COVALENT AND NON-COVALENT DERIVATIVES OF FULLERENE C\textsubscript{60} AND ITS BIOLOGICAL ACTIVITY

Aleksandar N. Djordjevic, Mirjana B. Vojinovic-Miloradov, Gordana M. Bogdanovic

This paper presents the basic chemical characteristics of buckminsterfullerene molecule C\textsubscript{60} and some of its biologically active derivatives. This new form of carbon with unique properties, discovered in 1985, is a very reactive species undergoing a variety of chemical reactions leading to a wide spectrum of possible covalent derivatives. Besides covalent compounds, fullerene reacts with different dopants to form non-covalent complexes, including endohedral, exohedral systems (intercalates). The review of the most significant papers on non-covalent and covalent derivatives and their biological activity is given, as well as our results in this field. Formation of intercalates of fullerene with small molecules of aliphatic alcohols is described and the importance of topology and size of dopants and the role of solvents in formation of intercalates is explained. Original synthesis of bromine derivative C\textsubscript{60}Br\textsubscript{24} and water-soluble polyhydroxy derivative C\textsubscript{60}(OH)\textsubscript{24} is described. Antioxidative effects of the latter compound were investigated in chemical model system and in vitro system of human neoplasmatic cells. The paper also gives theoretical explanation of addition C\textsubscript{60}R\textsubscript{24} derivatives together with assumption about antioxidative activity of fullerol.

KEY WORDS: fullerene C\textsubscript{60}, covalent derivatives, intercalate, fullerol, antioxidant activity, conformation C\textsubscript{60}R\textsubscript{24}

DISCOVERY OF BUCKMINSTERFULLERENE

Discovery of icosahedral buckminsterfullerene molecule has a long and very interesting history. For the experimental proof of the existence of fullerene molecule, in 1996 Nobel prize was awarded to Harold Kroto, Richard Smalley and Robert Curl, leaders of American-English research team that in 1985 synthesized this molecule as the most perfect symmetry form existing in nature (1).

Fullerene molecule possesses geometry of truncated icosahedron, which was already known to Leonardo and Duhler back in 1500, Fig. 1. (2).
In the middle of 20th century it was believed that there existed hollow carbon cages, but his hypothesis was not widely accepted. In 1971 Yoshida-Osawa research team suggested a spherical structure of an icosahedron for the ideally aromatic hydrocarbon-C_{60} (3). Although there are indications that Iijima and Huffman-Kreatchmer team synthesized this molecule, they could not recognize it in their experimental data (4).

Russian chemists Botchkvar and Gal’pern predicted the existence of C_{60} in 1972 on the basis of physical-chemical calculations, but their work remained unnoticed (5). It was “rediscovered” after experimental results of Crotto and Smolcny.

The shape of C_{60} reminds of a soccerball, and in the older literature it is referred to as “footballene”. The name “fullerenes” was given to the family of carbon clusters by Crotto and Smolcny after the American architect Richard Buckminster Fuller who designed geodesic domes (Fig. 2) in which pentagon was used to provide curvature.

![Fig. 1. Regular truncated icosahedron](image)

![Fig. 2. Geodesic dome designed for Montreal EXPO '67](image)

**STRUCTURE OF MOLECULE C_{60}**

Spherical molecule C_{60} and similar molecules from the class of fullerenes are cages in which carbon atoms are located in the vertices of a polyhedron. Bonds between them form two different types of rings-pentagons and hexagons.

In the symmetrical buckminsterfullerene, 60 C atoms are located in the vertices of a regular truncated icosahedron. All C atoms are equivalent, showed by a single peak in its {^{13}}C NMR spectrum.

Regular truncated icosahedron comprises 20 hexagonal and 12 pentagonal faces and 90 edges of the same length. Molecule C_{60}, strictly speaking, is not a regular truncated icosahedron. Although all C atoms are equivalent, three bonds of each atom are not equivalent and differ in length. The shorter C bond between two hexagons possesses higher electron density and can be considered double. In chemical reactions, the [6,6] double bonds with average length of 0.1370 nm act as electron-deficient polyolefins. The other two bonds are [5,6] single bonds between pentagon and hexagon, with an average length of 0.1448 nm.

Due to the closed-shell structure of C_{60} and other fullerenes, sp^2 hybridization is not planar as in graphite, but has a certain degree of pyramidalization (11.6°). In the molecular structure of C_{60} every pentagon is surrounded by five hexagons performing structure similar to corannulene. However, double bonds in corannulene are differently organized. Another structure
which can be recognized in molecule \( \text{C}_{60} \) is piracylene. It is consists of two pentagons and two hexagons which are placed in a way that structure contains non-adjointing pentagons. Considering piracylene as a part of molecule \( \text{C}_{60} \), isolated pentagon rule (IPR) can be applied. According to this rule, pentagons are placed in the longest interatomic distance which decrease curvature of molecule and increase stability of \( \text{C}_{60} \) sphere (1). In Fig. 3, molecules of corannulene and piracylene are presented.

**Fig. 3.** Structure of corannulene and piracylene

Graphite and diamond crystallize within 32 crystallization groups whereas molecule \( \text{C}_{60} \) forms crystal form which is, from symmetrical point of view, on the higher level of organization. Molecule \( \text{C}_{60} \) crystallize around a point. In order to obtain this structure, the five-fold axis has to be formed. This symmetry axis has not been known in crystallography before. Besides five-fold symmetry axis, which refers to pentagons, the molecule has three-fold axis (hexagonal), two-fold axis (double bond) and inversion point of symmetry (Fig. 4). Molecule \( \text{C}_{60} \) possesses icosahedral symmetry (Ih); this geometric solid has 12 vertices and 20 equilateral triangles and 30 edges. In icosahedron vertices there are centres of pentagons, while hexagons are the places between the pentagons.

**Fig. 4.** Icosahedral symmetries

Due to the five-fold symmetry axis, structure and energetic properties are determined by golden symmetry rule.

**PHYSICAL PROPERTIES OF FULLERENE \( \text{C}_{60} \)**

Experimental data show that the diameter of the molecule is 0.710 +/-0.007 nm, the outer diameter of \( \pi \) electron cloud can be estimated as 0.34 nm, inner diameter of \( \pi \) electron cloud can be estimated as 0.350 nm; bond energy per atom is 7.40 eV ( \( \Delta H = 2550.5 \text{ kJ/mol} \text{ C}_{60} \); electron affinity 2.65 +/-0.05 eV).

Pure fullerenes are black crystals, moderately soluble in non-polar organic solvents. Basic physical characteristics of fullerene \( \text{C}_{60} \) are given in Table 1 (6-9).
Table 1. Basic physical properties of fullerene C\textsubscript{60}

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average C-C bond length</td>
<td>0.144 nm</td>
</tr>
<tr>
<td>Average diameter of C\textsubscript{60} ball</td>
<td>0.683 nm</td>
</tr>
<tr>
<td>Outer diameter of C\textsubscript{60} ball</td>
<td>1.018 nm</td>
</tr>
<tr>
<td>Inner diameter of C\textsubscript{60} ball</td>
<td>0.348 nm</td>
</tr>
<tr>
<td>Specific density</td>
<td>1.72 g/cm\textsuperscript{3}</td>
</tr>
<tr>
<td>Molecular concentration</td>
<td>1.44 x 10\textsuperscript{-2} cm\textsuperscript{3}</td>
</tr>
<tr>
<td>Elastic modulus</td>
<td>14 GPa</td>
</tr>
<tr>
<td>Bond energy per atom</td>
<td>7.4 eV</td>
</tr>
<tr>
<td>Electron affinity</td>
<td>2065 eV</td>
</tr>
<tr>
<td>Energy gap (HOMO-LUMO)</td>
<td>1.7 eV</td>
</tr>
</tbody>
</table>

Buckminsterfullerene is practically insoluble in water and proton-acceptor solvents. It is soluble in halogen and alkyl-substituted benzen. Solubility of C\textsubscript{60} at 20°C in mg/ml: decalin 4.6; tetrachloromethan 0.32; tetrachloroethylene 1.2; 1,1,2,2-tetrachloethan 5.3; benzene 1.7; toluene 2.8; xylene 5.2; mesitylene 1.5; tetraline 16.0; bromobenzene 3.3; anisole 5.6; chlorobenzene 7.0; 1,2 dichlorobenzene 27.0; 1,2,4-trichlorobenzene 8.5; 1-methylnaphthalene 33.0; dimethylnaphthalene 36.0; 1-phenylnaphthalene 50.0 (10-11).

IR spectrum of C\textsubscript{60} contains four vibrational peaks (535, 880, 1190 and 1435 cm\textsuperscript{-1}). Solution of C\textsubscript{60} in benzene has characteristic maxima at 213, 257, 329 nm and minor maxima at 500, 540, 570, 600 and 625 nm (12). Presence of only one signal in \textsuperscript{13}C NMR spectra of the molecule C\textsubscript{60} at 143.3 ppm points to the perfect equality of all 60 C atoms placed on the sphere and confirms icosahedral symmetry.

**CRYSTALLINE STRUCTURE OF MOLECULE C\textsubscript{60}**

In solid state, molecules of C\textsubscript{60} crystallize in the form of face-centred cubic lattice (Fig. 5), with the lattice constant of 1.417 nm and the shortest interatomic distance of 1.002 nm (13). At temperatures below 225 K, the first phase transition occurs into simple cubic lattice.

**Fig. 5.** Face-centred cubic crystal lattice of buckminsterfullerene C\textsubscript{60}

**CHEMISTRY OF FULLERENES C\textsubscript{60}**

Fullerenes are highly electron-deficient systems and their chemical behaviour can be approximated with electron-deficient polyolefines (-RC=CR-)\textsubscript{n} while R stands for alkyl, acyclic or cyclic part of molecule (14-15).

Basic principles in the chemistry of molecule C\textsubscript{60} comprise various chemical transformations, together with theoretical investigations of C\textsubscript{60} and its derivatives. Using combinations of nucleophilic and electrophilic additions, cycloaditions and radical additions, it is possible to covalently bond any class of organic compounds to a fullerene core.
These structural and electronic characteristics of fullerene $C_{60}$ provide possibility for conducting six main types of chemical transformations:

1. Additions
2. Electron transfer chemical reactions yielding fullerene salts
3. Reactions of ring opening and degradation.
4. Formation of endohedral complexes $Cn@M$
5. Intercalation $[\text{guest}]_{n} C_{60}$
6. Formation of heterofullerene

High rate of pyramidalization indicates the sp$^2$ hybridization of C atoms in $C_{60}$. Strain energy (33.5 kJ/mol per C atom) makes about 80% of heat of formation. Driving force of addition reactions with small number of addends is a result of decreasing rather large inner strain of fullerene cage. Reactions which lead to sp$^3$ hybridized C atoms cause decrease of pyramidalization in the molecule $C_{60}$. This explains exothermal effects of most addition reactions. In reactions of total hydrogenation or halogenation $C_{60}X_n$ adducts are not the primary products obtained. In fact, they are rather unstable and the reaction of cage opening and molecule degradation is favoured. This lack of stability originates from another driving force in the fullerene chemistry. Introduction of new type of strain is further deviation of the tetrahedral sp$^3$ hybrid angle of fullerene C atoms in adducts. If addition product has a small number of addends on the $C_{60}$, sphere releasing strain force in fullerene cage is dominant and such addition products are relatively stable. Addition rate on molecule $C_{60}$ is a consequence of balancing these two opposite effects (16). Comparing reactivity of $C_{60}$ with another classes of compounds, it can be observed that $C_{60}$ behaves like systems with completely localized π-electrons and electron-deficient polyolefins. Analogously to olefins, $C_{60}$ undergoes a variety of cycloadditions.

1. Additions

Knowledge of rules and mechanism of addition to fullerenes makes possible the controlled synthesis and transformation of the side chains of addends, yielding monomers, polymers and copolymers with the potential application in medicine, pharmacy, technology, new materials, electronics, optics and catalytic processes and other applied sciences. Addition products can be of detergents and spheroid type. Nucleophilic additions include hydroalkylation and halogeneration with organic-Li and Grignard reagents (10). Owing to different positions of addends placed on the carbon sphere a number of regioisomers can be expected. Addition products of $C_{60}$ containing two addends could provide eight different products while triaddend derivatives could form 46 possible different regioisomers. Many of these addition reactions are not selective (17, 18). Radical additions to $C_{60}$ are similar to nucleophilic additions, in which organic radical R, is generated in situ (photochemically or thermally) yielding diamagnetic or paramagnetic adducts. Such are metallation of $C_{60}$ by pentacarbonyl-Re-radicals, hydrostannylation, or radical copolymerization (19, 20).

Reactions of hydrogenation of fullerene are the most common reactions of additions and provide necessary information on kinetics and thermodynamics of these addition products. Polyhydro fullerenes $C_{60}H_{13}n$ ($n = 7$-30) were obtained by standard methods using Brec-Hickel reductions with Li in liquid ammonia in the presence of t-BuOH (21, 22).

$$C_{60} + LiNH_2(t-BuOH) \rightarrow C_{60}H_{13}n (n = 7 - 30)$$

Polyhydro fullerenes with 24,26,28,30,32,34,36,38,40 and 56 hydrogen atoms were successfully identified by mass spectroscopy with the most intensive signal of $C_{60}H_{34}$. Characteristic colour of reaction mixture of $C_{60}H_7n$ is ivory. These results are in a good correlation with findings of the Monte Carlo method by which the presence of stable 1,4 addition products has been confirmed. Halogen derivatisations are applied in formation of intermediates of
C_{60}. Molecule C_{60} is halogenated by fluorine (23, 24), chlorine and bromine (25, 26). Halogen derivatives with fluorine, chlorine, bromine are very sensitive to reactions of nucleophilic substitutions (14).

Bromination in CS\textsubscript{2} or CHCl\textsubscript{3} yielded polybromine compound C\textsubscript{60}Br\textsubscript{8} (Fig. 6a), whereas bromination of C\textsubscript{60} in benzene resulted in C\textsubscript{60}Br\textsubscript{6} (Fig. 6b). These polybromine derivatives are unstable and C\textsubscript{60}Br\textsubscript{6} spontaneously disproportionates into C\textsubscript{60} and C\textsubscript{60}Br\textsubscript{8}.

![Fig. 6a. Molecular structure of C\textsubscript{60}Br\textsubscript{8}](image)

![Fig. 6b. Molecular structure of C\textsubscript{60}Br\textsubscript{6}](image)

Directed bromination of C\textsubscript{60} without solvent, at different temperatures yielded reaction mixture of polybromine derivatives, with low solubility in the most common organic solvents (27). This reaction mixture of polybromine derivatives served as a model system for substitution reactions (28). Bromination of C\textsubscript{60} with excess of elementary bromine in an atmosphere of nitrogen 8 days at room temperature with stirring yielded the compound C\textsubscript{60}Br\textsubscript{24} (29). Theoretical studies of bromine derivatives of C\textsubscript{60} showed that C\textsubscript{60}Br\textsubscript{24} differs in its symmetry and heat of formation comparing to C\textsubscript{60}Br\textsubscript{6} and C\textsubscript{60}Br\textsubscript{8} (30). The synthesized polybromine derivative has been studied by X-ray diffraction and it was shown that Br\textsubscript{2} molecules are occluded in a crystal structure of the molecule (C\textsubscript{60}Br\textsubscript{24}(Br\textsubscript{2})\textsubscript{x}) (31). Catalytic bromination of C\textsubscript{60} with elementary bromine yielded a single reaction product - C\textsubscript{60}Br\textsubscript{24} without occluded molecules of bromine in its crystal structure. The obtained structure is presented in Fig. 7 (32).

![Fig. 7. Structure of C\textsubscript{60}Br\textsubscript{24}](image)

Twenty four bromine atoms are symmetrically distributed over the C\textsubscript{60} sphere, which was confirmed by thermogravimetric analysis. IR spectrum of C\textsubscript{60}Br\textsubscript{24} (KBr) contains characteristic bands at: 1250, 1180, 1140, 1080, 1050, 960, 930, 850, 780, 760, 600, 560, 550 cm\textsuperscript{-1}. IR spectrum of the catalytically synthesized C\textsubscript{60}Br\textsubscript{24} is in accordance with the literature data (31).

The thermogravimetric curve contains only one sharp peak at the temperature of 165.2°C and the loss of mass is 72.59%. The sharp peak on the TGA curve (Fig. 8) shows that in the
process of thermal transformation all bromine atoms are lost, which is a characteristic of the completely symmetrical distribution of bromine over the C\textsubscript{60} molecule.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_8.png}
\caption{TG curve of C\textsubscript{60}Br\textsubscript{24}}
\end{figure}

Experimental loss of mass of 72.59\% is in accordance with the theoretical loss of 24 bromine atoms of 72.70\%. Experimental evidence of the symmetry of C\textsubscript{60}Br\textsubscript{24}, which is obtained by the thermogravimetric analysis, is completely in accordance with the theoretical predictions of the stability of this molecule.

The substitution of bromine atoms from C\textsubscript{60}Br\textsubscript{24} with hydroxyl groups yields the brown water-soluble fullerol C\textsubscript{60}(OH)\textsubscript{24} (33). IR spectrum (KBr) of fullerol is shown in Fig. 9.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_9.png}
\caption{IR spectrum of fullerol C\textsubscript{60}(OH)\textsubscript{24}}
\end{figure}

IR spectrum contains characteristic bonds for C-O, O-H, C=C and C-C vibrations. Wide peak at 3427 cm\(^{-1}\) is characteristic of stretching vibrations of the hydroxyl group. This value indicates that hydroxyl groups are not associated with intramolecular hydrogen bonds. Bands at 1627 and 1419 cm\(^{-1}\) represent stretching vibrations of the C=C and C-C bonds, and the band at 1080 cm\(^{-1}\) is characteristic of the C-O stretching vibrations. These results are in accordance with the literature data on the IR spectrum of fullerol (34-39). The \textsuperscript{13}C NMR (D\textsubscript{2}O) spectrum of fullerol contains peaks at 170 ppm and at 143 ppm which also refers to the sp\textsuperscript{2} C-atoms.

The structure of fullerol C\textsubscript{60}(OH)\textsubscript{24} is presented in Fig. 10.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_10.png}
\caption{Structure of C\textsubscript{60}(OH)\textsubscript{24}}
\end{figure}

Substitution of bromine atoms of polybromine derivative C\textsubscript{60}Br\textsubscript{24} with -OMe, -OEt, -OiPr, -OPr, -OBu resulted in formation of ether derivatives of the general formula C\textsubscript{60}(OR)\textsubscript{24} (16). Theoretical conformation analysis of fullerene derivative with 24 addends confirmed that conformation of the carbon cage does not change irrespective of the mentioned addends (-Br, -OH, -OMe, -OEt, -OiPr, -OPr, -OBu) (40).
Besides polybromine derivatives, polyamine derivatives play an important role as precursors in further synthetical paths. Synthesis of various fullerenes derivatives containing primary and secondary amines was performed (41-43). Theoretical and experimental data on chemical and physical characteristics of amino derivatives of C<sub>60</sub>(NH<sub>2</sub>)<sub>n</sub> is of great importance for the synthesis of polypeptide chains of the C<sub>60</sub>(NHICOR)<sub>n</sub> type which might be biologically active (44).

Fullerene polymers can be divided into two fundamental groups: homopolymers and copolymers. Homopolymers consist of one monomer species, bonded in a linear chain. Copolymerisation occurs between two monomers X and Y, but binding is rarely in random manner. Basic types of polymerisation are: “pearl necklace”, “charm bracelet”, “pendant on chain”, “pendant on surface”, “flagellens or dendrity”, “star polymers”, together with the dimers C<sub>116</sub>, C<sub>118</sub>. Functionalization with OH- groups and further derivatisation (Fig. 11) yield products that exhibit significant solubility in polar solvents (45, 46).

![polyesters](image)

**Fig. 11.** Forming of polyesters with chloride acids derivatives

1.1. Cycloadditions

Cycloadditions have been very well examined and some types of cycloadducts are exceptionally stable (12=400°). In these reactions, (6,6) bond always reacts as dienophile. The first synthesized crown ether with the molecule C<sub>60</sub> belongs to the group of cycloaddition products [4+2] (Fig. 12) (47).

![Crown ether C<sub>60</sub>](image)

**Fig. 12.** Crown ether C<sub>60</sub>

Reactions of C<sub>60</sub> with different diazomethans (48,15,45,46, 51,52, 53) diazoacetats (15, 54) and diazoamids (55) yielded a number of different fullerenes with methano bridge in their structure.

After treatment with diazomethane, initial methanofullerene C<sub>61</sub>H<sub>11</sub>, forms pyrazoline intermede which has been completely characterized. It has been shown that the first phase of this transformation is [3+2] cycloaddition of diazomethane, which occurs on the [6,6] double bonds. After releasing nitrogen, (photochemically or thermally) two different bridged products can be formed: closed ring system where is bridge placed in the position 1,2 and 1,6 isomer with open ring (Fig. 13). Compound with closed rings is by 25.1 kJ/mol more stable compared to the addition products with open ring structure (56).
Additions of another diazo-reagents e.g. substituted diphenyldiazomethane or alkyl-diazoacetate, leads to the mixture of two different isomers. In additions to symmetric diazo-compounds, four different isomers can be formed \( \text{A, B, C, D} \) (Fig. 14). These compounds are systems with closed and open rings with methylene bridges in the positions 1,2 (A and B) and 1,6 (C and D). Experimentally isolated compounds as well as computing of stability of different regioisomer addition products, confirm regularity regarding introduction of double bonds at the junction of pentagon and hexagon, which is proved to be energetically unstable.

Stable five-membered ring adducts could be formed in the [3+2] cycloaddition reactions of \( \text{C}_{60} \) with trimethylenemethanes (TMM) (57-59). Many chemical transformations of side chain of these compounds can be performed. The results achieved are important from many reasons: the chemistry of side chain provides a route for the synthesis of new potential bio-logically active fullerene derivatives. A series of addition derivatives of [3+2] cycloaddition are presented in Fig. 15.
Fig. 15. Chemical transformation of side chain of [3+2] cycloaddition product

Cycloaddition procedures for the [2+2] mode synthesis are presented in Fig. 16 (60,61).

Fig. 16. Addition procedures for s [2+2] mode synthesis on the molecule C\textsubscript{60}

Cyclopropanation of C\textsubscript{60} with diethylbromomalonate in toluene in the presence of NaH as an auxiliary base is occurring at room temperature. Monoaduct with 29 double bonds is dominant product of this reaction. By-products are unreacted C\textsubscript{60} and other adducts (62). Reaction scheme is presented in Fig. 17.

\[
C_{60} + \text{BrCH(\text{COOEt})\text{t}_{2} \xrightarrow{\text{NaH, } \Delta \ x 2}} C_{61}(\text{COOEt})\text{t}_{2} \text{Br} \xrightarrow{\text{NaBr, toluen}} C_{61}(\text{COOEt})\text{t}_{2}
\]

Fig. 17. Synthesis C\textsubscript{61}(\text{COOEt})\text{t}_{2}
Assuming that addition is symmetric and takes place exclusively on the [6,6] double bonds, it can be theoretically deduced that there are 8 possible isomer bisadducts, where 7 regioisomers have been isolated from the reaction mixture of C_{62}(COOEt)_4 (63). Since fullerenes are electron-deficient species, they require high anode potentials (in electrochemical oxidations) or strong oxidation agents, such as UV irradiation, O_2, O_3, or OsO_4 (64).

2. ELECTRON-TRANSFER CHEMICAL REACTIONS YIELDING FULLERENE SALTS

Chemical reactions with electron-donor reagents (nucleophilic agents) are characteristic for the molecule C_{60}. This molecule can be easily reduced by accepting electrons from nucleophilic reagents, which shows that this molecule is a weak oxidation agent. Molecule C_{60} is reduced in fullerides by accepting up to 6 electrons, forming various anions and radical-anions. Ability to undergo reduction has made possible the formation of C_{60}^- molecular superconductors (containing C_{60}^-) and “soft” ferromagnets (containing C_{60}^-) (65, 43).

3. NON-COVALENT DERIVATIVES OF FULLERENES, SUPRAMOLECULE REACTIONS (INTERCALATES) [guest]_{n-x-a}C[C60]

Besides the covalent chemistry of fullerenes, the synthesis and study of multicomponent molecular systems containing discrete C_{60} molecules is an important field of research. In such systems, the nature of the intermolecular (inter-C_{60}) contacts and their effect on the bulk properties is of particular interest. These inter-C_{60} contacts may be in all three dimensions, as in the fcc packing of pure C_{60}, or can be restricted to two dimensions in close-packed layers or one-dimensional structures.

Intercalates with no charge transfer are called clathrates. In a clathrate structure, the fullerenes appear on a sublattice, and other molecules reside either on other sublattices or at random lattice sites.

Fullerenes are a unique class of compounds in this aspect, because they can form different types of complexes:
1. Endohedral complexes are the systems in which dopant is located inside the fullerene cage.
2. Exohedral complexes (intercalates, Fig. 18) are the most common forms of fullerene complexes, in which dopant molecules are outside the cage, bonded to the C_{60} surface or located inside the cavities in the crystalline lattice of solid C_{60} (67).

![Fig. 18. Intercalate system of P_4 with fullerene](image-url)
When fullerenes interact with dopants without charge transfer, clathrates are formed as the systems in which dopants are distributed between the layers of C₆₀ molecules. These structures are stabilized by Van der Waals interactions between C₆₀ and dopants, usually solvents, or the mixture of solvents and the third molecular species. Since there is no charge transfer, physical properties of clathrate materials can be predicted from the properties of the constituents.

Intercalates with no charge transfer can be formed between C₆₀ and neutral molecules O₂, Sₓ, Iₓ, or n-pentane (68,69). It was also shown that small gas molecules such as O₂, CO and CO₂ can intercalate into C₆₀ lattice.

One of the best studied clathrates is C₆₀I₄, ordered crystal in which polarisable iodine molecules lie in the plane between ABA-layers of C₆₀ molecules, thus forming simple hexagonal cell (70).

Recently it has been shown that Ar C₆₀ clathrate can be obtained by interactions of Ar and solid C₆₀ in which argon atoms occupy octahedral positions (71).

Smaal et al. have shown that CO intercalates into C₆₀ lattice at 200 bar and 200°C. Dopant occupies octahedral holes of the close-packed cubic cell (72). Non-cubic crystals containing C₆₀ and solvent molecules were synthesized with aliphatic molecules (n-pentane, diethyl ether, 1,3-dibrom propane, cyclohexane and 1,4-hydroquinone). Water-soluble complex was obtained with y-cyclodextrine, in which C₆₀ is a “guest” molecule inside of a cluster of host molecules (73).

New non-covalent intermolecular fullerene system containing discrete C₆₀ and alcohol molecules have been prepared from the saturated solutions of fullerene in toluene and xylene with ethanol, methanol and isopropanol (66, 74). “Intercalation” refers to the (reversible) exohedral doping of mobile guest species into crystalline host lattice that contains an interconnected system of empty lattice sites (-) - interstitial positions - of appropriate size (66):

\[ X_{\text{guest}}^+ + x[C₆₀] \rightleftharpoons [\text{Guest}]_x[C₆₀] \]

Intercalation reactions are usually reversible and can also be characterized as topochemical processes, since the structural integrity of the host lattice is formally conserved in the course of the forward and reverse reactions.

The formation of intercalates with alcohols R-OH⊂C₆₀ (R=C₆₋C₆) has not been described in the literature yet. Pure intercalates R-OH⊂C₆₀ were obtained in the direct synthesis of C₆₀ in toluene with R-OH (methanol, ethanol and isopropanol). IR spectra of C₂H₅-OH⊂C₆₀ and C₆₋H₋O⊂C₆₀ showed no change compared to the pristine C₆₀. However, IR spectrum of the methanol intercalate contains additional peaks at 2920 and 3400 cm⁻¹ (Fig. 19).

![Fig. 19. IR spectrum of methanol intercalate C₆₀](image)

There has also been no change in their diffraction images concerning the d-values (the diffractograms of powder), indicating that the compounds are isostructural with C₆₀. The appearance of a very intensive d₁₀₁ peak in the powder diffractogram of the methanol derivative indicates that stacking disorder is probably eliminated, which can be correlated to the additional bands in the IR spectrum. Intermolecular interactions (of the type that exists in supramolecular systems) between C₆₀ and alcohol molecules C₆₋C₆ exist because of the specific energy surfaces of C₆₀ molecule (the consequence of its electronic, vibrational and rotational proper-
tics) and the size, inductive, spatial and geometrical characteristics of intercalates (alcohols). The size and shape of the alcohols which form intercalates with C\textsubscript{60} are such that they nicely fit within the intermolecular distances of C\textsubscript{60} molecules (0.293 nm) in a hexagonal lattice. Presence of methyl groups bonded to aromatic rings of solvent molecules is necessary in formation of described intercalates. The solvent plays an active role in the mechanism of intercalation, acting as a “tray” which brings molecules of alcohol in the suitable position to interact with electron-poor hexagonal face of the fullerene molecule (Fig. 20). Solvent molecules are not incorporated in the intercalate crystalline lattice, and binary systems of intercalates are obtained. Papers in this field published so far indicate the existence of exclusively ternary intercalate systems of the molecule C\textsubscript{60}.

Intercalates of C\textsubscript{60} with methanol, ethanol, and isopropanol are binary systems, and this was experimentally confirmed by GC/MS spectroscopy (66).

Theoretical investigation of these “host-guest” supramolecules is a contribution to intercalation chemistry as an important discipline related to solid-state chemistry, materials science, organic, inorganic and physical chemistry.

4. FORMATION OF ENDOHEDRAL CN(@M COMPLEXES

Based on the inner \(\pi\) electron cloud diameter of the molecule C\textsubscript{60} that is 0.350 nm and medium cage diameter is 0.711 nm, it is evident that atoms, ions, or molecules can be incorporated inside the ball. These compounds are called endohedral fullerenes and they are marked as X@C\textsubscript{n}, \(n \geq 24\) (Fig. 21).

In the studies of endohedral fullerenes, theoretical investigations were performed on X@C\textsubscript{60}(X = He, Ne, Ar, Kr, Xe, Ca, Mg, Li, Na, K, Sc, La, O) (75). Compounds with
Encapsulated noble gases have been the first discovered endohedral fullerenes (76). On the basis of the inner diameter of C$_{60}$ and theoretical calculations, He and Ne fit into the cage, whereas Ar, Kr and Xe form less stable structures due to much more overlapping of Van der Waals diameters (77, 78). When the atom of noble gas is encapsulated in the cage potential energy is decreasing and reaches minimum when the atom is centered; this energy is called endohedral complex energy (77). The most common experimental procedures to generate X@C$_{60}$ (X=metals) are based on formation of composite rods from the oxide of the metal whose intercalate is to be obtained, graphite rod and graphite cement rod. Molar ratio C/M in the composite rod is of primary importance for yield of X@C$_{60}$ and differs from the metal to metal one. These parameters could be experimentally obtained. Experimentally synthesized endohedral fullerenes are X@Cn (X=He, Ne, Ar, Kr, Xe, Li, Li, Na, K, Ca, Sc, Sc$_2$, Se, Mn, Ti, Zr, Hf, U, Y, Y$_2$, Eu, Gd, Gd$^{+}$, Cu, Zn, Mo, Ce, La, La$^{+}$, La$_2$, Ho, Ho$_{2}$, Ho$_3$) (75). Endohedral complexes for the time being can be synthesized only in small yields so physical and chemical characteristics are not completely defined. Chemical derivatisation of endohedral fullerenes into water-soluble molecules opens the possibilities for a potentially wide application in biomedicine as a diagnostic and therapeutic agents (79-81). In the future, endohedral fullerenes might have very large application in the environmental protection as a safe method for radioactive waste storage (82, 83).

5. BIOLOGICAL ACTIVITY OF FULLERENE C$_{60}$ AND ITS DERIVATIVES

Investigations of fullerene C$_{60}$ in vitro and in vivo models can be divided into two basic groups: undervatizcd C$_{60}$ and derivatized C$_{60}$. Potential application of the molecule C$_{60}$ in human organism is primarily limited by its hydrophobic characteristics. There are several fundamental methods aimed at overcoming the hydrophobic characteristics of C$_{60}$: encapsulation in special layers and chemical functionalisation with the aim to introduce hydrophilic group bonded to the molecule cage.

**Biological activity of underivatized fullerene C$_{60}$**

Encapsulated molecule C$_{60}$ can be solubilised in water as a complex with cyclodextrines (73, 84) calyxarenes (85,86) and polyvinyl pyrolidyne. (PVP). Hemolytic test performed on the sheep’s erythrocytes has not exhibited any effect of PVP-C$_{60}$ (87). Encapsulated C$_{60}$ can be fagocytosed by human leukocytes. (88, 89). Using irradiations, the molecule C$_{60}$ can be excited to the short-living singlet state $^1$C$_{60}^*$ (1.3 ns) which is almost quantitatively transformed into the long-living triplet state $^3$C$_{60}^*$ (50-100 ms) Assumed mechanisms of generating toxic oxygen radicals by photoexcitation of C$_{60}$ is presented in Fig. 22.

![Fig. 22. Possible mechanisms of generating toxic oxygen radicals by photoexcitation of C$_{60}$](image-url)
Triplet lifetime of the molecule C\textsubscript{60} is of fundamental importance for photoactivity in cells and plays practical role in cell cytotoxicity of triplet species with the lifetime higher than 100 \( \mu \text{s} \) \( (90) \). Some derivatives of C\textsubscript{60} also exhibit biological activity in the presence of light.

**Biological activity of derivatized fullerenes C\textsubscript{60}**

Potenitional application of the molecule C\textsubscript{60} in the human organism is primarily limited by its hydrophobic characteristics. The main chemical principles for conversion into water-soluble derivatives include mainly already mentioned synthetic methods, \textit{in vivo} biological behaviour of a water-soluble fullerene; \textit{\textsuperscript{14}C} labelling, absorption, distribution, excretion and acute toxicity. To assess potential activity of water-soluble fullerenes as drugs, studies on the \textit{in vivo} behaviour of fullerenes need a radiolabelled, water-soluble fullerene, in order to obtain data on the oral absorption, distribution and excretion of this class of compounds. In biological studies of fullerene derivatives one of the first steps is to examine their cytotoxicity. Cytotoxic activity of some derivatives is in a direct correlation with DNA cleavage \( (91) \).

One of the promising biologically active compounds of fullerene is p, p'-bis-succinimide derivative of p, p'-bis(aminooethyl)-phenyl fullerene C\textsubscript{60}, which is a potential cure for AIDS.

Active center of HIV-1 protease is quasispheric hydrophobic hole approximately sized 1 \( \text{nm} \). Concerning molecules modelling the first results were accomplished. It is showed that C\textsubscript{60} carbon sphere can almost perfectly fit inside the hydrophobic media of HIVP. The main idea for the synthesis more fullerene derivatives is based on the formation of strong hydrophobic interactions HIVP-C\textsubscript{60} derivative. It has been synthesized many of C\textsubscript{60} derivatives with anti HIV-1 activity and biological investigations \textit{in vitro} and \textit{in vivo} systems indicate the most potent p, p'-bis-succinimide derivative rather than p, p'-bis(aminooethyl)-phenyl fullerene C\textsubscript{60} \( (92-94) \). Structure of the molecule is presented in Fig. 23.

![Fig. 23. p,p'-bis-succinimide derivative of p,p'-bis (aminooethyl)-phenyl fullerene C\textsubscript{60}](image)

It has been shown that the compounds that act as radical sponges reduce though not completely, neuronal death caused by radical stress. Two derivatives formed in trisaddition of malonic units to fullerene C\textsubscript{60} exhibit antioxidative activity C \( (3) \)-tris-malonyl-C \( (60) \)-fuleren 1 and D(3)-tris malonyl-C(60)-fuleren 2 (Fig. 24)). They are highly soluble in water and are excellent radical scavengers \( (95) \).
Compound 1 increases also the lifetime of cells deprived of oxygen and glucose, without antagonism between 1 and NMDA (N-methyl-d-aspartat) receptors (96). Bis malonic acid adducts also express antioxidative properties (figure 25) (97).

Tetra malonic adducts turned to be less potent and exhibit smaller antioxidative activity than tris and bis adducts of C_{60} (98). Polyhydroxylated derivatives of C_{60} (fullerols, fullerol) of the general formula C_{60}(OH)_{24} belong to the group of water-soluble biologically active compounds extremely important for the application in biomedicine, pharmacy, and cosmetics. In the scientific literature, a variety of procedures for the synthesis of these water-soluble polyhydroxylated derivatives are described. Depending on the synthetic path and number of hydroxyl groups per molecule of C_{60}, they are named in several ways: fullerol-1, fullerol-2, fullerol (34, 99, 39, 37, 35, 100).

Endohedral radioactive nuclides in fullerol could be useful for therapeutic and diagnostic methods (79-81). Fig. 26 is presented theoretically modulated molecule $^{99m}$Te@C_{60}(OH)_{24}.

The first experimental in vivo results concerning endohedral fullerol Hox@C_{82}(OH)_{y} were published in 1999 (101). Radioactive holmium $^{166}$Ho is commercially used in diagnostics ($E_{\beta}^{\text{max}}=1.8\text{MeV}, t_{1/2}=26.8\text{h}$). Compound $^{166}$Hox@C_{82}(OH)_{y}, in the form of water solution, is intravenously injected into experimental rats and administration of $^{166}$Hox@C_{82}(OH)_{y} x=1,2...
in different tissues in different time schedule was monitored and compared to control compound Na\(^{166}\text{Ho}([\text{DTPA}][\text{H}_2\text{O}])\). Results indicated 20% excretion of intact \(^{166}\text{Ho}\text{ox}@C_{60} (\text{OH})\) within 5 days. 48 hours after administration, the compound was found in this tissues: brain (0.2%), lipid tissue (0.2%), blood (0.2%), skin (0.2%), muscles (0.3%), uterus/ovaries (0.5%), heart (1%), lungs (1.5%), spleen (6%), bone (12%), liver (15.2%), kidneys (33.5%). After 4 hours, concentration of \(^{166}\text{Ho}\text{ox}@C_{60} (\text{OH})\) had dropped significantly in all organs except for kidney, spleen, bone, and liver. Another possible application of fullerol could be referred to water-soluble drug carrier.

The acute toxicity of fullerol-1 was determined using mice pretreated intraperitoneally with polyhydroxylated \(C_{60}\) derivatives. The LD\textsubscript{50} value of fullerol-1 was estimated to be 1.2 g/kg. Pretreatment fullerol-1 decreased cytochromes P450 and \(b_5\) contents and NADPH-cytochrome P450 reductase, benzo[a]pyrene hydroxylase, 7-ethoxycoumarin-O-deethylase, aniline hydroxylase and erythromycin-demethylase activities in liver microsomes (102). Additions of fullerol-1 to mouse liver microsomes suppressed monoxygenases activities toward benzo[a]pyrene, 7-ethoxycoumarin, aniline and erythromycin. Fullerol-1 inhibited non-competitive and mixed-type inhibition in benzo[a]pyrene hydroxylation and 7-ethoxycoumarin-O-deethylating respectively. Additions of fullerol-1 to rat liver mitochondria resulted in a dose-dependent inhibition of ADP-induced uncoupling and markedly inhibited mitochondrial Mg\(^2+\) - ATPase activity with an IC\textsubscript{50} value of 7.1 \(\mu\text{M}\) (102). These results demonstrate that fullerol-1 can suppress the levels of the microsomal enzymes in \textit{vivo} decrease the activities of P450-dependent monoxygenase and mitochondrial oxydative phosphorylation in \textit{vitro}. Recently it has been reported the use of water-soluble fullerenols as free-radical scavengers for the absorption of superoxide radicals (\(O_2^-\)) generated by \textit{in vitro} xantine and xantine oxidase. Similar activities were also observed in removing highly reactive hydroxyl radicals (\(\text{HO}^-\)) generated by \(\text{H}_2\text{O}_2\) in the presence of \(\text{Fe}^{2+}\). Water-soluble fullerene derivatives have been demonstrated to scavenge oxygen radicals \textit{in vitro}. The reactivity of the fullerene derivatives is correlated to the intrinsically high electronegativity of \(C_{60}\) molecules. Combination of the moderate electron affinity of the fullerene derivatives and their allylic hydroxy functional groups makes fullerene derivatives candidates for the application as water-soluble antioxidants in the biological system. The antioxidative capabilities of administered fullerene derivatives in the respiratory system in vivo were examined by Yih-Loong Lai et al (103). In neuronal cultures, fullerenols reduce glutamate-induced neurotoxicity by about 80% at 50 \(\mu\text{M}\) which shows that eage fulleren molecules exert their neuroprotective functions by blocking glutamate receptors and lowering the intracellular Ca\(^{2+}\) (104). Fullerenols have been shown to be effective scavenger of free radicals produced during ischemia-reperfusion injury of the small intestine in dogs (105).

Bogdanovic et al. (106) evaluated cytotoxicity and antiproliferative activity of water-soluble derivative of fullerene \(C_{60}(\text{OH})_{24}\) against human tumor cell lines (K562, Raji, MCF) and animal cell lines (L929 and BHK). Preliminary results showed that generally low, but transient growth inhibition activity of water soluble derivative was obtained on the most of cell lines, regardless of their structure and features. Raji cells appeared to be resistant to growth inhibition activity.

The effect of fullerol \(C_{60}(\text{OH})_{24}\) on the growth of tumor cells in culture and its modulating effect on adriamycin (ADR) induced cytotoxicity in three different human breast carcinomas cells lines (107). Growth inhibition was evaluated by tetrazolium colorimetric WST1 assay. ESR spin “trapping” method was used to assay OH-radical scavenger activity of fullerol during Feuton’s reaction.

Fullerol \(C_{60}(\text{OH})_{24}\) inhibits human breast cancer cell lines in different manner depending on the cell type, dose and time of exposure. However, none of the fullerol concentrations induced 50% growth inhibition.

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Cell lines were simultaneously treated with ADR and fullerol in order to estimate modulating effect of fullerol on ADR-induced cytotoxicity (107). The cell growth was evaluated after 24, 48 or 72 h of continuous exposure to ADR (1 μmol/ml) and fullerol at a range of concentrations. The results showed that fullerol in combination with ADR, strongly inhibited ADR cytotoxicity for more than 50% in almost all concentrations, at each time point. However, distinct dose-dependent inhibition of ADR cytotoxicity was found in MDA-MB 231 cells after 48 h ranging between 25.67 and 71.87 % at extreme concentrations of fullerol. The rate of inhibition of ADR and fullerol combination was similar to, or lower than the inhibition induced by fullerol alone.

When we added fullerol at a concentration of 1.9 μg/ml, one hour before, simultaneously or one hour after ADR (0.1 μmol/ml) to MCF7 cells, we noticed extremely good cell survival after 48 h incubation. The survival fractions of MCF7 cells were 91.38, 87.73 and 87.47 % respectively.

According to the data that have recently appeared in the literature, polyhydroxylated derivatives of fullerene C_{60} have been proposed to be potent antioxidants (108). To established the effects of fullerol on the hydroxyl radical formation, fullerol, at a concentration of 0.5 μg/ml, was added prior to hydrogen peroxide in the described reaction mixture.

The trapping of hydroxyl (OH*) radical by fullerol led to a decline in the formation of the PBN-hydroxyl radical adduct (PBN-OH). The scavenging activity of fullerol was estimated by the percentage of decrease of the relative intensity (RI) of the PBN-OH adduct signal, with a reference to the control without fullerol. All the experiments were repeated three times. RI of ESR signals of PBN-OH adduct in the presence of 0.5 μg/ml of fullerol decreased by 88%. (Fig. 27a, b). The results showed that fullerol was a potent hydroxyl radical scavenger of the radicals formed in Fenton’s reaction.

![Fig. 27. ESR spectra of PBN-hydroxyl radical adduct obtained during Fenton’s reaction](image-url)
The obtained results suggest that the antiproliferative effect of fullerol and its protective effect on ADR cytotoxicity might be mediated through antioxidative and hydroxyl-radical scavenger activity of fullerol but additional investigations are necessary for better understanding of these mechanisms, as well as antioxidative and prooxidative properties of C_{60}. Speaking of hydroxyl (OH) radical it is shown that antioxidative ability is decreasing with increasing number of addends. This conclusion is confirmed by the experiments with bis-malonic adducts of malonic acid of C_{60} and fullerols, where bis adducts exhibit higher scavenger activity (109). On the other hand, produce of singlet oxygen species is not correlated to the addends nature but decreases with the number of saturated double bonds in the molecule C_{60} (110).

Although water-soluble fullerenes are not toxic, they are retained in the body for long periods, raising concerns about chronic toxic effects. The fact that fullerenes distribute rapidly in many tissues suggests that they may be useful to deliver highly polar drugs through membranes to a target tissue and they may even have applications in the delivery of drugs to the brain. Recent advances in the fullerene synthetic chemistry can enable control over fullerene absorption/excretion profiles in the future.

CONCLUSION

Spherical carbon clusters, fullerenes C_{n} (n≥24), of which the most significant is C_{60}, are new allotropic modifications of carbon. In difference to graphite and diamond, fullerenes are very reactive species. Molecule C_{60} undergoes covalent reactions (yielding addition derivatives) and non-covalent chemical transformations (yielding endohedral and exohedral derivatives). Derivatization of fullerenes gives various addition products with specific physical and chemical properties. If polar functional groups (–OH) are bonded onto the C_{60} molecule, water soluble derivatives - fullerols C_{60}(OH)_{n} (n=10-24) - are obtained. Two-step synthesis via C_{60}Br_{24} intermediate, including total substitution of bromine atoms, yielded fullerol C_{60}(OH)_{24}, which exhibits antioxidative effects in both chemical model system and in vitro system. Molecule C_{60} forms isostructural intercalate binary systems with small aliphatic alcohols (C1-C3). Due to its specific symmetry, physical and chemical properties, fullerene C_{60} represents a specific scientific and technical challenge and has wide research and application potentials - thus opening the new, fullerene era.

REFERENCES

Proceeding.


**КОВАЛЕНТНИ И НЕКОВАЛЕНТНИ ДЕРИВАТИ ФУЛЕРЕНА C\text{60} И ЊИХОВА БИОЛОШКА АКТИВНОСТ**

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У раду су описана основе карактеристике бакуменстерфулерена, молекула C\text{60}. Ова нова форма угљеника са јединственим особинама, открivena 1985. је веома реакциона врта која подлеже низу хемијских реакција (адицијама, циклоадицијама, електрохемијским и хемијским редукцијама, деградацијама итд.), у којима настaje широк спектар могућих ковалентних деривата. Поред ковалентних јединења, фулерен реагује са различитим допантима при чему настају нековалентни комплекси, укључујући ендоедарске, егзоедарске и супстанциона дереивате. Дат је преглед најзначајнијих радова о ковалентним и нековалентним дериватах и њиховоj биолошкоj активности, као и преглед раних радова из ове области. Описана је настајање фулеренских интеркалате са низом алифатичних алкохола и математичким моделовањем објашњен значај топологије и величине допантa, као и улога раствараца у процесу интеркалатије. Описана је оригинална синтеза бромног деривата C\text{60}Br\text{24} и водорастворног деривата C\text{60}(ОН)\text{24}. Испитане су антиоксидативни својства полицикливог дереивата у хемијском модел системима и у \textit{in vitro} систему хуманих неоплазматских ћелија.

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