Medicinal applications of fullerenes

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Abstract: Fullerenes have attracted considerable attention in different fields of science since their discovery in 1985. Investigations of physical, chemical and biological properties of fullerenes have yielded promising information. It is inferred that size, hydrophobicity, three-dimensionality and electronic configurations make them an appealing subject in medicinal chemistry. Their unique carbon cage structure coupled with immense scope for derivatization make them a potential therapeutic agent. The study of biological applications has attracted increasing attention despite the low solubility of carbon spheres in physiological media.

The fullerene family, and especially C60, has appealing photo, electrochemical and physical properties, which can be exploited in various medical fields. Fullerene is able to fit inside the hydrophobic cavity of HIV proteases, inhibiting the access of substrates to the catalytic site of enzyme. It can be used as radical scavenger and antioxidant. At the same time, if exposed to light, fullerene can produce singlet oxygen in high quantum yields. This action, together with direct electron transfer from excited state of fullerene and DNA bases, can be used to cleave DNA. In addition, fullerenes have been used as a carrier for gene and drug delivery systems. Also they are used for serum protein profiling as MELDI material for biomarker discovery. In this review we report the aspects of medicinal applications of fullerenes.

Keywords: fullerenes, antioxidants, photosensitizer, drug delivery, diagnostic

Introduction

An important area of research in modern material nanoscience concerns carbon-based materials, among which fullerenes take one of the first places. Since their first detection and bulk production, they have gained a prime role on scientific scene, reaching the climax when 1996 Nobel Prize for Chemistry was awarded to Kroto, Curl and Smalley for their seminal discovery (Kroto et al 1985). [60] Fullerene, the most abundant representative of the fullerene family was produced for the first time on a preparative scale in 1990, by resistive heating of graphite (Kraetschmer et al 1990). Fullerene molecules are composed entirely of carbon, in form of a hollow sphere, ellipsoid or tube. Spherical fullerenes are also referred to as buckyballs. An important property of C60 molecule is its high symmetry. There are 120 symmetrical operations, like rotation around the axis and reflection in a plane, which map the molecule onto itself. This makes C60 the most symmetrical molecule (Taylor et al 1990). The C60 fullerene surface contains 20 hexagons and 12 pentagons. All the rings are fused; all the double bonds are conjugated. In spite of their extreme conjugation, they behave chemically and physically as electron-deficient alkenes rather than electron rich aromatic systems (Fowler and Ceulemans 1995). The unique physical and chemical properties of these new forms of carbon led many scientists to predict several technological applications. However, the difficult processibility of fullerenes has presented a major problem in hectic search for medicinal applications. C60 are insoluble in aqueous media and aggregate very easily (Prato 1997). There have been
several attempts to overcome the natural repulsion of fullerenes for water. The most widely used methodologies are:

(a) Encapsulation or micro-encapsulation in special carriers like cyclodextrins (Youle and Karbowski 2005), calixarenes (Shinkai and Ikeda 1998), polyvinylpyrrolidone (Yamakoshi et al 1994), micelles and liposomes (Bensasson et al 1994). In addition, the combination of fullerenes and lipid membranes has led to very interesting results. Lipid bilayers are dynamically mobile structures, partially ordered and of biopharmaceutical interest for covering biocompatible surfaces or for the controlled release of drugs (Hetzer et al 1997);

(b) Suspension with the help of co-solvents by saturating fullerenes in benzene solutions poured into THF. The resulting mixture is added dropwise to acetone, and then water is slowly added. A yellow suspension is formed and solvents are evaporated to a final known volume of water (Scrivens et al 1994);

(c) Chemical functionalization to increase the hydrophilicity eg, with amino acid, carboxylic acid, polyhydroxyl group, amphiphilic polymers etc (Hirsch et al 1994; Bianco et al 1996; Brettreich and Hirsch 1998; Chen et al 2001).

The current list of fullerene derivatives covers practically all known classes of chemical compounds, demonstrating both high chemical activity and a broad versatility of chemical reactions. This outstanding chemical appearance generates great interest for their practical applications in creating novel materials for medical use. In this review we are reporting the medical applications of fullerenes, including, antiviral activity, antioxidant activity and their use in drug delivery. In addition, the powerful photoinduced biological activities as a potential scaffold for photodynamic therapy and diagnostic applications are highlighted.

**Antiviral activity**

Compounds with antiviral activity are generally of great medical interest and different modes of pharmaceutical actions have been described. Replication of the human immunodeficiency virus (HIV) can be suppressed by several antiviral compounds, which are effective in preventing or delaying the onset of acquired immunodeficiency syndrome (AIDS). Fullerenes (C60) and their derivatives have potential antiviral activity, which has strong implications on the treatment of HIV-infection. The antiviral activity of fullerene derivatives is based on several biological properties including their unique molecular architecture and antioxidant activity. It has been shown that fullerene derivatives can inhibit and make complex with HIV protease (HIV-P) (Friedman et al 1993; Sijbesma et al 1993). Dendrofullerene 1 (Figure 1) has shown the highest anti-protease activity (Brettreich and Hirsch 1998; Schuster et al 2000). Derivative 2, the trans-2 isomer (Figure 1), is a strong inhibitor of HIV-1 replication. The study suggests that relative position (trans-2) of substituents on fullerenes and positive charges near to fullerenes cage provide an antiviral structural activity.

Fulleropyrrolidines with two ammonium groups have been found active against HIV-1 and HIV-2 (Marchesan et al 2005). 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. Figure 2 shows the derivatives, which are employed in lymphocyte CEM cell
cultures infected with HIV-1 or HIV-2. The results reveal that trans fullerenes derivatives are more active than cis-counterparts whereas the equatorial one is totally inactive.

Fullerenes C60 derivatized with two or more solubilizing side chains have also been active, when tested in lymphocyte CEM cell cultures infected with HIV-1 and HIV-2 (Bosi et al 2003). Although derivatization of fullerenes with pyrrolidinium nitrogen enhances their solubility, the practical use of this method is limited by the generation of different isomers, whose separation is difficult. Activity results prove that these derivatives are completely inactive against HIV-2, whereas the derivatives with corresponding quaternary salts at the pyrrolidine nitrogen are impinging against HIV-1. This finding is important to understand the electrostatic interactions and the structure of compounds with antiviral activity.

Amino acid derivatives of fullerene C60 (ADF) are found to inhibit HIV and human cytomegalovirus replication (Kotelnikova et al 2003). The selected fullerene derivatives are rendered water soluble by attachment of amino acids to C60: C60-L-Ala, C60-L-Ser, C60-L-Gly, C60-L-Arg and C60-d-Arg, C60-ACA (C60-ε-aminocaproic acid), C60-ACNa (sodium salt of C60-ACA), C60-ABA (C60-γ-aminobutyric acid) and C60-ABNa (sodium salt of C60-ABA) (Romanova et al 1994). The mechanism is based on penetration of ADF carrying bivalent metal ions through lipid bilayer of liposomes, insertion to the hydrophobic domains of proteins and changing their functions of membrane bound enzymes. The observation that fullerenes and C60 derivatives are not immunogenetic further supports their potential as pharmaceutical compounds.

On the other hand, water-insoluble fullerene (C60) derivatives have antiviral activity against enveloped viruses. After visible light illumination for 5 h of semliki forest virus (SFV, Togaviridae) or vesicular stomatitis virus (VSV, Rhabdoviridae) (Kaesermann and Kempf 1997) in the presence of C60, the infectivity of these viruses is lost. This effect is attributed to the generation of singlet oxygen and is equally effective in solutions that contained proteins. Several dyes are available that allow singlet oxygen generation (Rywkin et al 1995). Figure 3 shows the time-dependent loss of infectivity of SFV after illumination in the presence of C60 and oxygen. The light induced inactivation

![Figure 2 Structures of Fulleropyrrolidines. Copyright © 2005. Reproduced with permission from Marchesan S, Da Ros T, Spalluto G, et al. 2005. Anti-HIV properties of cationic fullerene derivatives. Bioorg Med Chem Lett, 15:3615–18.](image-url)
can be suppressed by removing oxygen from the solution by flushing argon through the suspension.

Cationic, anionic and amino acid type fullerene derivatives inhibit HIV-reverse transcriptase and hepatitis C virus replication (Mashino et al 2005). Anionic fullerene derivatives show antioxidant properties whereas cationic derivatives have the antibacterial and antiproliferative activities. The amino acid type derivatives are found to be the most active out of all the derivatives of fullerenes. All the fullerene derivatives tested are more active than the non-nucleoside analog of HIV-RT inhibitor. Derivatization of fullerenes with long alkyl chains had a negative effect on their antiviral activity. The two important targets for anti-HIV characteristics are the HIV-protease and HIV-reverse transcriptase. Studies based on molecular modeling data suggest that the C60-core can penetrate into the hydrophobic binding site of HIV protease. However, the hypothesis that HIV-protease inhibition is the main mechanism of the anti-viral activity of fullerene and its derivatives still lacks direct experimental proof.

The suggested mode of action of fullerene derivatives as anti-HIV compounds is further investigated by studies of the spatial hydrophobic relationship between C60 and the cavity regions of HIV-protease, which is a homodimeric enzyme that belongs to the class of aspartic proteases (Zhu et al 2003). The study is based on simulations of molecular dynamics, free energy techniques and simulations of the effect of C60 on water content of the cavity. The hydrophobic interactions between C60 and cavity regions hold the inhibitor tightly and as a result cause the release of water, which provides indirect evidence for inhibitor presence. Some fullerene derivatives are found to have an inhibition constant of 53 μM and desolvation happens over 333–352 Å of active cavity. The free energy profiles prove a connection of opening and closing of flaps in the cavity to the potential inhibitor binding.

The synthesis and characterization of fullerene derivatives as inhibitors of HIV aspartic protease enzyme holds great potential for the development of novel anti HIV drug (Marcorin et al 2000). The active region of HIV protease is a cylindrical hydrophobic cavity (diameter ~10 Å), which contains two amino acid residues, aspartate 25 and aspartate 125, whose binding causes suppression of protein slicing and inhibits viral replication. The study is carried out with a water-soluble fullerene derivative on peripheral blood mononuclear cells (PBMC). The mode of action is based on electrostatic and/or hydrogen bond interactions of fullerene derivative side chains with Asp 25 and Asp 125, as shown in Figure 4.

**Figure 3** Kinetics of the photodynamic inactivation of SFV by C60. SFV was illuminated with visible light in the presence of C60 under constant stirring and O2 bubbling (●). Mean values and standard errors from three independent experiments are shown. Controls include the incubation of SFV with C60 without illumination (○), the illumination of SFV without C60 (○) and the illumination with C60 under constant stirring and flushing with argon (▲). Copyright © 1995. Reproduced with permission from Rywkin S, Ben-Hur E, Reid ME, et al. 1995. Selective protection against IgG binding to red cells treated with phthalocyanines and red light for virus inactivation. Transfusion, 35:414–20.

**Fullerenes as photosensitizers**

Another potential medical application of C60 is related to the photoexcitation of fullerenes. In fact, fullerene can be
excited from ground state to $^1\text{C}_{60}$ by photoirradiation. This short-lived species is readily converted to long-lived $^3\text{C}_{60}$ via intersystem crossing. In presence of molecular oxygen, the fullerene can decay from its triplet to ground state, transferring its energy to $\text{O}_2$, generating singlet oxygen $^1\text{O}_2$, known to be highly cytotoxic species. In addition, the high-energy species $^1\text{C}_{60}$ and $^3\text{C}_{60}$ are excellent acceptors and in the presence of a donor, can undergo a different process, being easily reduced to $\text{C}_{60}^\cdot$ by electron transfer. Again, in the presence of oxygen, the fullerene radical anion can transfer one electron, producing a superoxide anion radical $\text{O}_2^\cdot$ and hydroxyl radical $\cdot\text{OH}$ (Yamakoshi et al 2003). The excited fullerene can be reduced under biological conditions in the presence of biological reducing agents eg, guanosin. On the other hand, singlet oxygen and superoxide radical anions are well known reactive species towards DNA (Da Ros et al 2001). This property of fullerenes renders them potential photosensitizers for their use in photodynamic therapy (PDT). Many fullerene conjugates with different functional groups possessing biological affinity to nucleic acids or proteins, are being investigated for anticancer activity. In particular, conjugates of C60 and, acridine or complementary oligonucleotide, which interact with nucleic acids, have been synthesized with the objective of increasing cytotoxicity (An et al 1996; Yamakoshi et al 1996). Cytotoxicity of dendritic C60 monoadduct and malonic acid C60 trisadduct was investigated on Jurkat cells, and upon exposure to UV light, the cell number was found to drop by approximately 19% within two weeks (Rancan et al 2002). Ji et al also studied the biodistribution and tumor uptake of C60(OH)$_x$ in five kinds of tumor models by radiotracer 125I-labeled C$_{60}$(OH)$_x$ (Ji et al 2006).

Iwamoto and Yamakoshi introduced a highly water soluble C60- N vinylpyrrolidone copolymer as agent for photodynamic therapy (Iwamoto and Yamakoshi 2006). C60 was incorporated covalently into poly (vinylpyrrolidone) chain via radical polymerization. This nanoparticle was the most water-soluble fullerene yet reported and aqueous solutions of concentrations even higher than reported for saturated C60 in toluene could be generated with this method.

Liu et al (2007) demonstrated the use of poly ethylene glycol (PEG)-conjugated fullerene containing Gd$^{3+}$ ions for photodynamic therapy in combination with magnetic resonance imaging (MRI). The authors demonstrate through experimental data that tumor PDT effect was significantly promoted by photosensitizer tumor targetability and MRI activity. C60-PEG-Gd was injected into tumor bearing mice. The MRI activity was introduced into C60-PEG of PDT.
photosensitizer. The chelate incorporation of Gd\(^{3+}\) ions could convert C60-PEG derivative to a photosensitizer with both the diagnostic and therapeutic functions (Liu et al 2007).

Recently, Mroz et al (2007) investigated the photodynamic activity of fullerenes derivatized with hydrophilic and cationic groups against a range of mouse cancer cell lines. They found that, monocationic fullerene is highly effective photosensitizer for killing cancer cells by rapid induction of apoptosis after illumination.

**Antioxidant activity**

Results published in 1999 have shown that fullerenes have a potential as biological antioxidants. The antioxidant property is based on the fact that fullerenes possess large amount of conjugated double bonds and low lying lowest unoccupied molecular orbital (LUMO) which can easily take up an electron, making an attack of radical species highly possible. It has been reported that up to 34 methyl radicals have been added onto a single C60 molecule. This quenching process appears to be catalytic. In other words the fullerene can react with many superoxides without being consumed. Due to this feature fullerenes are considered to be the world’s most efficient radical scavenger and are described as radical sponges (Krusic et al 1991). The major advantage of using fullerenes as medical antioxidant is their ability to localize within the cell to mitochondria and other cell compartment sites, where in diseased states, the production of free radicals takes place.

Experiments on rats done by Najla Gharbi and co-workers proved this remarkable trait. They showed that aqueous C60 suspensions prepared without using any polar organic solvent, not only have no acute or sub acute toxicity in rodents, but also protect their livers against free-radical damage (Gharbi et al 2005). Rats are intoxicated with CCl\(_4\), which led to the formation of trichloromethyl radical CCl\(_3\)^•, causing severe damage to the liver on reaction with oxygen. Trichloromethylperoxy radicals CCl\(_3\)OO^•, a highly reactive species which rapidly initiates the chain reaction of lipid peroxidation (Slater et al 1985), is formed. C60 is able to scavenge a large number of these radicals per molecule leading to the result that rats pre-treated with C60 and intoxicated with CCl\(_4\) showed no liver damage. Considering the histopathological examinations and biological tests, pristine C60 can be considered as a powerful liver-protective agent when used in a dose-dependent manner.

When fullerene is derivatized with polar groups, as in case of polyhydroxylated fullerenes (fullerenol) and C60 tris(malonic)acid, they become water soluble enabling them to cross the cell membrane and localize preferentially to mitochondria (Foley et al 2002; Youle and Karbowski 2005), which generate great masses of cellular oxygen free radicals. This phenomenon makes them useful for a variety of medical applications (Tsai et al 1997; Lotharius et al 1999; Bisaglia et al 2000). These radical scavengers have shown to protect cell growth from various toxins that can induce apoptotic injuries in vitro (Lin et al 1999; Lin et al 2002; Chen et al 2004) in different cell types such as neuronal cells (Dugan et al 1997; Bisaglia et al 2000), hepatoma cells (Huang et al 1998), or epithelial cells (Straface et al 1999).

Apoptosis is of critical importance for variety of physiological and pathological phenomenon which led numerous scientists to design experiments in this regard. Daniela Monti et al investigated the protective activity of this drug against oxidative stress-induced apoptosis. 2-deoxy-D-ribose (dRib) or TNF-α plus cycloheximide were used as agents to trigger apoptosis in human peripheral blood mononuclear cells (PBMCs) by interfering with the redox status of cell and mitochondrial membrane potential. It was found that carboxyfullerenes, also known as C60 tris(malonic)acid, was able to protect quiescent PBMCs against apoptosis by preserving the mitochondrial membrane potential integrity, which is the early stage of apoptosis (Monti et al 2000). Other interesting results showing that fullerenes have potential as biological antioxidants were also published by Dugan et al. The authors treated transgenic mice carrying a defective copy of the gene encoding for human superoxide dismutase (SOD1), which led to amyotrophic lateral sclerosis (ALS), with C60 tris(malonic)acid. SOD1 knock out mice treated with C60 developed symptoms of disease 10 days later and lived 8 days longer than untreated control mice (Dugan et al 1997).

Fullerenes are also used for cytoprotective action against UVA irradiation (Xiao et al 2006). The ultraviolet A radiation (320–400nm) generates reactive oxygen species, which have a biological effect on human skin cells, leading to cell damage or cell death. Once again the radical scavenging nature of water soluble fullerene derivative namely Radical Sponge® (C60 with poly(vinylpyrrolidone)) was utilised to protect human ore mammalian cells against oxidative stress, through catalytic dismutation of superoxide. The ability of Radical Sponge® to enter into depth of human skin epidermis due to its stability towards oxidative decomposition makes it more reliable than Vitamin C and enables the prevention of both UV skin-injuries and skin aging, without photosensitization and cytotoxicity.
Water soluble fullerenes namely fullerenols and malonic acid derivatives of C60 have attracted great attention in the field of neurosciences. The brain contains a number of different unsaturated fatty acids, and underlies aerobic metabolism, and has limited ability to regenerate damaged tissues, making it a very sensitive organ towards oxidative damage caused by free radicals. These reactive oxygen species being O$_2^*$ (superoxide), •OH (hydroxyl) radicals and closed shell H$_2$O$_2$ molecules (Halliwell 1992). Fullerene derivatives have the ability to inhibit the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicals, stopping them from attacking adjacent fatty acid chains or membrane proteins, which would lead to glutamate-receptor-mediated excitotoxicity and apoptotic cell death. In cell culture experiments, C60 tris(malonic)acid rescued cortical neurons from a broad range of insults and was furthermore found to show robust neuroprotection in a number of other cell culture models of neurological disease including Parkinson’s disease (Dugan et al 1997).

**Fullerenes in drug and gene delivery**

The direct delivery of drugs and biomolecules through cell membrane into cells has attained increasing attention and has put a main focus on the development of efficient and safe carriers to transport genes or drugs. Transport of any compound into the nucleus of an intact cell is a major challenge, as transfer is limited by at least three membrane barriers which are the cell membrane, the endosomal membrane and the nuclear membrane. Hence it is important to fully understand the mechanism through which carriers enter cells. There are four major groups of drug and gene carriers which are organic cationic compounds, viral carriers, recombinant proteins and inorganic nanoparticles (Azzam and Domb 2004; Xu et al 2005). A large number of nanoparticles can be potentially used as carriers for the cellular delivery because of their versatile properties, including good biocompatibility, selective targeted delivery and controlled release of carried drugs. Fullerenes belong to the class of inorganic nanoparticles and show wide availability due to their small size (~ 1 nm) and biological activity. The activities of this allotropic form of carbon rest upon the properties of both, the fullerene core and its chemical modification. The fullerene core is very hydrophobic, while the functional groups attached to the core add further complexity to the behaviour of fullerene molecule. By attaching hydrophilic moieties, fullerenes become water-soluble and are capable of carrying drugs and genes for the cellular delivery. Derivatized fullerene can cross the cell membrane and bind to the mitochondria as demonstrated by Foley et al (Foley et al 2002). Moreover DNA-functionalized fullerenes are able to enter the COS-1 cells and show comparable or even better efficiency than that of commercially available lipid-based vectors (Isobe et al 2001; Nakamura and Isobe 2003). Biochemical studies on the mechanism of transfection indicate that the fullerene reagent forms a protective sheath around bound DNA, which increases the lifetime of DNA in endosomes and thus supports their chromosomal incorporation (Isobe et al 2006a). For the attachment of DNA-sequences preferably aminofullerenes are employed. The detachment of DNA in the cytoplasm can be achieved either through loss of its amino groups or loss of the binding ability of amines by transformation into neutral compounds (Isobe et al 2006b). A lipophilic slow-release drug delivery system which employs fullerene derivatives to enhance therapeutic efficacy in tissue culture was designed by Zakharian et al (2005). Modified fullerenes have the potential to provide such a lipophilic slow-release system and is comprised of significant anticancer activity in cell culture as demonstrated with C60-paclitaxel conjugate. Furthermore the ability of fullerenes to penetrate through intact skin is widening their application in cellular drug and gene delivery (Ryman-Rasmussen et al 2006). A fullerene-based peptide was synthesized by Rouse et al and its ability to penetrate through flexed and unflexed skin was observed (Rouse et al 2007). For this study porcine skin was used as a model for human skin. It was demonstrated that mechanical flexion which alters the structural organization of skin, increases penetration by compromising the permeability barrier of epidermis. Less is known about the toxicity of fullerenes in cell culture and living organism. Some studies were carried out on the biological efficacy of water-soluble fullerenes in vitro (Tsuchiya et al 1995; Dugan et al 1997) and in vivo (Yamago et al 1995; Satoh et al 1997) which indicated low toxicity. Another study was designed to determine the genotoxicity of fullerenes (a mixture of C60 and C70) in bacterial reverse mutation assay including the chromosomal aberration test in hamster lung cells followed by the acute oral median lethal dose of fullerenes when applied to rats (Mori et al 2006). The results revealed that fullerenes did not have the ability to induce acute oral toxicity or in vitro genotoxicity. Although water-soluble fullerenes are not acutely toxic, they are retained in the organism for long periods, raising concerns about chronic toxic effects (Yamago et al 1995). However there is striking evidence that hydrophilic functional groups on the surface of fullerenes dramatically decrease toxicity of raw C60 molecule (Sayes et al 2004). Underivatized fullerenes aggregate in water where they are supposed to cause oxidative damage to cellular membranes even at relatively low concentrations (20 ppb level).
Diagnostic application

The nature of a fullerene cage as a potential “isolation chamber” recommends the possibility to carry an unstable atom, for instance a metal atom, within the interior of the molecular cage forming so-called endofullerenes/metallofullerenes that would be able to isolate reactive atoms from their environment. Several studies have already shown that fullerene cages are relatively non-toxic and resistant to body metabolism (Moussa et al 1997; Chen et al 1998). Biodistribution studies with water soluble derivatives of C60 demonstrate that these compounds are primarily localised in the liver and their clearance is very slow (Moussa et al 1997). Metallofullerenes introduce no release of the captured metal atom under in vivo conditions, in contrast to metal chelates, they have a potential in diagnostic application. Endofullerenes can be applied as magnetic resonance imaging contrast agent MRI, X-ray imaging agent and radiopharmaceuticals.

Fullerol, which is highly water soluble, was chosen for radiolabeling with 67Ga3+. The results show that radiolabeling yields could reach 97% under the best applied conditions. The radiochemical purity of 67Ga-C60(OH)x solution kept at 37 °C remained at 88% after 212 hours. Results from biodistribution studies provide evidence for localization of this compound to macrophages, because the fullerene derivative localized predominately to bone marrow, liver and spleen with slow clearance and negligible amounts in the blood (Li et al 2005). The distribution and metabolism of these newly designed derivatives were also extensively investigated. It was found that holmium metallofullerol molecules could significantly accumulate in liver; moreover, they could be detected in the bone. The localization of the metallofullerol in bone can bring an important conclusion that these species are selectively targeted to tissues rich in macrophages and might be useful chemotherapeutic agent for treatment of leukemia and bone cancer (Thrash et al 1999).

In addition, evidence was provided for the formation of 99mTc@C60 and 99mTc@C70, the first direct encapsulation of a radionuclide during fullerene formation. These results have already shown the utility of ultra low level radioactivity detection methods for the identification of trace levels of endofullerenes. As it has been mentioned, such encapsulation of a radionuclide would facilitate their inert transport through biological systems. Applying the proper antibody label, the radionuclide could be transported to the region of interest without considerable interaction between radiolabel and the antibody (Karam et al 1997).

For quite long time, bigger fullerenes (C80 or C82) were believed to restrict the incorporation of more than two, larger lanthanide elements. It has already been reported by Cao et al (2002) that a dimetallic species of titanium was incorporated into C80 cage. It is worth mentioning that the encapsulation of a lanthanide metal inside C82 cage, the metal atom typically introduces a trivalent oxidation state in order to partially fill the lowest unoccupied molecular orbitals (LUMOs) of fullerenes, thus creating an open-shell electronic state (Liu and Sun, 2000). Only few lanthanide elements among others cerium and praseodyum were found to form higher symmetry (Ih) C80 cage encapsulates (Ding et al 1996).

Interestingly, Iezzi and his co-workers recently synthesized an endohedral metallofullerene by trimetallic nitride template process that allowed the formation of a stable high symmetry (Ih) [Lu3N]6+[C80] cage (Iezzi et al 2002). In comparison with Sc3N@C80 metallofullerene yields were found to be considerably lower for lutencium metallofulleren than for scandium metallofullerene (Stevenson et al 1999). Due to the similarities between Sc3N@C80 and Lu3N@C80 in spectroscopic data and chromatographic behavior, one can conclude that the trimetallic nitride cluster has a minimal influence on properties of fullerene cage.

In another approach, fullerene derivatives were used as a carrier for serum protein profiling, which is a powerful tool for the identification of protein signatures for pathologies and biomarker discovery, using material-enhanced laser desorption/ionisation (MELDI) technique. MELDI is a new form of laser desorption/ionisation and introduced in 2005 in our laboratory is (Feuerstein et al 2006; Rainer et al 2006). Three fullerene compounds in particular: dioctadecylmethano-[C60]fullerene (DOMF), [C60]fullereneacetic acid (FAA) and copper(II) iminodiacetic acid-[C60]fullerene (IDAF) were tested for their ability as MELDI carrier materials using human serum (Vallant et al 2006). The fullerene materials are incubated with serum and the absorbed proteins and peptides are analyzed with MALDI-TOF MS. Each of the three fullerenes gave reproducible mass spectra, but their spectra were notably different, reflecting the different components trapped by the diverse adsorption behaviors. For screening the proteins in serum, direct laser irradiation of the adsorbed proteins was possible over the range m/z 2000–30,000. However, for identification and fractionation, it was necessary to elute the adsorbed compounds and subject them to MALDI-TOF MS analysis. Figure 5 illustrates the influence of fullerene derivatizations on the MELDI protein profile patterns in the m/z range of 2300–6300 (A, B and C) and 10200–20000 (D, E and F); (A and D) dioctadecyl methano[60]fullerene, (B and E) [C60]fullereneacetic acid, (C and F) Cu(II)-IDA-[C60]fullerene. In the low-molecular-weight
region at m/z 1000–4000, where most potential biomarkers are expected to be found, FAA gave several peaks in the spectrum, whereas DOMF gave none whatsoever. Unfortunately, the signal intensities in case of FAA were too low for compound identification by MS/MS analysis, so again, the compounds needed to be eluted for identification.

**Conclusion**

Since fullerenes were discovered at the end of last century, many new findings and important aspects on these carbon molecules have been accumulated to form a new exciting scientific field. Recent developments suggest that many of proposed fullerene applications are to be practical technologies in a wide range of areas such as IT devices, diagnostics, pharmaceuticals, environmental and energy industries. The direct application of fullerene and their derivatives to biological targets is now yielding promising applications in medicine. Such attention to them is caused by unique chemical and physical properties of the fullerene core, including of their photodynamic properties. The hydrophobic spheroid and the radical sponge character of fullerene are responsible for the activity in different fields. Fullerenes have unusual redox chemistry and may be reversibly reduced by up to six electrons. These, along with the low toxicity detected so far in fullerenes, are sufficient to stimulate researchers in chemistry and in biology to unite their efforts and systematically investigate the biological properties of these fascinating molecules. A wave of research and development activities all over the world has led to large number of application-oriented patents, spanning a very broad range spectrum of potential commercial applications, including: anticancer anticancer drug delivery systems using photodynamic therapy, HIV drugs, and cosmetics to slow down the aging of human skin.

The fullerene field is going to contribute to industries. In the past, high production cost of fullerenes has been the main obstacle in the development of fullerene market. We are now seeing a very rapid decline in price which will open the door to a host of other applications. In addition, many industrial applications of fullerenes are now being commercialized.

**Abbreviations**

ADF, Amino acid derivatives of fullerene C60; AIDS, Acquired immunodeficiency syndrome; ALS, Amyotrophic lateral sclerosis; DOMF, Dioctadecylmethano[C60]fullerene; FAA, [C60]fullerenoacetic acid; IDAF, Iminodiacetic acid-[C60]fullerene; HIV, Immunodeficiency virus; HIV-RT, Immunodeficiency virus reverse transcriptase; HIV-P,
Immunodeficiency virus protease; LUMO, lowest unoccupied molecular orbits; MRI, Magnetic resonance imaging; MELDI, Material-enhanced laser desorption/ionization; PDT, Photodynamic therapy; PBMCs, Peripheral blood mononuclear cells; PEG, Poly ethylene glycol; SFV, Semiliki forest virus

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