electrophiles, fullerenes react with a host of nucleophiles such as Grignard reagents and organolithium reagents. Reactions of C_{60} with methylmagnesium chloride stops quantitatively at the penta-adduct with the methyl groups centered around a cyclopentadienyl anion which is subsequently protonated [10]. Another nucleophilic reaction is the Bingel reaction [11] (Fig. (**2**)). The malonate (functionalized with the halide atom) is often obtained *in situ* in a mixture of base and tetrachloromethane or iodine. The reaction is also known to take place with the ester groups replaced by alkyne groups in dialkynylmethanofullerenes.

Fullerenes undergo Friedel Crafts alkylation with chlorobenzene yielding 1,2 addition adduct (Ar-CC-H) [12]. The [6,6] bonds of fullerenes react as dienes or dienophiles in cycloadditions, for instance Diels Alder reactions. 4-membered rings can be obtained by [2+2] cycloadditions for

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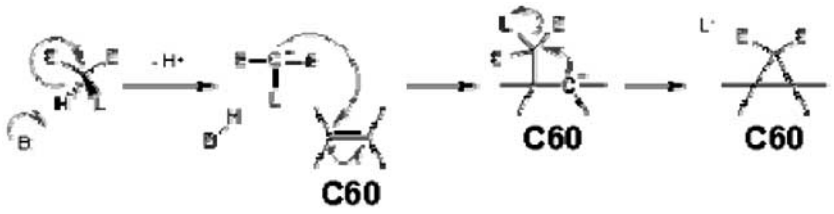


Fig. (2). Bingel reaction mechanism: E strong electron withdrawing group, L leaving group.

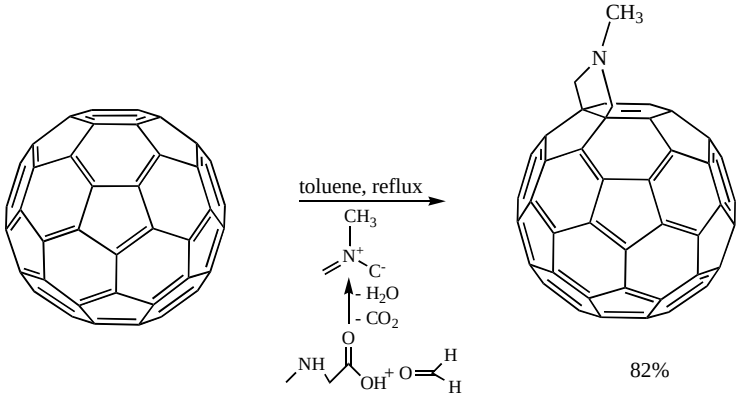


Fig. (3). Prato reaction.

example with benzyne. Prato reaction (Fig. (**3**)) is an example of a 1,3-dipolar cycloaddition to a five membered ring [13].

Fullerenes are easily hydrogenated by several methods, with $C_{60}H_{18}$ and $C_{60}H_{36}$ being the most studied hydrofullerenes. However, completely hydrogenated $C_{60}H_{60}$ is only hypothetical because of large strain. Highly hydrogenated fullerenes are not stable and prolonged hydrogenation of fullerenes by direct reaction with hydrogen gas at high temperature conditions, results in collapse of cage structure with formation of polycyclic aromatic hydrocarbons. Although more difficult than reduction, oxidation of fullerene is possible with oxygen and osmium tetraoxide. Fullerenes react in electrophilic additions as well. The reaction with bromine can add up to 24 bromine atoms to the sphere [14, 15]. The maximum for fluorine addition is $C_{60}F_{48}$ [16]. According to *in silico* predictions, the as yet elusive $C_{60}F_{60}$ may have some of the fluorine atoms in endo positions (pointing inwards) and may resemble a tube more than it does a sphere [17]. Fullerenes react with carbenes to give methanofullerenes. A part of fullerene research is devoted to the so-called open-cage fullerenes, whereby one or more bonds are removed chemically exposing an orifice [18]. In this way it is possible to incorporate small molecules such as hydrogen, helium or lithium. The first such open-cage fullerene was reported in 1995 [19]. The diverse reactions possible for fullerenes are shown in (Fig. (**4**)) [20].

Solubility

Fullerenes are sparingly soluble in many solvents. This is the only allotropic form of carbon that can be dissolved in common solvents at room temperature. The solubility of C_{60} is about 2.8 mg/ml in toluene at room temperature [21]. As the size increases, the solubility of fullerene decreases. Good solvents for fullerenes are carbon disulphide, o-dichlorobenzene, toluene and xylene [22, 23]. Some fullerene structures are not soluble because they have small band gap between the ground and excited states. The examples include small fullerenes like C_{28} [24], C_{36} and C_{50}.

To overcome the natural repulsion of fullerenes for water, the most widely used methodologies are [25]:

(a) Encapsulation or microencapsulation in special carrier.

(b) Suspension with the help of co-solvents.

(c) Chemical functionalization for introduction of solubilising appendage.

TYPES OF FULLERENES

The fullerenes can be categorized in a number of ways as in Fig. (**5**).

BIOLOGICAL APPLICATIONS OF FULLERENES

The fullerenes with their distinguished chemical and physical characteristics, coupled with low toxicity in solutions [31], are promising agents to exhibit a wide range of biological activities [32-34]. Some of the applications possessed by fullerenes are discussed here.

Neuroprotector

Many neurodegenerative disorders such as Parkinson's, Alzheimer's and Lou Gehrig's diseases are due to hyperproduction of oxygen and nitric oxide radical species, probably due to the over- excitation of glutamic acid receptors.

The neuroprotective activity of fullerenes is based on their capability to react with oxygen radical species (ROS)

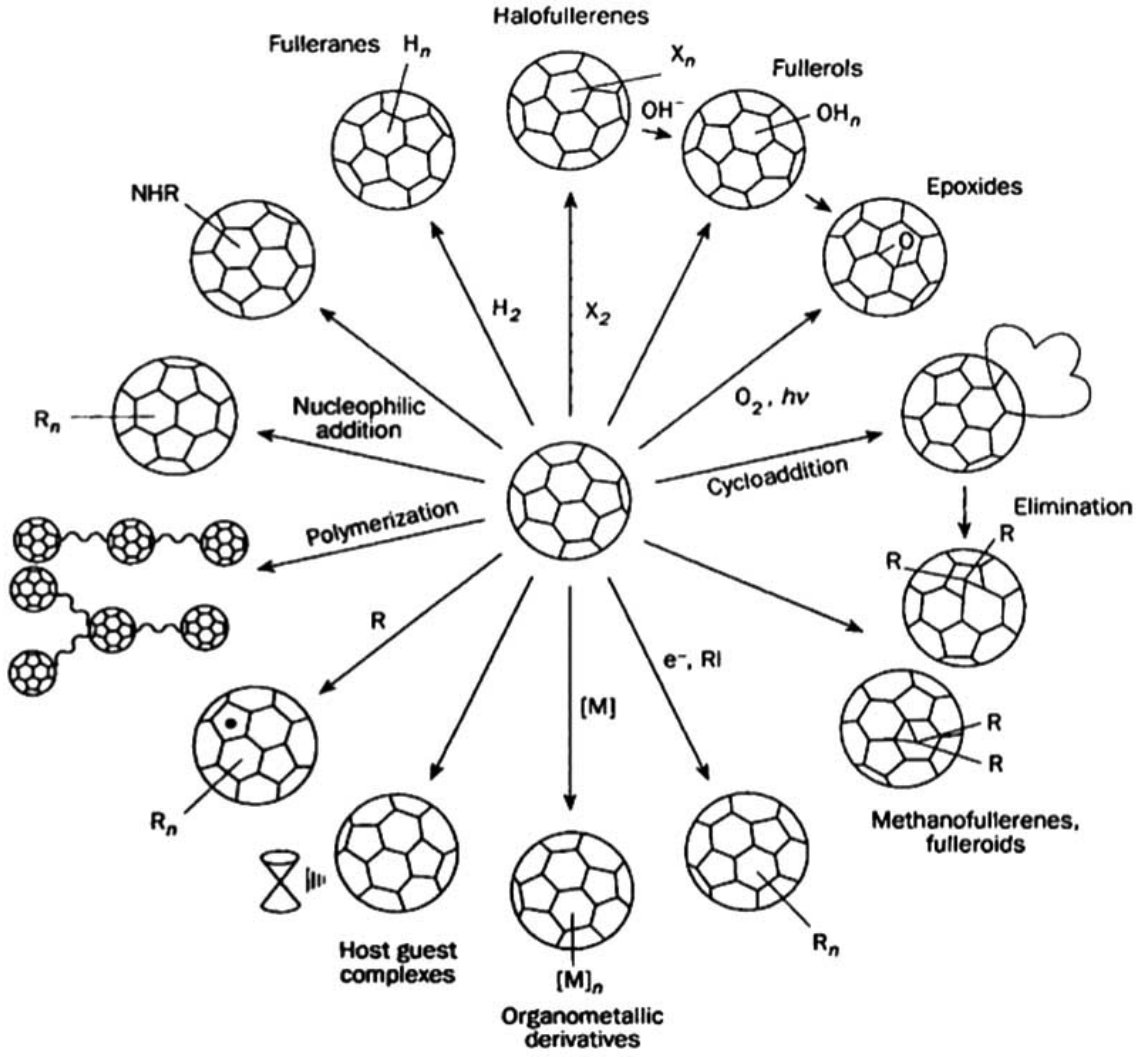


Fig. (4). Diverse reactivity of Fullerenes.

such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($^{\cdot}OH$) radicals, which attack lipids, proteins, DNA, and other macromolecules. In particular, poly-hydroxylated fullerenes named fullerenols or fullerols [C_{60} $(OH)_n$] have proved to be excellent antioxidants, reducing apoptosis in neuron culture; with their high solubility and their ability to cross blood brain barrier. Fullerols have also been shown to absorb many oxygen radicals per fullerene molecule and reduce the toxicity of free radical damage on neuronal tissue [35]. Administration of aqueous solutions of hydrated C_{60} fullerenes (C_{60}HyFn) with C_{60} concentration of 30nM as a drinking water during chronic alcoholization of rats not only protects the tissues of central nervous system (CNS) from damage caused by oxidative stress with high efficacy but also prevents the pathological loss of both astrocytes (the main cells of CNS) and astrocytic marker, glial fibrillary acidic proteins (GFAP). Thus, as a consequence, due to its adaptogenic effects, C_{60}HyFn significantly improves behavioral response and eliminates emotional deficits induced by chronic alcohol uptake [36].

Earlier, it was shown that carboxyfullerene attenuates the cortical infarction in rat brain [37]. Malonic acid C_{60} derivatives are capable of eliminating both superoxide anion and H_2O_2 and are effective inhibitors of lipid peroxidation (LPO) in the nervous tissue [38]. Carboxyfullerenes have been demonstrated to manifest powerful neuroprotection against cytotoxic, apoptotic, and metabolic insults in cortical cell cultures [39, 40]. Fullerenol has been found to block H_2O_2-induced inhibition of neuronal signals [41]. Recently, it has been reported that intracerebroventricular injection of hydrated C_{60} fullerenes prevented the impairment of performance of the cognitive task induced by amyloid-$\beta_{25\text{-}35}$ as well as inhibited Aβ fibrillization *in vitro* [42].

1,2-(dimethoxymethano)fullerene has been shown to inhibit amyloid peptide aggregation by binding to the central hydrophobic part of the peptide. This application keeps fullerene in the class of really promising Alzheimer's disease therapeutics [43].

Antioxidants

Fullerene antioxidants bind and inactivate multiple circulating free radicals, giving them unusual power to stop free radical injury and halt the progression of diseases caused by

TYPES OF FULLERENES

The fullerenes can be categorized in a number of ways as in Fig. (**5**)

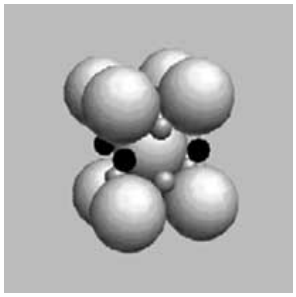
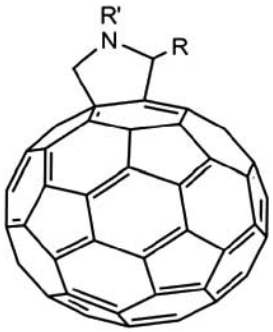
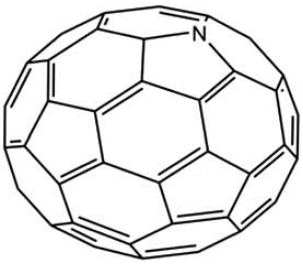
Alkali doped fullerenes 	As the fullerene molecule is highly electronegative, it readily forms compounds with electron donating species, e.g. alkali metals leading to formation of a new class of compounds called alkali doped fullerides wherein alkali metal atoms fill in the space between buckyballs and donate valence electron to the neighboring C_{60} molecule. If the alkali atoms are potassium or rubidium, the compounds are superconductors and they conduct electric current without any resistance at temperatures below 20-40K [26] e.g. K_3C_{60}, Rb_3C_{60}.
Endohedral fullerenes: 	When some other atoms can be enclosed inside the hollow shell of fullerenes, it results in the formation of inclusion compounds known as endohedral fullerenes. When the atom trapped inside is a metal, they are called metallofullerenes [27]. Most of the 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 noble gases. As it is very difficult to open up carbon cage molecules to enclose a foreign atom inside, endohedral material must be synthesized while formation of cage itself [28]. e.g. La@C_{82} and Sc_2@C_{84}.
Exohedral fullerenes: 	It is the most important and most versatile of all species of fullerenes where molecules are formed by a chemical reaction between fullerenes and other chemical groups. Fullerene derivatives are also known as functionalized fullerenes. Due to presence of conjugated π-system of electrons, two main types of primary chemical transformations are possible on fullerene surface: addition reactions which lead to covalent exohedral adducts and redox reactions yielding salts, respectively [29]. As fullerenes are insoluble in water, numerous derivatives of fullerenes have been synthesized with improved solubility profile.
Heterofullerenes: 	Heterofullerenes represent another fundamental group of modified fullerenes. They represent heteroanalogues of C_{60} and higher fullerenes; one or more carbon atoms of the cage are substituted by hetero-atoms. The novel hetero fullerenes $C_{58}X$ (X= S, Se, Te) have been reported. They all have odd number of hetero atoms in the heterofullerene cage. According to HOMO-LUMO gaps and heat of formation, they are less stable than C_{60} and stability follows the order $C_{58}S < C_{58}Se < C_{58}Te$ [30].

Fig. (5). Chemical modifications of fullerenes.

excess free radical production. As free radical scavengers, the protective effect of fullerene derivatives has been demonstrated in various systems, including reduced injury or ischemia reperfusion intestine [44], protecting cell types from undergoing apoptosis [45] and reduced free radical level in organ perfusate [46].

Fullerenes provide effective defense against all damaging forms of reactive oxygen species (ROS): hydrogen peroxide, hydroxyl radicals and superoxide. C_{60} has thirty conjugated C=C, all of which can react with a radical species. The capture of radicals by fullerenes is too fast to measure and is referred to as “diffusion controlled” [47].

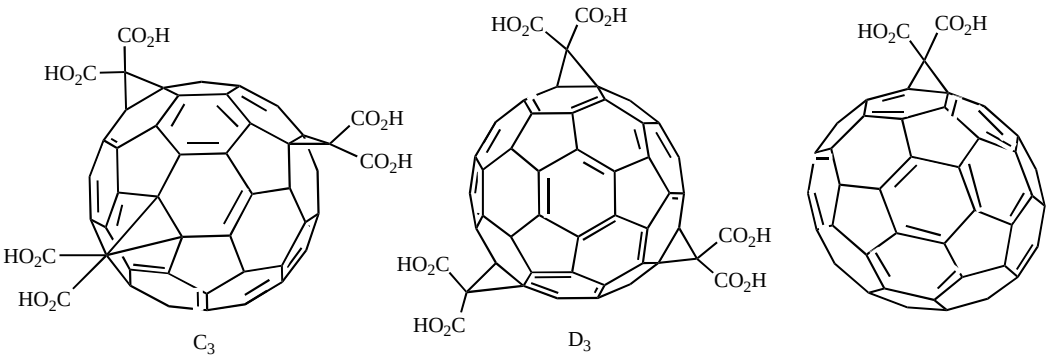


Fig. (6). Carboxyfullerenes (tris and mono derivatives).

C_{60} fullerene reacts easily with radicals because its orbital electron cloud possesses six low- energy empty orbitals, which are configured to accept extra electrons. C_{60} includes four additional unique mechanisms that optimize fullerene antioxidant behaviour:

- Formation of a very stable and relatively unreactive fullerene radical.
- The energy level of the first empty fullerene orbital is the same as the energy level of the electron orbital in superoxide. Thus, the electrons can easily hop from superoxide to the fullerene orbital.
- Reaction of fullerene molecule with many superoxides without being consumed.
- Fullerene antioxidants localize within cells to mitochondria and other sites where excess free radical production occurs in disease states [47].

$C_{60}(ONO_2)_{7+2}$ have been used to attenuate ischemia reperfusion induced lung injury by antioxidant action [48] and other compounds, such as a water soluble hexasulphonated derivative, have demonstrated to be effective against oxidative stress *in vivo*.

Fullerene has prominent potential to attenuate toxicity and to eliminate the increase in the $OH^{\bullet}$ radical induced by co-administration of metamphetamine plus morphine as a free radical scavenger [49]. Wang *et al* compared the antioxidant activity of C_{60} derivatives (Fig. (**6**)) and tocopherol (vitamin E) against $^1O_2$ and $OH^{\bullet}$ respectively generated from enzymatic xanthine/xanthine oxidase and Fenton reactions. C_{60} proved to be the most effective among the considered liposoluble compounds i.e. C_{60}, carboxyfullerenes and tocopherol [50]. ROS can cause oxidative damage to cellular components [51] and such entities are scavenged by fullerenes.

Anti-HIV Activity

HIV Protease (HIVP) is the fundamental enzyme for the virus survival. It is an aspartic protease enzyme similar to mammalian proteases like renin, but is specific for HIV proteins and does not cross react with human proteases. The HIVP cleaves a polyprotein shortly after viral budding. The effect of this cleavage is to activate reverse transcriptase (RT), RNAse H, integrase and protease itself. The latter completes the life cycle of HIV-1 and without this step it is not possible to infect new CD4 cells. The protease has been predicted to be one of the potential targets for antiviral therapy and HIVP inhibitors are currently the mainstay in clinical medicine. Fullerene derivative was tested for antiviral activity in cells acutely and chronically infected with HIV-1 and in cell free systems [52]. The authors reported a selective binding of fullerene derivative when used along with azidothymidine to CD4 receptors in the virus.

The active site of HIVP is a quasispherical hydrophobic cavity, whose diameter is about 10 Å. On its surface, two amino acid residues, aspartate 25 and aspartate 125, catalyze the hydrolysis of the substrate [53]. Friedman and coworkers hypothesized that since C_{60} derivatives have approximately the same diameter as the cylinder that describes the active site of HIVP, so fullerene can be accommodated inside the hydrophobic cavity present in the enzyme and its location might prevent the interaction between the catalytic portions of the HIVP and the virus substrates. Inhibition of HIVP in presence of C_{60} was demonstrated through molecular modeling studies and the experimental observations [54]. Cationic and amino acid type fullerene derivatives (Fig. (**7**)) inhibit HIV-reverse transcriptase and hepatitis C virus replication. Among the fullerene derivatives, the amino acid-type fullerene derivative was the most efficient in human immunodeficiency virus-reverse transcriptase inhibition [55].

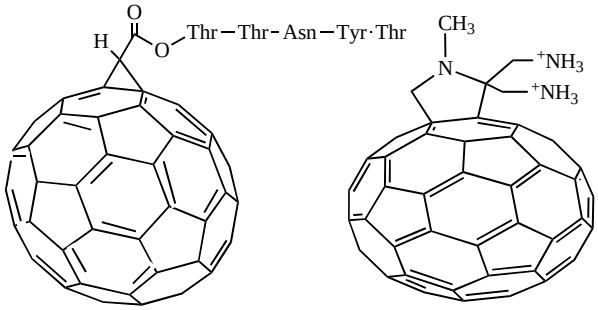


Fig. (7). Amino acid and cationic derivative of fullerenes.

Enzyme Inhibition

A fullerene derivative has shown inhibitory activity against various enzymes such as cysteinic proteases (papaine, catepsine) and serinic proteases (tripsine, plasmine,and thrombone) [56].

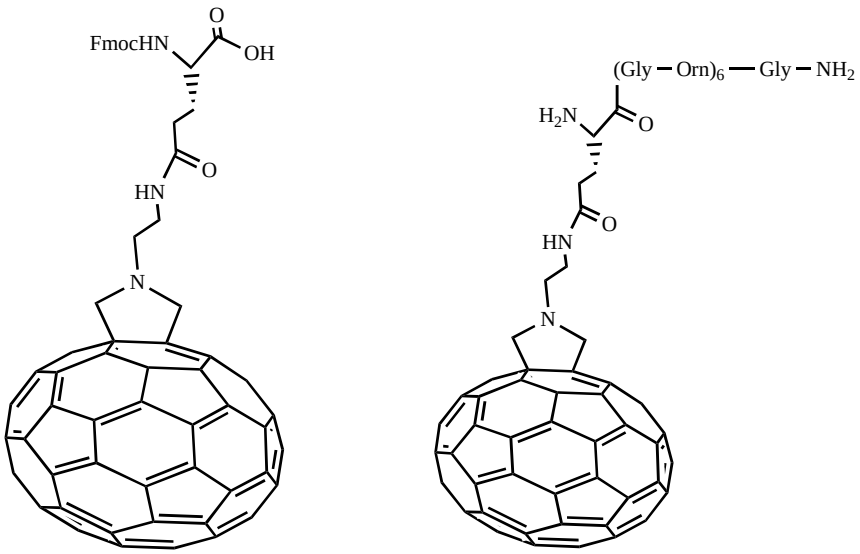


Fig. (8). Fulleropeptides.

The unique characters of hydrophobicity and electrophilicity together with the high reduction potential are probably the key elements for this activity but the mechanism is still unknown. Inhibition towards glutathione-S- transferase has been reported [57] and in case of fullerols, towards P450-cytochrome dependent monooxygenases, plasmatic reticulum enzymes of hepatic cells and mitochondrial ATPase in the process of oxidative phosphorylation [58].

A relevant development in enzyme inhibition by fullerene derivatives relates to nitric oxide synthase (NOS). Nitric oxide is a very reactive radical molecule and it is an important physiological, almost ubiquitary messenger. However, at high concentration it becomes toxic. It has been observed that fullerols are able to decrease bronchospasm induced by the system xanthine/xanthine oxidase, inhibition of all three forms of Nitrous oxide systems (NOS), neuronal, epithelial and inducible, have been effected by trimalonic derivatives of C_{60}, mainly C_3 and D_3 (Fig. (**7**)). The inhibition is multisite and positively cooperative and it seems that C_3 inhibits the inter-subunit transfer of electrons, presumably by a reversible distortion of the dimer interface [59].

Durdagi *et al* developed for the first time 3D-QSAR of fullerene-based molecules and proposed that these models could serve as a basis for the design of novel fullerene-based HIV-1 PR inhibitors with enhanced activity [60].

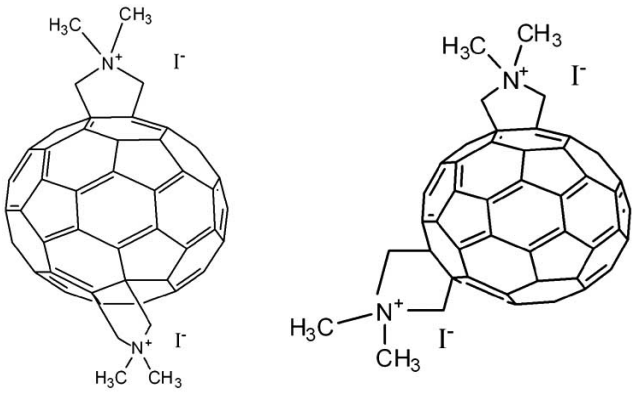
Antimicrobial Activity

Fullerenes have been reported to have potential antimicrobial activity against microbes such as *Candida albicans, Bacillus subtilis, Escherichia coli* and *Mycobacterium avium* [61, 62].

Monomethoxy triethylene glycol (mTEG) substituted fulleropyrollidines showed complete inhibition of *Mycobacterium avium* at a dose of 260 μg/ml and *Mycobacterium tuberculosis* at a dose of 50 μg/ml [63]. Carboxyfullerene derivatives have been found to inhibit gram positive and gram negative bacteria. As these derivatives insert in to gram positive bacteria, disrupt the cell wall structure and cause bacterial death whereas the cell-wall of gram negative bacteria having an outer membrane consisting of lipoproproteins, polysaccharides and phospholipids, is not susceptible to access by fullerenes [64]. This suggests that carboxyfullerenes could be considered as new antimicrobial agents against gram positive cocci. Carboxyfullerenes have been found to inhibit *Escherichia coli* induced meningitis by reducing the damage caused by infiltering neutrophillic blood brain barrier.

Fulleropeptides are a group of fullerene derivatives with potential antibacterial activity (Fig. (**8**)) [65]. The fullerene has been used for the first time in the solid phase peptide synthesis (SPPS) to prepare peptide derivative [66].

Mashino *et al* utilized two isomers (trans-2 and trans-4) of C_{60}-bis (N,N dimethylpyrrolidinium iodide) (Fig. (**9**)) to study the bacteriostatic effects of fullerene derivatives on *Escherichia coli* and they attributed this to the inhibition of energy metabolism by two opposite dose dependent mechanisms. At low fullerene concentration, the oxygen uptake is decreased, on the contrary at high concentration the oxygen



Trans 2 **Trans 4**

Fig. (9). C_{60}-bis (N,N dimethylpyrrolidinium iodides).

uptake is increased and oxygen is converted to H_2O_2. These fullerene concentrations also inhibit the respiratory chain activity [67].

Anti Ageing Agent

Serum depletion induces apoptosis associated with an increased production of free radicals. Apoptosis induced by amyloyl peptide $A\beta_{1-42}$ was also inhibited by these fullerene derivatives [36]. Apoptosis is a scheduled cell death that is mainly due to the transferring of the growth factor TGF-β, a dimeric protein of 25kDa. In this process, ROS species are released and one way to stop the damage, or at least to decrease it, is an antioxidant treatment. Huang *et al.* demonstrated that fullerene derivatives can prevent apoptosis in hepatic tumor cells Hep_3B by neutralization of the TGF-β induced reactive oxygen species [68]. Hexa (sulfobutyl) fullerene show antiapoptotic effects in kidney cells exposed to an oxidative stress induced by ischemic event and following reperfusion [69]. The decrease in apoptotic cell death level is strictly related to the neutralization of ROS both *in vitro* and *in vivo*. Carboxyfullerene was found to reduce the effect of apoptosis on the peripheral blood mononuclear cells (PBMC) induced by 2-deoxy-D-ribose or TNFα with cycloheximide [70].

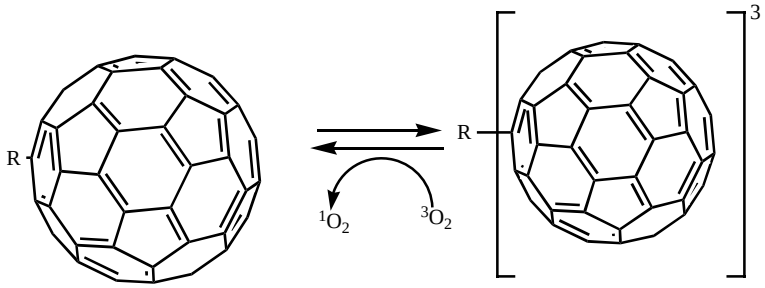
Anticancer Activity

Another potential biological application of C_{60} is related to the easy photoexcitation of fullerenes on the basis of which fullerene based photodynamic compounds are being developed for the treatment of cancer [71]. A water soluble fullerene carboxylic acid derivative was found to be cytotoxic when exposed to visible light. Cytotoxicity of C_{60} derivatives was mediated by its ability to cleave DNA (Fig. (**10**)) [72]. Fullerene can be excited from ground state to $^1C_{60}$ by photo irradiation. This short lived species is readily converted to the long lived $^3C_{60}$ *via* intersystem crossing. In the presence of molecular oxygen, fullerene can decay from its triplet to the ground state, transferring its energy to O_2, generating $^1O_2$, known to be a highly cytotoxic species. In addition, the high energy species $^1C_{60}$ and $^3C_{60}$ are excellent acceptors and, in the presence of a donor, can undergo a different process, being easily reduced to C_{60}^- by electron transfer. Again, in the presence of oxygen, the fullerene radical anion can transfer one electron, producing O_2^-, and hydroxyl radical $OH^\cdot$ [73].

The excited fullerene can be reduced in the presence of the guanosine residue present in the DNA. Hydrolysis of oxidized guanosines followed by DNA cleavage is a consequence of the electron transfer from Guanine (G) to C_{60}. This property of fullerenes makes them potential photosensitisers for the photodynamic therapy (PDT). $^1O_2$ acts by [4+2] or [2+2] cycloaddition on G and these modifications increase the instability of the phosphodiesteric bond that becomes easily susceptible to alkaline hydrolysis. The triplet state of fullerene is formed by intersystem crossing from the singlet state, produced by photoirradiation. The triplet state can lead to formation of intra and intermolecular charge transfer complexes among C_{60} and aromatic rings or tertiary amines [74, 75]. Generally, DNA cleavage occurs at guanine (G) residues but without differentiation among various G sites [76]. More complex conjugates can recognize specific G residues. A C_{60} fullerene derivative, conjugated with 14 deoxynucleotides was synthesized and found to possess high affinity for both single and double stranded DNA [77]. The observed increased reactivity at specific G sites was tentatively attributed to the action of singlet oxygen. Iwamoto and Yamakoshi introduced a highly water soluble C_{60}-N vinylpyrrolidine copolymer as an agent for PDT. C_{60} was incorporated covalently into poly (vinylpyrrolidone) chain *via* radical polymerization [78]. Molecules containing a fullerene core and a 4-aryl-1,4-dihydropyridine was found to be a good cytotoxic agent when tested against adenocarcinoma A549 cells. This action seems to be related to inhibition of ATP hydrolysis [79].

Metallofullerene materials have been reported for various applications, including the treatment of cancer tumors by delivering radioactive atoms directly to diseased tissues. As a result an increased therapeutic potency and a decreased adverse effect profile for radiation treatments can be achieved. Fullerenes are ideally suited for this goal because of their size and resistance to biochemical attack from within the body. Thus, radioactive atoms may readily be transported within the buckyballs, and any fear of stray radiation damaging otherwise healthy tissue is minimized [80].

Liu *et al.* demonstrated the use of poly ethylene glycol (PEG) - conjugated fullerene containing Gd^{3+} ions for PDT in combination with magnetic resonance imaging (MRI) [81]. Mroz *et al.* investigated the photodynamic activity of fullerenes derivatized with hydrophilic and cationic groups



R= Single strand of DNA (DNA Cleavage) or cancer cell (Photodynamic Therapy)

Fig. (10). Applications of C_{60} mediated formation of $^1O_2$. First $^3C_{60}$ is formed by photoexcitation. Second, $^1O_2$ is generated by the fullerene as a result of sensitization of $^1O_2$ by $^3C_{60}$ as the fullerene decays back to the ground state. Finally either cleavage of a DNA strand or destruction of a cancer cell could occur as a result of interaction with $^1O_2$.

against a range of mouse cancer cell lines. They found that monocationic fullerene is very effective photosensitiser for killing cancer cells [82].

Recently, Injac *et al* examined Fullerenol $C_{60}(OH)_{24}$ as a potential organo-protector for anticancer therapy (radio- and chemo-), as well as a synergistic agent in tumor-inhibitory Doxorubicin treatment in rats and reported that fullerenol is effective at a lower dose of 25 mg/kg [83].

Diagnostic Applications

Endohedral metallofullerenes are the fullerenes with metal ion trapped inside fullerene cage. They have shown potential applications in diagnostics. As for example,

- Magnetic Resonance Imaging agents - $M@C_{82}(OH)_{30}$ where $M=Gd^{3+}$ [84]
- X-Ray contrast agents- $M@C_{82}(OH)_{30}$ where $M=^{166}Ho^{3+}$ [85]
- Radiopharmaceuticals- $M@C_{82}(OH)_{30}$ where $M=^{166}Ho^{3+}$ and $^{170}Tm^{2+}$ [86, 87]

$^{166}Ho^{3+}@C_{82}$ $(OH)_{30}$ has been reported as radioactive tracer for imaging of diseased organs and for killing cancerous tumours. The radioactive metal is trapped inside the carbon shell, which is very stable and resistant to metabolism by body. The metallofullerenes have been found to be non-toxic, and they stay in the body for approximately one hour, allowing imaging of circulatory system.

Anti Osteoporotic

Bone tissue is an especially appealing target for vectored pharmaceuticals because its primary inorganic component, hydroxyapatite (HAP), offers a multitude of binding sites for structurally suitable compounds. It is well established that biphosphonate compounds are bone active and bone seeking drugs useful in treatment of osteoporosis and other bone disorders. In addition fluoride ions are useful in treatment of osteoporosis where it improves bone strength and helps prevent fractures. However, biphosphonate drugs are not efficiently absorbed in the gastrointestinal tract, while the fluoride ions are toxic. So there is need for more effective and less toxic bone active drugs, particularly biphosphonates and F^- or their equivalents [88, 89].

Tissue vectored biphosphonate fullerene $C_{60}(OH)_{16}$ AMBP [4,4-bisphosphono-2-(polyhydroxyl-1,2-dihydro-1,2-methanofullerene[60]-61-carboxamido)butyric acid], designed and evaluated *in vitro* [90] to target bone tissue, reduces hydroxyapatite mineralization by 50% at a concentration of 1mM.

Antifullerene Monoclonal Antibodies

Practical applications of fullerene as biological or pharmacological agents require that dosage and serum levels be capable of measurement, preferably by sensitive and simple immunological procedures. This requires that specific antibodies to the fullerene be produced. Bioengineering of antibodies either as a whole or as specific binding fragments are currently used in the armamentarium of cancer therapeutics and of other proliferative diseases including arthritis.

Immunization of mice with a water-soluble C_{60} fullerene derivative conjugated to bovine thyroglobuln yielded a population of fullerene-specific antibodies of the IgG isotype, showing that the immune system is diverse enough to recognize and process fullerenes as protein conjugates. The resulting antibodies population included a subpopulation cross reacting with C_{70}, as it was confirmed from immune precipitation techniques and ELISA assays [91]. It is important to consider that only very small amount of fullerenes are needed to stimulate an immune response and therefore, to produce workable amounts of monoclonal antibodies. As fullerene derivatives become useful clinically, anti fullerene antibodies are ideally suited for serum assays.

Anti Arthritic Activity

It has been reported that there is generation of ROS and depletion of cellular antioxidants in degenerated articular cartilage [92-94]. The degeneration of articular cartilage is partially mediated by oxygen derived free radicals [95]. The mechanical stress on articular cartilage stimulates excess production of ROS from chondrocytes, leading to depolymerisation of hyaluronic acid and chondrocyte death [96]. Yodoh and coworkers reported the use of water soluble fullerene as a therapeutic agent in the protection of articular cartilage against degeneration in osteoarthritis. Fullerene, being a radical sponge efficiently acts as free radical scavenger and has several hundred folds higher antioxidant activity than other antioxidants [97]. Tsuchiya *et al* reported the role of fullerene in promoting chondrogenesis [98]. C_{60} has potential to decrease friction on cartilage surface. Fullerene acts as lubricant in friction and is called a "Molecular bearing" with superlubricity [99, 100]. Due to reduction in friction on joint surfaces, further cartilage degeneration can be prevented [101]. The authors demonstrated *in vitro* and *in vivo* protective activity of water soluble C_{60} fullerene against catabolic stress-induced degeneration of articular cartilage in osteoarthritis. They also compared the inhibitory effect of C_{60} on cartilage degeneration with that of Hyaluronic acid in rabbit model of osteoarthritis and found this inhibitory effect of C_{60} to be better than that of Hyaluronic acid alone. Combined treatment of C_{60} and Hyaluronic acid showed much superior efficacy to treatment with C_{60} or Hyaluronic acid alone.

Fullerenes as Drug Delivery Systems

The quest for effective novel drug delivery system is still on [102]. A drug delivery system is generally designed to improve the pharmacological and therapeutic profile of a drug molecule [103]. The ability of functionalized carbon nanotubes (fCNTs) to penetrate into the cells offers the potential of using fCNT as vehicle for the delivery of small drug molecules [104, 105]. The ability of fullerenes to penetrate through intact skin is widening their application in cellular drug and gene delivery [106]. Rouse *et al* synthesized fullerene based peptide and observed its ability to penetrate through flexed and unflexed skin [107]. Thus fullerene based system can be used in topical drug delivery.

Gene Transfection

In the recent years, attempts of using fullerenes in different pharmacological fields have greatly increased. A very

important application includes utilization of derivatized fullerene as gene transfection agent. DNA functionalized fullerenes are able to enter the COS-1 cell lines and show comparable or even better efficiency than that of commercially available lipid-based vectors [108-109]. The role of fullerene in gene transfection is based on the fact that the fullerene reagent forms a sheath around bound DNA resulting in an increase in life time of DNA in endosomes which supports their chromosomal incorporation [110]. Zakharian *et al* designed a lipophilic slow release drug delivery system which employs fullerene derivatives to enhance therapeutic efficacy in tissue culture [111].

Cardiovascular and Hepatoprotective Activity

Hexasulfobutyl [60] (FC_4S) is composed of six sulfobutyl functional groups covalently bound on a C_{60} cage [C_{60}-($CH_2CH_2CH_2CH_2SO_3Na$)]. The authors have reported that FC_4S is effective as an antioxidant against ion dependent or ion independent LDL oxidation. The primary mechanism for water soluble derivatives involves protection of lipoprotein from oxidation by scavenging of aqueous free radicals prior to their attack on the lipoproteins, lipids and or sparing and regenerating lipoprotein associated antioxidants [112].

Fullerenol play a hepatoprotective role in doxorubicin-induced hepatotoxicity *via* its antioxidant properties Current *in vivo* results confirm that intracellular fullerenol protects hepatocytes against doxorubicin toxicity, but the levels of malondialdehyde (MDA) and different glutathione (GSH) forms show very significant cellular injury. Therefore, the pro-oxidant influence of fullerenol on healthy hepatocytes *in vivo* or *in vitro*, as well as the *in vivo* irritability of the peritoneum and abdominal tissue caused by very low fullerenol solubility, could give dark light on the potential cardio- and hepatoprotector effects [113].

OTHER APPLICATIONS

Superconductors

When K_3C_{60} is cooled, its resistivity begins to drop sharply at about 18K indicative of superconductivity, that is to say they lose all resistance to electric current flow when they are cooled sufficiently. Molecular donor –acceptor charge transfer complexes can present considerable interest as the initial compounds for the manufacture of superconductors (superconductivity transitions up to 40 K have been observed) [114].

Carbon Nanotubes and Nanowires

It has been discovered [115] that graphitic carbon needles grew on the negative carbon electrode of the arc-discharge apparatus used for the mass production of C_{60}. The needles ranged up to 1 mm in length and consisted of nested tubes (concentric cylinders) of rolled graphite sheets. The smallest tube observed was 2.2 nm in diameter, which corresponds roughly to a ring of 30 carbon hexagons. Some of the needles consisted of only two nested tubes, while others contained as many as 50. The separation between the tubes was 0.34 nm (3.4 angstroms), which matches the separation of the sheets in bulk graphite. The tips of the needles were generally closed by caps that were curved or cone-shaped [116]. Carbon nano tubes (CNTs) are used as devices for the controlled release of therapeutic agents, using the inner cavities of CNTs for nanochannel fluidic delivery.

Catalysis

Fullerene C_{60} participates in catalyzed processes as either a part of the catalyst or a substrate, which modifies the action of the catalyst. The complex n_2-$C_{60}Pd$ $(PPh_3)_2$ has been used as the catalyst in the homogenous and the heterogenous hydrogenation reactions with high yields comparable to hydrogenation with Pd/C. Alkenes were completely reduced [117, 118].

Polymerization Reactions

When C_{60} fullerene pellets are exposed to a pressure of 1.2 GPa at 600K for 5h [2+2], cycloaddition occurs to give polymers, including linear polymers, in which the C_{60} fragments are linked by cyclobutane rings. The C_{70} fullerene is more difficult to polymerize. During polymerization of ordinary monomers, C_{60} can be introduced both in the backbone and in the side chain of the polymer formed. High pressure (0.1 to 0.5 GPa) polymerization of styrene in the presence of C_{60} gives rise to a mixture containing products both soluble and insoluble in chloroform. Modification of polymers by C_{60} fullerene fragments changes markedly their physiochemical properties [119].

Fullerenes in Nanomedicines

Since fullerene is an excellent anti-oxidant material (Fig. (**11**)), vitamin C_{60} Bioresearch Corporation (VC_{60}) started development of the world's first cosmetic ingredient with fullerene named "Radical sponge (RS)" [120]. The fullerenes have been reported to increase hair growth in human skin

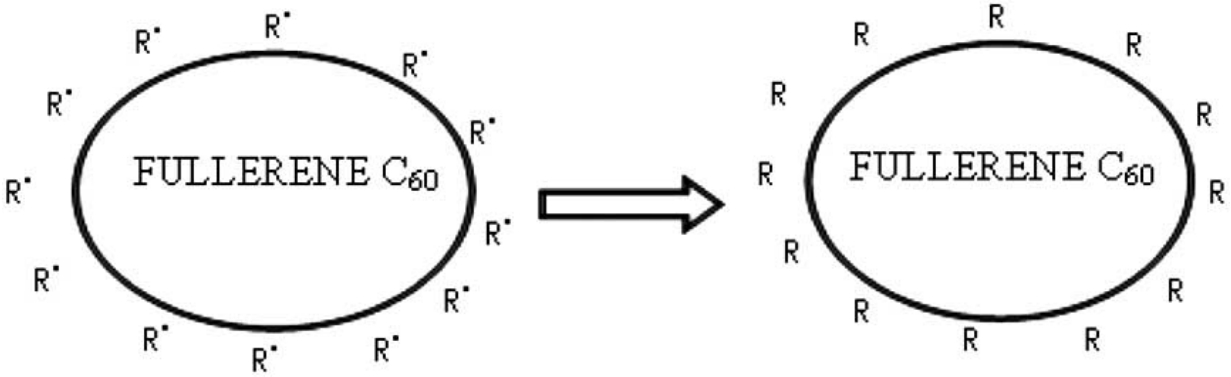


Fig. (11). Free radical capturing by fullerenes.

sections maintained in culture. These studies have wide-ranging implications for the conditions leading to hair loss, including alopecia, chemotherapy, and reactions to various chemicals [121].

Recently, Foldvari *et al* have explained the use of carbon nanotubes as functional excipients for nanomedicines and envisaged their use as building blocks for novel drug delivery systems, dosage forms, and biomedical substrates [122].

Great interest has been generated in fullerenes in general, but especially in CNTs and carbon nanohorns (CNHs) as biologically compatible materials and drug carriers mainly because of their distinct architecture, hollow interior and cagelike structures.

Human mast cells (MC) and peripheral blood basophills (PBB) are responsible for initiation and propagation of several inflammatory conditions. These conditions are mainly due to type I hypersensitivity. Type I hypersensitivity is due to B cells produced specific IgE antibody (IgE Ab) to common normally inocous antigens Ags. Ryan *et al* have reported the role of fullerenes as negative regulator of allergic mediator release that suppress Ag-driven type I hypersensitivity. The authors reported that Human MC and PBB exhibited a significant inhibition of IgE dependent mediator release when preincubated with C_{60} fullerenes. Fullerenes alone could not induce MC or PBB mediator release. However, when cells were incubated with water soluble fullerenes with optimal concentration of Ag (100 ng/ml) there was significant inhibition of both degranulation and cytokine production as compared to skin MC and PBB not incubated with the fullerenes [123].

Razeyat *et al* designed porphyrin adducts of cyclohexyl fullerene-C_{60} as nanocationite particles for targeted delivery of the paramagnetic magnesium stable isotope to the heart muscle. The authors reported achievement of about 80% recovery of the tissue hypoxia symptoms in less than 24 hours after a single injection (0.03–0.1 LD_{50}) [124].

FULLERENES - STRUCTURE ACTIVITY RELATIONSHIP

Fullerenes have been structurally modified to yield various derivatives. These derivatives have not only increased the solubility of, hitherto, insoluble fullerenes but also have demonstrated a range of biological activities.

Oligo and polyfunctionaled C_{60} derivatives can be better neuroprotective agents than monofunctionaled fullerenes. For example, C3 malonic acid derivatives act as superoxide dismutase (SOD) mimetics. As the number of carboxylic acids attached to fullerenes is increased, the neuroprotective activity increases. The symmetry of the distribution of carboxylic groups over C_{60} core also affects the activity. More clustered the malonic acid groups, better is the neuroprotective activity [125].

Derivatization of fullerene with polar groups such as polyhydroxylated fullerenes and tris (malonic) acid, increases water solubility and enables them to cross cell membrane and localize preferentially to mitochondria generating larger volumes of cellular oxygen free radicals [126-127]. Fulleropyrrolidines with ammonium groups have been found active against HIV-1 and HIV-2. The relative positions of side chains on fullerenes have a strong influence on antiviral activity. A series of fullerene derivatives have been synthesized to elucidate the structural parameters that affect antiviral activity of fullerenes [128]. Microinjection of C_{60} complexed with poly (N-vinyl-pyrrolidone) into hippocampus prevented amnesia elicited by protein synthesis blockade in rats [129].

Buckminsterfullerenes are capable of adding multiple radicals per molecule, the addition of as many as 34 methyl radicals to single C_{60} sphere [130]. As a result, fullerenes can inhibit the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicals.

Unfunctionalized C_{60} is cytotoxic in certain systems whereas addition of functional side chains to carbon skeleton diminishes the level of cytotoxicity [131-133]. Monocationic fullerene is very effective photosensitiser for killing cancer cells [134]. Water soluble carboxyfullerene is cytotoxic when exposed to light and thus has anticancer activity. When conjugated with 14 Deoxynucleotides, C_{60} gets higher affinity for single and double stranded DNA. Water soluble C_{60} derivative in conjunction with bovine thyroglobulin yields fullerene specific antibodies of IgG isotype. A C_{60} fullerene derivative, conjugated with 14 deoxynucleotides was synthesized and found to possess high affinity for both single and double stranded DNA [135].

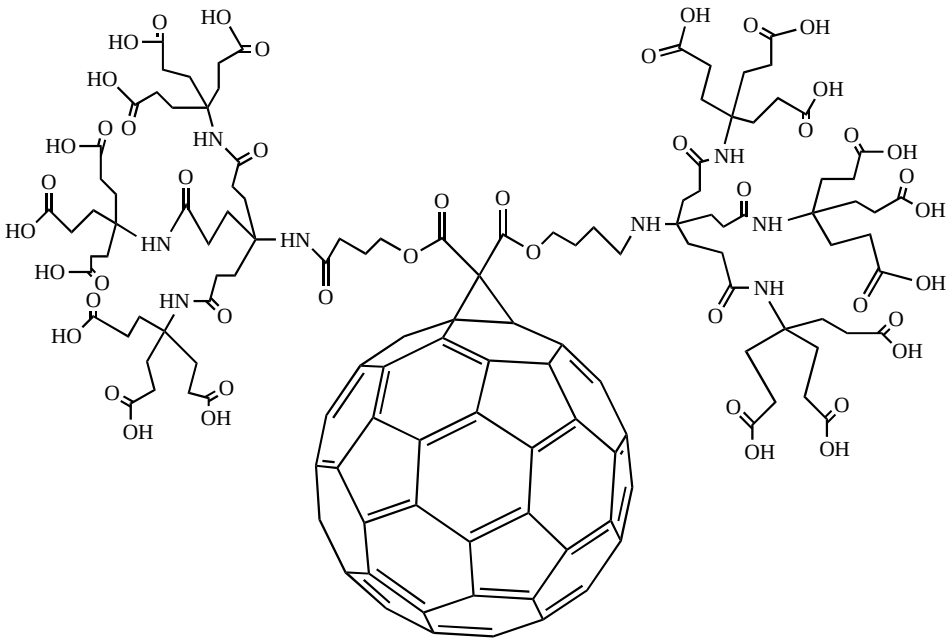
C_{60} fullerene derivatized with two or more solubilising side chains are active against HIV-1 and HIV-2 [136]. Among the alkyl derivatives, anti HIV activity is inversely proportional to length of alkyl chain. A dendrofullerene derivative has highest anti-protease activity (Fig. (**12**)) [137, 138].

Trans isomer is a stronger inhibitor of HIV-1 replication as compared to cis derivatives whereas equatorial one has no activity. However, among trans derivatives, relative position has effect on the activity. For instance, trans 2 of substituents on fullerenes and positive charges near fullerene cage greatly enhance activity (Fig. (**13**)) [139]. Amino acid derivative of C_{60} fullerene (ADF) inhibits HIV and Cytomegalovirus replication [140].

Tissue vectored biphosphonate fullerene $C_{60}(OH)_{16}$ AMBP [4,4-bisphosphono-2-(polyhydroxyl-1,2-dihydro-1,2-methanofullerene[60]-61-carboxamido)butyric acid], designed and evaluated *in vitro* to target bone tissue, reduces hydroxyapatite mineralization by 50% at a concentration of 1mM. An amide bisphosphonate addend, in conjunction with multiple hydroxyl groups, confers a strong affinity for the calcium phosphate mineral hydroxyapatite of bone [141].

Fullerene derivatives bearing sugar moieties such as D-mannosyl – fullerols [142] avoid the erythrocyte aggregation and thereby act as possible antiplatelet agents [143].

A recent study shows that C_{60} fulleropyrrolidine-xanthine dyads could express synergistic immunomodulatory effects during inflammations. These agents hold promise for future development of a new generation of potent anti-inflammatory agents. Fullerenes bearing a xanthine moiety



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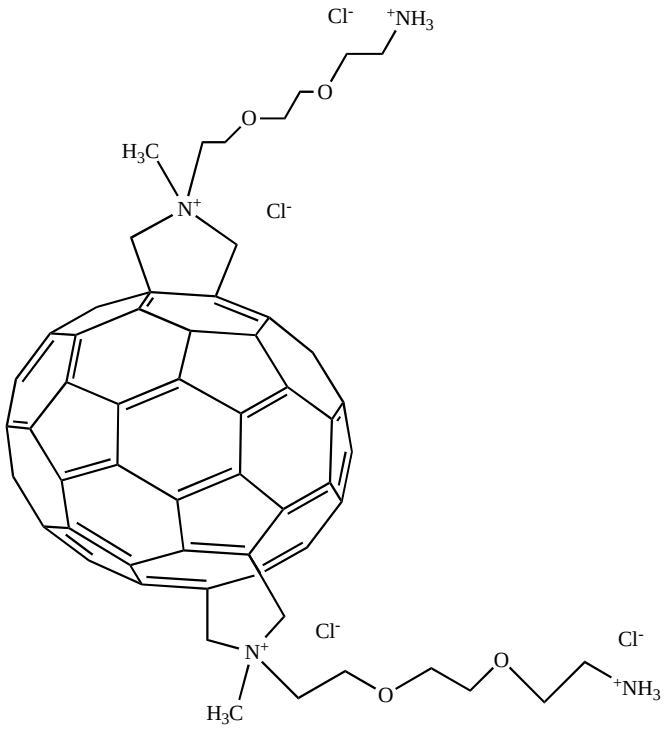

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Fig. (12). Structure of dendrofullerenes.

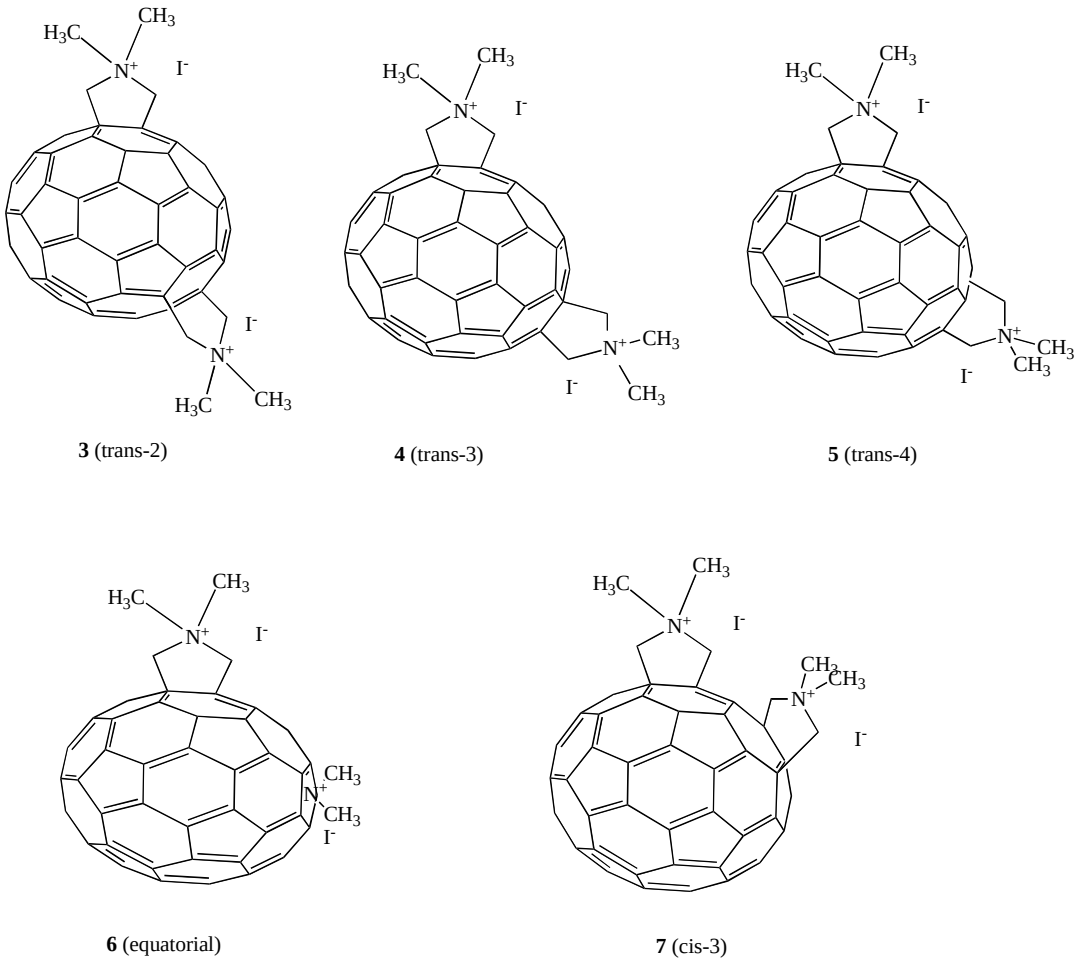
are potential double action anti-inflammatory agents, capable of simultaneous inhibition of lipopolysaccharides (LPS) induced nitric oxide (NO) and tumour necrosis factor TNF-α production [144].

Hexasulfobutyl fullerene, a water soluble derivative has been reported to prevent atherosclerosis by protecting low density lipoprotein (LDL) from oxidation [145].

Increase in hydrophilicity of core enables fullerenes to carry drugs and genes for cellular delivery as derivatized fullerenes can cross the cell membrane and bind to the mitochondria [146].

CONCLUSION

The fullerenes are allotropes of carbon having unique properties. They are the only allotrope of carbon which can



3 (trans-2) **4** (trans-3) **5** (trans-4)

6 (equatorial) **7** (cis-3)

Fig. (13). Structure of fulleropeptides.

be solubilized. The various forms of fullerenes- alkali-doped, exohedral, endohedral, nanopeapods and heterofullerenes present a variety of structures to the medicinal chemists for modifications and manipulations to achieve molecules of desired structures and activities. The fullerenes have become one of the most researched areas and the results are highly encouraging, with a wide range of biological activities being reported. The nano size of the fullerenes has offered a potential tool for nanomedicine. Thus, the fullerenes have the potential of becoming the future of chemistry and medicine. Further research may soon give the world what it direly needs - the nanobuckymedicine.

ABBREVIATIONS

ADF = Amino acid Derivative of Fullerene

AMBP = Amide Biphosphonate addend

CNH = Carbon Nanohorns

CNT = Carbon Nanotubes

DNA = Deoxyribonucleic acid

fCNT = Functionalized Carbon Nanotubes

HIVP = Human Immuno Virus Protease

HOMO = Highest Occupied Molecular Orbitals

HPLC = High Performance Liquid Chromatography

IgEAb = Immunoglobulin E Antibody

LDL = Low Density Lipoprotein

LPO = Lipid Peroxidation

LPS = Lipopolysaccharide

LUMO = Lowest Unoccupied Molecular orbital

MC = Mast Cells

NO = Nitric Oxide

PBB = Peripheral Blood Basophills

PBMC = Peripheral Blood Mononuclear Cells

ROS = Reactive Oxygen Species

RT = Reverse Transcriptase

TNF = Tumour Necrosis Factor

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