

IMPLICATIONS OF THE NANO-CAGED STRUCTURE OF C₆₀ IN MOLECULAR TOXICITY AND BIOCOMPATIBILITY AMONG DIFFERENT BIOSYSTEMS

Submitted in partial fulfilment of the requirements for the award
of Master of Technology degree in Biotechnology

By

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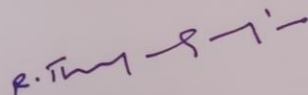
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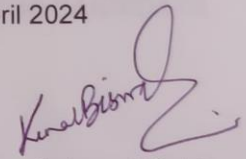
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **NIROLIN NANCY S (42810006)** who carried out Project entitled "**Implications of the nano-caged structure of C₆₀ in molecular toxicity and biocompatibility among different biosystems**" under our supervision from November 2023 to April 2024


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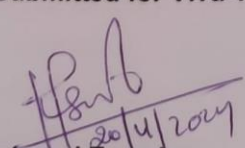

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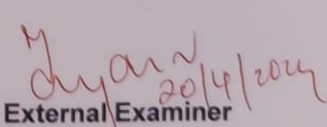
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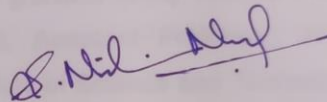

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DECLARATION

I, Nirolin Nancy S (42810006), hereby declare that the Project Report entitled **"Implications of the nano-caged structure of C₆₀ in molecular toxicity and biocompatibility among different biosystems"** done by me under the guidance of Dr. R. Thyagarajan, and Dr. Kunal Biswas at the International Research Centre, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Master of Technology degree in Biotechnology.

DATE: 20.04.2024



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ABSTRACT

C₆₀ has a distinctive nano-caged structure, also known as fullerene. Because of its remarkable qualities, the nano-caged structure of fullerene C₆₀ has generated a lot of attention in biological research. Its unique structure—60 carbon atoms organized in a spherical form—offers a wide range of possible uses in treatment, imaging, and drug administration. However, because of possible toxicity and problems with biocompatibility in different biological systems, questions have been raised about its safety. It is essential to comprehend the molecular processes behind these effects in order to safely and effectively use C₆₀ in cancer therapies and other biological applications. The many special characteristics of C₆₀ are revealed by its synthesis and characterisation. Research on the effects of C₆₀ on cells, organs, and organisms was examined to determine its toxicity mechanisms and assess its biocompatibility in various biosystems, with a focus on cancer cell lines. The toxicity mechanisms of C₆₀ include ROS scavenging, oxidative stress, and cell damage. The interplay between C₆₀'s properties and the biological milieu modulates its biocompatibility, with variations observed in MCF-7 cancer cell lines. To minimize dangers and optimize C₆₀'s therapeutic potential, a thorough grasp of its molecular toxicity mechanisms and interactions with biological systems is necessary. To guarantee the safe and efficient use of C₆₀ in biomedical applications, future research efforts should concentrate on improving biocompatibility evaluation techniques and clarifying the long-term consequences of C₆₀ exposure.

Keywords: C₆₀ Fullerene, Biosystem, Molecular Toxicity, Bio-Compatibility

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List of Abbreviations

BMMC	Bone Marrow-derived Mast Cells
CNC	Cellulose Nano Crystal
DCF	Di-Chloro Fluorescin
DCFH-DA	2',7'-Di- Chloro Fluorescin Di-Acetate
DFT	Density Functional Theory
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
EDTA	Ethylene Diamine Tetra acetic Acid
FCC	Face Centered Cubic
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infra-Red
HMME	Hematoporphyrin Mono-Methyl Ether
HUVEC	Human Umbilical Vein Endothelial Cells
MESP	Molecular Electro-Static Potential
MRI	Magnetic Resonance Imaging
NO	Nitric Oxide
PDT	Photo Dynamic Treatment
PEG	Poly Ethylene Glycol

PS	Photo Sensitizers
PVP	Poly Vinyl Pyrrolidone
ROS	Reactive Oxygen Species
UV-Vis	UV- Visible
XRD	X-Ray Diffraction

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CHAPTER 1

INTRODUCTION

1.1 STRUCTURE OF C₆₀

Fullerenes are a third carbon allotrope that are large carbon cage molecules that are assumed to be three-dimensional equivalents to benzene [Kroto *et al.*, 1985]. The most prevalent type of fullerene is Buckminster fullerene (C₆₀), which has 60 carbon atoms arranged in a spherical pattern. It is the molecule with 12 pentagons and 20 hexagons, shaped like a soccer ball and is called a truncated icosahedron. Through sp² hybridization, each carbon atom forms a bond with three other neighbouring atoms [Krätschmer *et al.*, 1990]. One of its most crucial biological characteristics of C₆₀ is that, it has a capacity to function as a "free radical sponge" and quench different free radicals more effectively than traditional antioxidants [Krusic *et al.*, 1991]. Illuminating C₆₀ with visible or UV light, on the other hand, it promotes its transition to a stable triplet excited state, where energy is then transferred to the molecule of oxygen to produce extremely reactive singlet oxygen [Markovic., 2008].

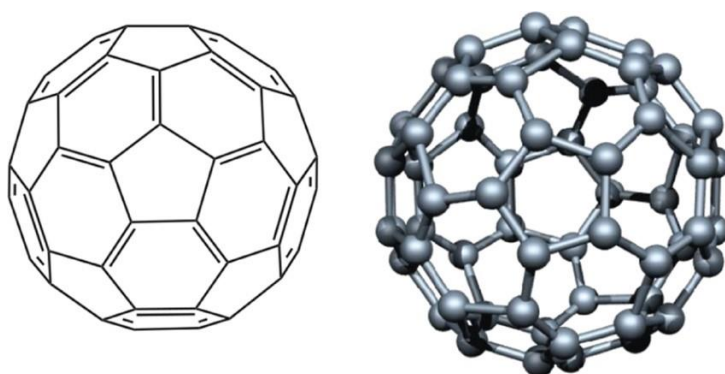


Figure 1.1: 2D and 3D schematic structure of the C₆₀ fullerene
(Source: Porto L.S *et al.*, 2020)

1.2 REACTIVE OXYGEN SPECIES (ROS)

Singlet oxygen and other reactive oxygen species (ROS) are recognized to be involved in both cellular signaling and cell damage [Briviba *et al.*, 1997]. C₆₀'s ability to either quench or create cell-damaging ROS could thus be used to develop it into a cytoprotective or cytotoxic anticancer/antimicrobial drug. The biocompatibility of fullerene-based drug delivery or tissue scaffolding systems, as well as the predicted widespread usage of C₆₀ in consumer goods, may be threatened by the potential ROS-dependent toxicity of C₆₀. Research into these problems has been hampered by extremely low water solubility of C₆₀ and the fact that derivatization of the fullerene core with different functional groups and other solubilization processes can change the photophysical properties and ROS-generating/quenching capacity of C₆₀. Additionally, even though the fullerene molecule's diameter of 0.7 nm it does not match the requirements for nanoparticle size [Williams, 2008]. Reactive Oxygen species are signaling molecules. Radicals, ions, or molecules containing one unpaired electron in their outermost electron shell are examples of reactive oxygen species. ROS are extremely reactive as a result of this trait [Langenstroer *et al.*, 1992]. High levels of reactive oxygen species can be caused by increased metabolic activity, mitochondrial dysfunction, increased cellular receptor signaling, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxygenases, and thymidine phosphorylase, or cross-talk with infiltrating immune cells [Lee *et al.*, 2006].

Numerous clinical conditions include hypertension, ischemia-reperfusion injury, diabetes, and cytotoxic brain injury [Yamakoshi *et al.*, 2003] are caused by NO with superoxide anion. Reactive oxygen species (ROS), which are extremely reactive molecules produced by photoexcited fullerene C₆₀ through energy and electron transfer, are what cause the interaction with the NO radical and the eradication of NO-derived relaxation [Mirkov *et al.*, 2004]. When NO is liberated from sodium nitroprusside solution, fullerene C₆₀ displays scavenging activity. Water-soluble fullerene derivatives have been shown to have antioxidant and antibacterial properties, as well as free radical scavenging and anti-tumor activity [Sato *et al.*, 2006]

1.3 BIOFUNCTIONALIZATION

Hydroxylated fullerenes and carboxyfullerenes are powerful antioxidants and free radical scavengers in a variety of biological model systems (such as cultured cells, mice, and rats) which performs decreasing lipid peroxidation, regulating apoptosis, and scavenging both the superoxide anion and H_2O_2 . According to reports, polyhydroxylated fullerlenols regulate macrophages for cancer adoptive immunotherapy and significantly suppress tumor spread. To create improved nanotherapeutics for treating pancreatic cancer, Glycofullerenes are being studied as non-receptor tyrosine kinase inhibitors. In General, the creation of multiple derivatives of different fullerenes increases the original fullerenes' functionality and increases the spectrum of their applications, from industry to medical. In addition to a variety of anti-tumor capabilities, fullerene and its derivatives have also been demonstrated to possess traits that are absent from typical features such as enormous specific surface area, highly specialized nanostructures, and electron affinity caused by surface chemistry. As a result, fullerene compounds have better water solubility, lower toxicity, and more effective tumor targeting when compared to other conventional anti-tumor medications [Ye *et al.*, 2021].

With such tremendous applications, the toxicological aspect of fullerene also needs to be analyzed. When present in high quantities, fullerenes are expected to have inflammatory, cell-damaging, and carcinogenic capabilities, giving them a "two-edged sword" quality. The pristine fullerene exhibits significantly superior safety. Following pulmonary exposure, C_{60} fullerenes seem to be capable of inducing localized reactions that are neither inflammatory nor toxic in character, but rather anti-inflammatory. During the inhalation exposure period, C_{60} fullerene particles have shown no significant inflammation or tissue injury. Meanwhile, the immune system-related genes have been up-regulated by particles of C_{60} fullerene [Fujita *et al.*, 2009].

They are highly symmetrical and extremely stable. C_{60} fullerenes can pass through cell membranes and blood-brain barriers (BBB). Fullerenes are

bioresorbable and readily enter cells, which can make them attractive candidates for tumour therapy. Fullerene with amphiphilic and self-assembly characteristics [Chen & Elimelech, 2006] which is highly desirable for efficient drug delivery and tumour imaging [Naz *et al.*, 2019]. Fullerenes in pristine and doped forms have high antineoplastic efficiency [Skivka *et al.*, 2018]. In addition, their spherical shape means that they are less likely to damage healthy tissues than other drug-delivery vehicles that are commonly used in chemotherapy. Depending on whether the molecule has a surplus or shortage of unpaired electrons, fullerenes can act as both donors and acceptors of unpaired electrons, attaching them to the double bonds [Shytikov *et al.*, 2021]. Fullerenes are biocompatible with strong free radical scavenging and antioxidant potential [Shytikov *et al.*, 2021] [Prylutska *et al.*, 2014]

1.4 APPLICATIONS

Nanoconjugates of fullerene and anticancer drugs are proven to be more effective at reducing toxicity, enhancing efficacy, and controlling drug release. The bioavailability of the drug has been significantly improved by fullerenes. In addition, their spherical shape means that they are less likely to damage healthy tissues than other drug-delivery vehicles. C₆₀ due to its cage-like structure makes it useful in drug delivery applications. Due to their adaptability, nanoparticle-based drug delivery systems have emerged as the perfect platform for the treatment of cancer. As its goal is to restrict the proliferation of tumour cells, the nano-carrier has demonstrated great potential in the treatment of lung cancer [Sharma *et al.* 2019]. Fullerene as a nanocarrier of anticancer drugs including cisplatin, gemcitabine, docetaxel, paclitaxel, doxorubicin, landomycin, fluorouracil etc... has shown a tremendous decrease in toxicity *in vivo* and *in vitro*. Research on delayed drug release with fullerene nanovesicles has synthesized three amphiphilic functionalized fullerenes C₆₀R₅Cl (R = methyl ester of 4-aminobutyric/glutamic acid or phenylalanine) for effective drug release through regulation of vesicle polarity and size by amino acid methyl ester

groups [Lin *et al.*, 2017]. There are several possible approaches to cancer treatment with fullerenes and their nanoconjugates as anticancer agents.

1.4.1 Tumour suppression activity

Among the biological and pharmacological characteristics of water-soluble fullerene derivatives is their antioxidant activity. However, it has been shown that a water-soluble fullerene colloid called nano- C₆₀ produced reactive oxygen species (ROS) inside cells, which in turn caused lipid peroxidation and cell death [Bozzini, G., *et al.*, 2012]. Yamawaki and Iwai found that hydroxyl fullerene C₆₀ (OH)₂₄ promoted autophagic cell death and induced an accumulation of polyubiquitylated proteins in human umbilical vein endothelial cells (HUVECs), but did not appear to promote apoptosis. Furthermore, Isakovic, A *et al.*, 2006 and his colleagues showed that in mouse fibrosarcoma (L929), rat glioma (C6), and human glioblastoma (U251) cell lines, nano- C₆₀ triggered the caspase-dependent and ROS-independent pathways of C₆₀ (OH)_n-mediated death. When exposed to the C₆₀ derivative, the human cancer cell line may experience apoptosis due to ROS generation. As apoptotic regulators, ROS are essential to the upstream/downstream signaling system. The cationic C₆₀ derivative induced apoptotic cell death in HL-60 cells, which was at least partly mediated by ROS. Anti-cancer medications have the ability to induce apoptosis, which allows phagocytosis to remove dead cells without triggering inflammation in the surrounding tissue. A C₆₀ derivative is a novel possibility for anti-cancer medications [Nishizawa, C., *et al.*, 2009].

CHAPTER 2

REVIEW OF LITERATURE

2.1 PROPERTIES OF C₆₀

According to [Giacalone, F. and Martin, N., 2006], Since the preparation of fullerene C₆₀ in multigram amounts in 1990, a wide variety of chemically modified fullerenes have been synthesized and outstanding structural, magnetic, superconducting, electrochemical, and photophysical properties were reported. This unabated interest in fullerenes has allowed exploring the many possibilities that these carbon nanostructures have in biological applications and particularly, materials science as well as in the emerging field of nanoscience and nanotechnology. Because of their original structure, three-dimensional fullerenes as well as polymers are intrinsically useful scaffolds for construction of high molecular weight structures. Therefore, combination of both systems has led to a wide variety of new materials showing appealing features based on the possibility of tuning their properties by modifying the chemical nature of the components or the chemical linkage between them. Several reviews have previously been reported in many literatures.

C₆₀ molecule is composed of 60 carbon atoms which are arranged as 12 pentagons and 20 hexagons [Taylor R. and Walton D.R., 1993]. All rings are fused and double bonds are highly conjugated. The reason for high symmetry of this molecule is 120 symmetrical operations found in molecule like rotation around the axis and reflection in plane maps the molecule onto itself. When a carbon atom is placed at each vertex of molecule which all valences satisfied by two single bonds and one double bond, stabilized by resonance gives structure of C₆₀ molecule, appears to be aromatic.

2.1.1 General Properties

Pure fullerenes have better close packing than impure one. Derivatives of C₆₀ show range of solubility [Taylor R *et al.*, 1990]. Fluorinated derivatives are more soluble and some bromo derivatives are much less soluble. Fullerenes have low density (1.65 g/cc) relative to diamond (3.51 g/cc). Fullerenes are stable up to temperature of 1000°C. As the cage is entirely of sp² - hybridized carbons it gives electron withdrawing negative inductive effect so fullerenes are strongly electron-attracting i.e., fullerenes can readily react with nucleophiles. High strain energies contribute to reactivity. Fullerenes can undergo various reactions [Taylor R., 1999] such as reduction, oxidation, hydrogenation, halogenation, nucleophilic reactions, radical reaction, transition metal complex reaction, regioselective reaction and so on. Fullerenes are susceptible to degradation or decomposition in presence of light [Arbogast J.W *et al.*, 1991] and oxygen. The intersystem crossing of singlet excited state to energetically lower triplet excited state results in decaying of C₆₀. The triplet excited states are prone to many deactivation processes such as ground state quenching, quenching by molecular oxygen and transfer of electrons to molecules [Mohan Gokhale, M. and Ravindra Somani, R., 2015].

2.1.1.1 Stability

Localization of electrons and ring strain affects stability of fullerenes. By applying huckel rule [Taylor R., 1991] to different fullerene derivatives like C₅₀, C₆₀, C₇₀, C₈₀ and C₈₄, among these only C₅₀ and C₇₀ were predicated to be aromatic and C₆₀ was less aromatic. As Huckel's rule gives aromaticity in spherical systems than planar systems, only C₆₀ was found to be more aromatic and highly stable. The highest stability of C₆₀ is due to reduced strain in ring as twelve pentagons are isolated from each other [Kroto H.W., 1987].

When there are minimum bond orders in five-membered rings it indicates that all double bonds are in six-membered rings and none in five-membered rings. When meta position (i) of any two rings is fused with hexagonal ring,

as in Figure 2.1, this arrangement permits minimization of bond orders in five-membered rings. The degree of instability in C_{60} will occur when requirement of minimization of bond orders is not satisfied as there is para relationship between two pentagonal rings. It is significantly seen in higher fullerenes like C_{70} , it is less stable than C_{60} which contains five arrangements of type (ii). This indicates that as number of carbon atoms increases, stability is going to decrease [Mohan Gokhale, M. and Ravindra Somani, R., 2015]

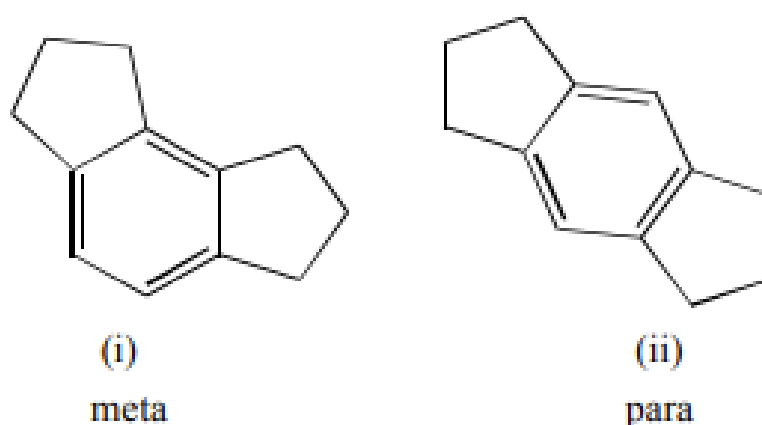


Figure 2.1: Stability of Fullerenes

(Source: Mohan Gokhale, M. and Ravindra Somani, R., 2015)

2.1.2 Characteristics properties of C_{60}

While [Ghasemi, A.S and friends, 2019] studying the properties of C_{60} fullerenes they found that, DFT analysis was used to examine how the anticancer medication gemcitabine interacted with Al-doped C_{60} and C_{70} fullerenes. It is important to note that the medication gemcitabine did not exhibit any binding to the C_{60} and C_{70} pristine cage surfaces. Calculations were made for quantum chemical and physicochemical factors. The results obtained for geometrical characteristics and molecular analysis indicated that the drug was adsorbed by both Al-doped C_{60} and C_{70} fullerenes, which also had negative energies. This characteristic can be understood as these Al-doped fullerenes' capacity to release gemcitabine.

When two distinct fullerene complexes are compared, it can be seen that AIC69Gem has a higher gap energy, more stability, and less chemical reactivity than AIC59Gem. AIC69Gem also has a lesser chemical hardness and a larger chemical potential. Another equally significant and fascinating characteristic of fullerenes is their ability to successfully compete as competing energy acceptors in addition to acting as electron acceptors. The results of this competition mostly depend on the experimental circumstances [Guldi, D.M *et al.*, 2000].

Singlet oxygen can be generated effectively by C₆₀. It's extremely high yield of singlet oxygen and resistance to photooxidative oxidation point to a significant risk of photodynamic harm to biological systems. The level of C₆₀ contamination in the environment thus becomes a crucial issue. The mechanism of quenching by C₆₀ is still unknown; chemical reaction is unlikely because hundreds of laser pulses under oxygen at either excitation wavelength result in neither the loss of starting material nor the synthesis of new product (as seen in UV-visible absorption spectra) [Arbogast J.W., *et al.*, 1991].

2.1.3 Antioxidant properties

The antioxidant capacity of Fullerenols and their derivatives have been measured by DMPO-spin trap/ESR methods, and by using the β -carotene and DPPH assays [Kato, S *et al.*, 2009]. However, the kinetics and radical scavenging mechanisms of these compounds are still not completely understood. Density functional theory (DFT) calculations suggest that fullerenols with 6 and 12 hydroxyl groups are the most stable derivatives [Wang, Z *et al.*, 2014].

Ueno H *et al.*, 2014 studied the antioxidant activity of several hydroxylated fullerenols with 8, 10, 12, 24, 26, 36, and 44 hydroxyl groups by a β -carotene bleaching assay. The relative radical scavenging rate of fullerenols toward radical species derived from linoleic acid under autoxidation conditions indicated that C₆₀(OH)₁₂ and C₆₀(OH)₄₄ reacted

1.62 and 1.54 times faster than β -carotene, respectively. Based on the analysis of the possible products of both fullerenols and C₆₀ with radical species generated from methyl linoleate under autoxidation conditions, the authors proposed two antioxidant mechanisms dependent on the number of hydroxyl groups.

Srđenović, B.U *et al.*,2005 studied the influence of C₆₀(OH)₂₄ on Chinese hamster ovary cells (CHO-K1). Their results showed strong antioxidant properties influencing the cellular redox state and enzyme activities. Therefore, they reduce cell proliferation and were proposed as cytoprotective agents. Improved free radical scavenger properties were reported by Awan *et al.*,2016 as a result of functionalization of C₆₀(OH)₃₀ on the surface of cellulose nanocrystals (CNCs). The immobilization appears to be due to the covalent interaction between the -OH groups on the CNC and π -conjugation of the fullerenols. It was proposed that this results in a reduced tendency to form clusters and greater accessibility to the free radicals

2.1.4 Immunological properties

Funakoshi-Tago *et al* 2016., demonstrated that bis-malonic acid fullerene derivative compound 51 (Fig. 12) suppressed the interleukin 33 (IL-33) induced expression of IL-6 in bone marrow-derived mast cells (BMMC). IL-33 works both intra- and extracellularly as a nuclear factor regulating gene transcription and as a traditional cytokine, respectively. Intracellular IL-33 is thought to be released after cell necrosis to alert the immune system of tissue damage or stress, and extracellular IL-33 coordinates immune defence and repair mechanisms while also initiating differentiation of helper T cells as the adaptive immune response is triggered. This has attracted attention as a new target for the treatment of inflammatory diseases [Wang, S. *et al.*, 2012.].

Pacor S *et al* 2015., studied the interaction between fullerene derivatives 52 and 53 and cells of the immune system and their results stress the important role of fullerene derivatives in the control of inflammatory and cancer diseases in figure 2.2.

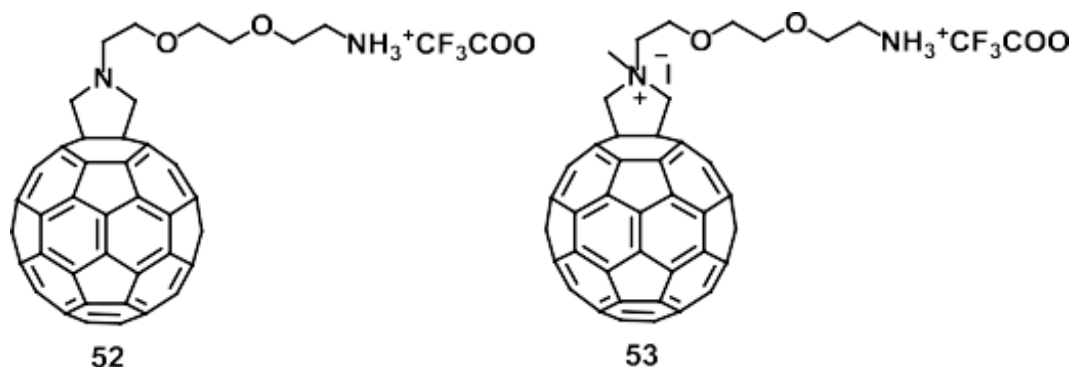


Figure 2.2: Fullerene derivatives with immunological properties

(Source: Castro, E et al., 2015)

52 showed biological properties that make it compatible with monocytes and macrophages, remarking its potential use to deliver compounds capable of modulating the immune responses or as a vehicle to deliver anticancer drugs. Compounds 52 and 53 were more active on neoplastic proliferating cells than on circulating monocytes. Compound 53 is more toxic than compound 52 against the tested cells. At active doses on tumor cells, 53 was found in the cell cytoplasm without altering the cell membrane permeability. It was not clear why 53 was toxic to macrophages, but it can be attributed to the high concentration to which the cells were exposed rather than a negative effect on mitochondrial function.

2.2 IMPLICATIONS OF C₆₀

2.2.1 C₆₀'s nano-caged structure

According to [Hazrati, M.K. and Hadipour, N.L., 2016] finding, several possible configurations were selected for 5-FU approaching the side-wall of fullerene. On a C₆₀ fullerene a few highly symmetric adsorption sites can be considered. Pentagons and hexagons of the cage and the top side directly above the carbon atom were chosen as active sites for the C₆₀ nanocage.

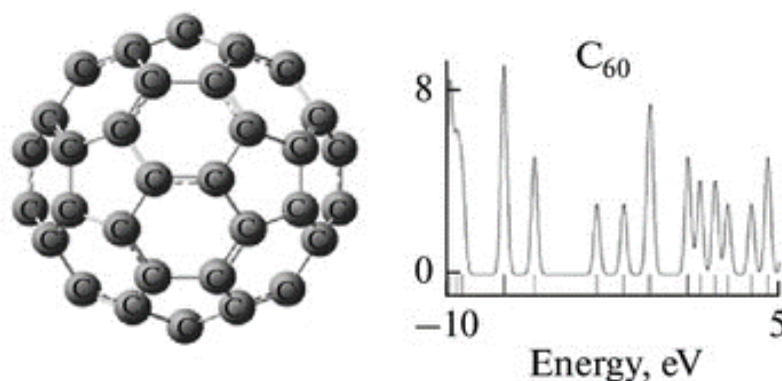


Figure 2.3 Schematic structure of C_{60} nanocage with its density of state plots

(Source: Baei, M.T et al., 2017)

Computed electrostatic potentials on the molecular surfaces of a single 5-FU molecule are showed that the aromatic ring, O (1), O (2), and F atoms might be considered as probable active sites. All the selected dopant atoms, including boron, silicon and aluminum, have more metallic characteristic than carbon. Doping boron, silicon and aluminum in C_{60} cage reduce the E_g of C_{60} by 9%, 16% and 14% for the most stable complexes, respectively. It has been found that 5- FU prefers to be adsorbed via its carbonyl group located between two $-NH$ groups to the doped atom in C_{60} cage. The weak parallel displaced π - π stacking interaction of C_{60} and 5-FU was altered to strong chemisorption by doping the nanocage [Mitschker, J. and Klüner, T., 2012]. Nanocage structure helps to carry the drug more effectively.

2.2.2 Role of ROS

Singlet oxygen and other reactive oxygen species (ROS) are known to be involved in cellular signaling and cell damage and interact with a variety of biological targets [Markovic *et al.*, 2008, Briviba *et al.*, 1997]. Simon H.U *et al.*, 1997 found that ROS plays dual role, Inflammatory cells proliferate in both allergic and non-allergic inflammations, produce ROS. Proteins as well as DNA are both damaged by ROS. Consequently, it is not surprising that ROS

constitute a component of the defensive system against bacteria. Since ROS may potentially kill tissues, which is a scenario that actually occurs under severe inflammatory conditions, antimicrobial activity must be highly selective [Vachier *et al.*, 1994].

Additionally, ROS play a role in the immune system's defensive mechanism because too many of them are created by phagocytes, which in turn boost intracellular signal transduction cascades in T cells and lower their activation threshold [Dröge., 2002]. There is proof that ROS play a part in neuronal apoptosis, a crucial step in brain development [Sorce S *et al.*, 2009].

According to the mitochondrial free radical theory of aging, ROS damage to macromolecules that causes aging. In light of this, it has been suggested that ROS regulate aging through modulating the stress response to age-dependent damage [Hekimi *et al.*, 2011]. Additionally, ROS play a part in how brain cells or CNS cells communicate with one another. For instance, it has been demonstrated that ROS regulate neuronal ion channels, kinases, and transcription factors [Thomas M.P *et al.*, 2007, Brieger K *et al.*, 2012].

2.2.2.1 Role of ROS in apoptosis

ROS have been shown to be significant regulators of pathways that preserve cellular homeostasis, such as cell cycle and death, cellular senescence, and arrest, in addition to their role as potential oncogenic transformation mediators [Chung *et al.*, 2003]. ROS can be viewed as an important participant in the process of programmed cell death since it is a crucial facilitator of stress signaling cascades that can inhibit cellular transformation by inducing cell cycle arrest, apoptosis, and cellular senescence [Curtin *et al.*, 2002, Macip S *et al.*, 2002]. In molecular pathways associated with the growth and spread of tumors, ROS generally appear to be important regulators, providing prospective targets for new therapeutics [Behrend *et al.*, 2003].

ROS is an aggressive chemical oxygen species that can quickly react with any adjacent biomolecules, killing any nearby cells through apoptosis or

necrosis. This includes both normal and pathological cells. Free radicals produced from fullerenes can be produced by two key mechanisms, both of which have anti-cancer effects. In type 1 photochemical mechanisms, Fullerenes receives electrons from reducing agents like NADH or NADPH that is present in the cell causing them to contain an additional unpaired electron and behave like a radical anion C_{60}^- . This transfers an electron to oxygen during the reaction, creating ROS like superoxide or hydroxyl radical. In a type 2 reaction, energy is directly transferred to the oxygen molecule to create exceptionally cytotoxic singlet oxygen (Benedicta *et al.*, 2022).

2.2.3 Quenching of C_{60}

One of C_{60} 's biologically significant characteristics is its capacity to quench different free radicals by acting as a "free radical sponge" [Krusic *et al.*, 1991]. C_{60} 's ability to either quench or produce cell-damaging ROS could thus be used to transform it into a cytotoxic anticancer drug. With increased C_{60} functionalization, an abnormally fast rate of increase in singlet oxygen-quenching capacity may also occur, giving highly functionalized fullerenes outstanding O_2 deactivation capabilities [Markovic., 2008].

It has also been noted that the PVP/ C_{60} and PEG/ C_{60} complexes, as well as the γ -cyclodextrin/ C_{60} complex, effectively quench superoxide and hydroxyl radicals by Xiao *et al.*, 2005. According to the structure-activity connection, C_{60} derivatives with stronger O_2 -quenching groups (like -OH) exhibit less photodynamic activity than those with the same number of weaker O_2 -quenching groups (like -CH) [Mroz P *et al.*, 2007]. The majority of the biological and toxic effects of fullerenes are caused by their capacity to quench highly reactive ROS. This ability is particularly sensitive to the physico-chemical changes brought by the solubilization techniques used to produce fullerene nanoparticles. [Markovic., 2008]

Alzheimer's disease (AD) synergy therapy using C_{60} 's ROS-quenching characteristics to target amyloid- β peptide ($A\beta$), $A\beta$ aggregates, which are essential for the pathogenesis of AD [Dugan, L.L *et al.*, 1997, Gordon, R *et*

al., 2017]. After C₆₀ and the peptide KLVFF were conjugated, up conversion nanoparticle (UCNP@C₆₀-pep) was used as the A-targeted theragnostic platform for treating AD [Li, M *et al.*, 2013]. To reduce possible phototoxicity at nontarget locations and preserve intracellular redox equilibrium, UCNP@C₆₀-pep may quench excessive ROS production. The overproduction of ROS can be quenched by UCNP@C₆₀-pep in the dark, which could alleviate oxidative stress and lessen negative effects [Du, Z *et al.*, 2018] and other fullerene derivatives and their functions are tabulated in Table 2.1.

TABLE 2.1 Tabular column indicates the different fullerene derivatives and their quenching activity

SI.NO	Fullerene Derivative	Quenches	Reference
1	PVP/C ₆₀ and PEG/C ₆₀ , as well as g-cyclodextrin/C ₆₀ complex	efficiently quench superoxide and/or hydroxyl radicals	Guldi <i>et al.</i> , 1999
2	polyhydroxylated C ₆₀ (fullerol) or malonic acid (carboxy) derivatives of C ₆₀	Quench ROS	Hu Z <i>et al.</i> , 2007
3	fullerol	directly quench nitric oxide (NO)	Yamakoshi <i>et al.</i> , 2003
4	SDS-solubilized C ₆₀	a potent superoxide scavenging activity	Markovic., 2008
5	bis-malonic C ₆₀	The singlet oxygen-quenching	Hu Z <i>et al.</i> , 2007
6	PEG-modified fullerene, hydroxy-fullerene and is stearate-mixed fullerene as efficiently as ascorbic acid (Asc)	Effectively quenches hydroxyl radicals	Xiao L <i>et al.</i> , 2005

2.3 TOXICITY

Because of the widespread usage of nanotechnology, there are worries about the compound's safety and toxicity after human interaction. Several studies have demonstrated that fullerenes are extremely beneficial in many aspects despite being harmful due to a variety of reasons including physicochemical features, contact with the environment, pharmacokinetics, and pharmacodynamics [Holmannova *et al.*, 2022].

2.3.1 *In-vivo study*

The capacity of both undoped and doped C₆₀ fullerene to induce inflammatory responses *in vivo* has been identified as one of the potential dangers of chronic fullerene exposure [Trpkovic *et al.*, 2012]. A study with freshwater fish, *Anabas testudineus*, has demonstrated the potential of fullerene C₆₀ to cause genotoxicity even at sublethal concentrations [Sumi & Chitra, 2019]. In C₆₀ exposure experiments even after 48 hours, *Daphnia magna* were still unable to eliminate C₆₀ from their digestive tracts, exhibiting rapid absorption rates but even slower disposal rates [Freixa *et al.*, 2018]. C₆₀ has shown hepatotoxicity and cardiotoxicity in an analysis using rats. The inactivation of Kupffer cells (macrophages of liver cells) upon C₆₀ interaction has been observed [Elshater *et al.*, 2017]

At higher exposure concentrations, increased pulmonary inflammation and cytokine levels have been observed in an experiment involving nose-only inhalation using rats [Sayers, B.C *et al.*, 2016]. It has been demonstrated through research on rabbits that C₆₀ fullerene nanoparticles can alter the mechanical characteristics of red blood cell membrane, making it "softer" than usual and more brittle [Halenova *et al.*, 2020]

2.3.2 *In vitro study*

C₆₀ Nanoparticles have significantly accelerated the growth of melanoma in experimental animals, in contrast to its powerful cytotoxic and

antiproliferative effects on melanoma cells [Zogovic *et al.*, 2009]. An experiment on the toxicity of the pristine C₆₀ fullerene aqueous colloid solution (C₆₀FAS) with human embryonic kidney (HEK293) cells revealed no toxic effects at concentrations of 75-150mg/kg, yet haematological effects were observed at higher levels. Using kidney human embryonic (HEK293) cells, the toxicity of the C₆₀-Cis-Pt nanocomplex in comparison to the undoped Cis-Pt had been investigated. The examined results show that the nano complex with Cis-Pt equivalent concentrations has no harmful effect. It has been reported that Cis-Pt's toxic effect against normal cells is reduced by its complexation with C₆₀ fullerene [Skivka, L.M. *et al.*, 2020].

2.4 BIOCOMPATIBILITY OF C₆₀

Very little is known about engineered nanoparticles and how they interact with cells or human organisms. Once a molecule is below 100 nanometers it begins to interact with proteins and can result in entirely unintended consequences. Particles below 50 nm can translocate into cells relatively easily and are able to interact with channels, enzymes, and other cellular proteins. By changing the surface coatings, the nanomaterial toxicity can almost be completely altered. For example, changing the surface features can change a hydrophobic particle into a hydrophilic one.

According to Rancan, F *et al.*, 2002 interactions between cells and nanoparticles are mediated by the surface characteristics of both the material and the target cells. On one hand, proteins, extra-cellular matrix and cell recognition play an important role. On the other hand, the physico-chemical properties of nanoparticles play an important role in inducing inflammatory and/or cytotoxic effects. Inhaled nanoparticles can translocate from the lung to the blood and reach all target cells of the cardiovascular, hepatic system, and kidney, as well as interact with the pool of immune competent cells.

Several cells and receptors on these organs have been found to be sensitive to nanoparticles and nanoparticles can disturb normal cell function and its autocrine or paracrine signalling in various ways [Borm, P.J. and

Kreyling, W., 2004]. So, while these materials can be safe under laboratory conditions a more reliable or environmentally relevant endpoint is to assess the behaviour of these compounds under environmental conditions.

2.4.1 C₆₀ interaction with cell lines

One of the most efficient drug transporters today is C₆₀ fullerene [Montellano. A *et al.*, 2011]. A typical method to impart C₆₀ fullerene-specific characteristics for enhanced formulation has been the complexation of C₆₀ fullerene with well-known medicinal compounds. Thus, it has been shown that a combination between the anticancer medicine doxorubicin (Dox) and C₆₀ fullerene improves the drug's therapeutic action and boosts the effectiveness of its targeted delivery to malignant tumors [Panchuk, R.R *et al.*, 2015].

Due to their nano dimension and hydrophobicity, the pristine C₆₀ molecules can be incorporated into both a model bilipid membrane (BLM) [Prylutska, S.V *et al.*, 2007, Schuetze, C *et al.*, 2011] and a cellular plasma membrane (PM) [Prylutska, S *et al.*, 2012]. These incorporations have strong antioxidant [Gharbi N *et al.*, 2005, Prylutska, S.V *et al.*, 2011,] and antitumor [Corrie, P.G., 2008] properties while having no acute toxic effects on normal cells. On a model BLM and PM of tumor cells (for instance, the HeLa cell line), the effects of C₆₀ fullerene complexed to Dox. Long acknowledged as a streamlined but effective structure to represent cell membrane (PM), artificial lipid membrane (BLM). In comparison to 1 hour of incubation, 3 hours of incubation with the C₆₀ + Dox mixture results in a more extensive accumulation of drug-fullerene complexes in HeLa cells. After 24 hours of incubation with the C₆₀ + Dox mix, all cells perish. [Montellano. A *et al.*, 2011].

Despite having multiple side effects that have a substantial negative impact on the patient, chemotherapy is one of the most popular cancer treatments [Corrie, P.G., 2008]. Cisplatin (Cis- [Pt (II)(NH₃)₂Cl₂], Cis), a water-soluble derivative of inorganic, divalent platinum, is one of the most widely used anticancer chemotherapeutics. Cis works through a number of different methods, one of which is to hinder DNA synthesis and cellular

proliferation. But almost all of the side effects listed above also occur together with these symptoms [Florea, A.M. and Büsselberg, D., 2011]. C₆₀ fullerene (FC₆₀) as a potential substitute delivery platform for Cis. Pristine FC₆₀ has been demonstrated to have antioxidant activity [Prylutska, S.V *et al.*, 2008, Prylutska, S.V *et al.*, 2011]

2.4.2 C₆₀ interaction with Mouse

Skin lesions are a common feature of atopic dermatitis (AD), a chronic inflammatory skin condition that primarily affects youngsters. The condition frequently coexists with asthma and allergic rhinitis. Numerous findings point to oxidative stress as a mediator of AD [Nakai, K *et al.*, 2009, Kapun, A.P. *et al.*, 2012, Koren Carmi, I. *et al.*, 2015] Reactive oxygen species (ROS) are mostly produced intracellularly by mast cells. The stratum cornea suffers from oxidative protein degradation brought on by the production of ROS, which compromises barrier function and worsens AD. Some of the compounds of C₆₀ fullerene are strong antioxidants that can effectively inactivate ROS [Prylutska, S.V *et al.*, 2011].

In a mouse AD model, nC₆₀ dramatically reduces the generation of particular IgE. Mice were sensitized with EC to cause skin irritation, and subcutaneous and epicutaneous C₆₀ was used to administer C₆₀ therapy. Additionally, it was demonstrated that IL-4 and IL-5 levels considerably dropped following EC and SC C₆₀ administration. It was found that EC C₆₀ therapy significantly upregulates IL-12 and IFN- production by switching the immunological response from Th2 to Th1. Foxp3 and FLG expression is elevated when nC₆₀ is used in the EC route. As a result, there is a decrease in AD susceptibility when Foxp3 and filaggrin expression are increased together. Using nC₆₀ in the form of EC application is a successful alternative therapy method for reducing allergic inflammation. [Shershakova, N, *et al.*, 2016]

In Figure 2.4, using the mouse model, the new C₆₀ and Cis in vivo anticancer potential was evaluated. The tumor volume in the spleen was the

first factor to be evaluated. When compared to the control group, all therapies led to a reduction in tumor volume. On day 20 of the trial, the mice in the FC₆₀ and Cis alone treatment group had 15% less tumor volume than the animals in the control group. The C₆₀+Cis complex treated animals showed the most anticancer impact, with a 75% reduction in tumor volume compared to the control group. The body weight of the mice given FC₆₀ or the C₆₀+Cis complex was considerably lower than that of the untreated animals. The reduction in body mass loss, nevertheless, allowed it to only reach 22% and 15%, respectively. The findings point to a potential tumor therapy using an FC₆₀ and Cis complex as a potentially successful strategy for creating new anticancer treatments for pre-clinical trials [Prylutska, S.V *et al.*, 2017].

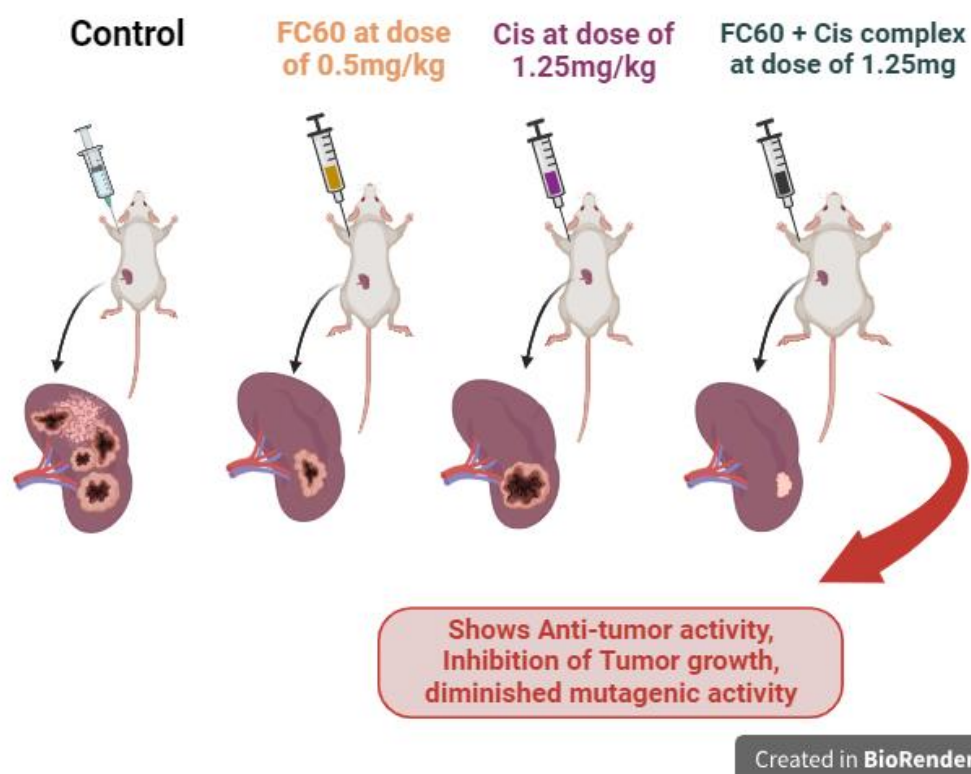


Figure 2.4: In-vivo studies with C57BL/6J in male mice as a model reveal a corresponding increase in the antitumor activity of FC₆₀, Cis and FC₆₀ complexed with Cis

2.4.3 Biomacromolecules

Fullerenes (C_{60}) and its derivatives may be used in biosensors, drug delivery systems, and other products. The genotoxicity of these compounds is a significant barrier to their use in biological applications. Therefore, it is essential to comprehend how fullerenes interact with biomolecules in order to uncover the cause of their toxicity and make them safe for use. Fullerenes' antiviral action was proven through the suppression of the HIV protease [Friedman, S.H *et al.*, 1993, Marcorin, G.L *et al.*, 2000]. The prediction that fullerene derivatives could be able to fit into the HIV protease's catalytic cavity was specifically supported by binding and inhibition studies. There have also been reports of fullerene interactions with a few other proteins, including a fullerene-specific antibody, human serum albumin (HSA), and bovine serum albumin (BSA) [Belgorodsky, B *et al.*, 2005, Rozhkov, S.P *et al.*, 2003]. It was discovered that the three binding sites for HSA type 1, HSA type 2, and HIVP have an intriguing and significant pattern of physicochemical characteristics. The great degree of similarity between the patterns identified for the binding sites of NS C_{60} and CF from a computational perspective is encouraging and demonstrates the consistency of the docking and aligning methodologies. [Benyamini, H *et al.*, 2006].

CHAPTER 3

AIM AND SCOPE

3.1 AIM

To explore the multifaceted implications of fullerene C₆₀'s unique nano-caged structure in the context of molecular toxicity and biocompatibility across diverse biological systems.

3.2 OBJECTIVES

- To analyse the physicochemical properties of C₆₀ Nanomaterials.
- To produce potential anti-cancerous activity of C₆₀ molecules in *In-vitro* conditions.
- To explore the toxicological and ROS dependent studies in cancer cells.
- To check the Bio-Compatibility aspects of functionalized and non-functionalized C₆₀ molecules in healthy cells and cancer cells.
- To evaluate the pharmacokinetics and insilico studies of C₆₀, cancer cells interface studies.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

The chemicals used in the current experiments were: Pristine Graphite (CAS no: 7782-42-5) were procured commercially from Sisco Research Laboratory, India of 99.9% purity with no further purifications.

Table 4.1 Specifications of C₆₀ provided by Sisco Research Laboratory

ITEMS	RESULT
Appearance (Colour)	Black
Appearance (Form)	Rod
Purity	99%
Diameter	40mm
Length	300mm

4.2 METHODS

4.2.1 Sample preparation

4.2.1.1 Synthesis of C₆₀

Kratchmer and Huttman developed this technique in 1990. In an inert environment, an electric arc is created between graphite rods to generate a powdery condensate known as soot. The fullerenes that can be extracted with toluene are present in a portion of this fluffy condensate. Following solvation in a tiny amount of toluene, the fullerenes in the soot are extracted. The solvent toluene is then eliminated by evaporation, and the resulting solid mixture—which consists primarily of C₆₀ with a little number of higher fullerenes—is next exposed to sonication. Method for obtaining pure C₆₀. It

is one of the bottom-up process involving graphite to produce C_{60} fullerenes and it is named as Arc-discharge method (Nimibofa, A., L *et al.*, 2018).

With slight difference in the Arc-discharge method, we are subjecting the commercially procured graphite in the muffle furnace at 1200°C for 3-4 hours. Because Scott, L.T., 2004 proposed that bending flat molecules using heat is possible. Likewise, the commercially procured graphite is exposed in extreme heat of 1200°C for 3 hours that further produces soot composed of large and small fullerene molecules.

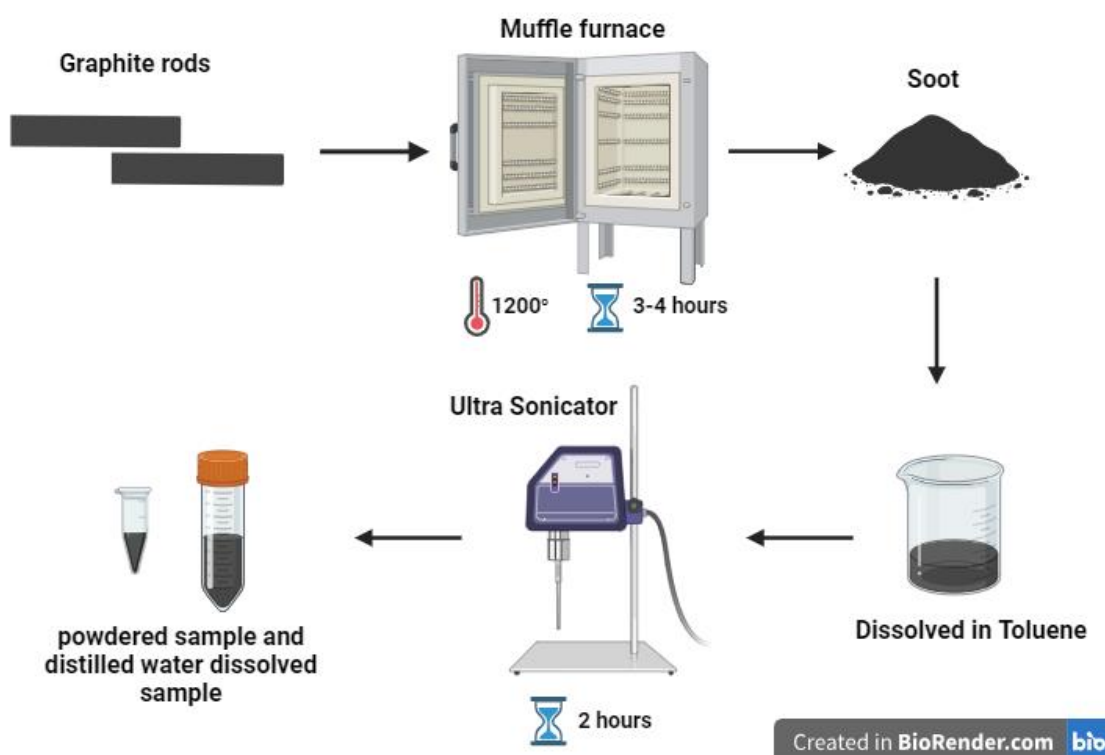


Figure 4.1: Schematic diagram of synthesis of C_{60} sample

4.2.1.2 C_{60} sample preparation

The obtained soot was then dissolved in the solvent called toluene and processed using sonochemical method, which is to subject the dissolved soot in ultrasonic processor FS-250N three times for 40 minutes to create the suspensions. Those ultra fine C_{60} in the suspensions were then diluted to the necessary concentrations in double distilled water.

4.2.2 Cell culture and treatment

The tests employed MCF7 human epithelial human adenocarcinoma cells, which were purchased from NCCS, Pune. With 10% v/v fetal bovine serum and 100 U/mL penicillin, 100 µg/mL streptomycin added as supplements, Dulbecco's modified Eagle's medium/high glucose media with additives was used to sustain cell lines in tissue culture flasks. The cells were grown at 37 °C and 5% CO₂ in 95% air humidified incubator and divided when they reached high confluence (mostly twice a week). In the instance of the MCF7 cells, cells were seeded in several well plates using 8.5 ×10⁴ cells/cm². The cells were incubated with prepared pristine C₆₀ solutions at various concentrations (0.2, 0.4, 0.6, 0.8, 1.0µg/ml) for 24, 48, or 72 h as required. Control cells were incubated with double distilled water alone. The C₆₀ exposure was estimated as µg/cm² growth area rather than µg/ml culture volume since culture containers of various volumes were employed during the studies.

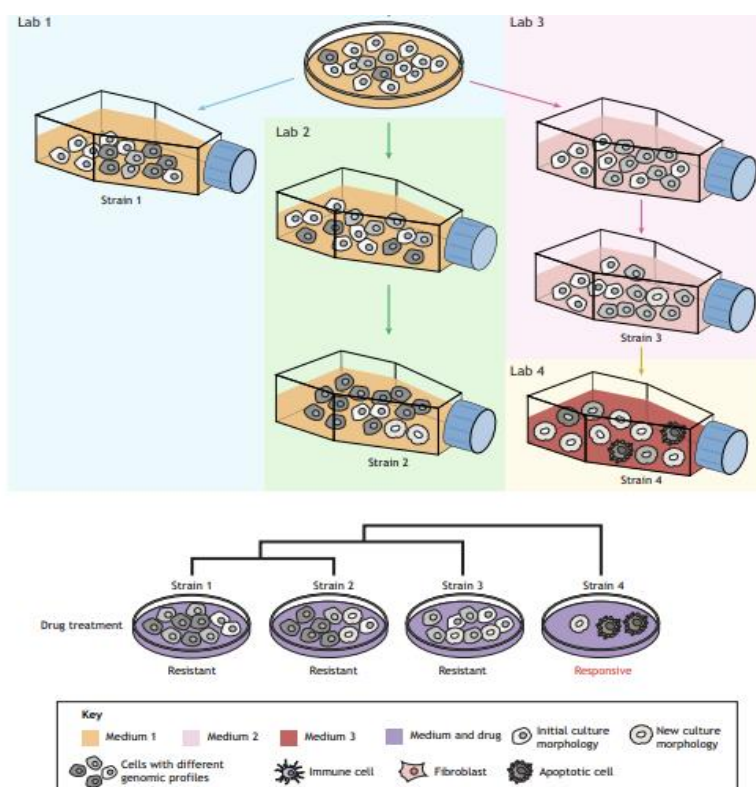


Figure 4.2: Schematic diagram of Cancer cell line culture

(source: Hynds, R.E et al., 2018)

4.2.3 Characterization studies

4.2.3.1 UV-vis spectroscopy

Absorption or reflectance spectroscopy in the ultraviolet-visible spectral band is referred to as ultraviolet-visible spectroscopy. This indicates that it makes use of light in the visible and nearby ranges. The immediate appearance of absorption or reflectance in the visual range influences how the substances involved are perceived to be coloured. Atoms and molecules go through electronic transitions in this area of the electromagnetic spectrum. This spectroscopy aids in determining whether nanoparticles are present. Over the spectrum of wavelengths, the nanoparticles provide distinctive absorption spectra (Kumar, 2013; Pinheiro *et al.*, 2004).

A JascoV-630 double-beam UV-vis spectrophotometer operating in the 200–1200 nm wavelength range was employed. In a 1 cm quartz cell, the supernatant phase adsorption for water was determined at 25 °C. Throughout the experiment, these dispersions were diluted to maintain their recorded absorbance values in the 0.1–2 range.

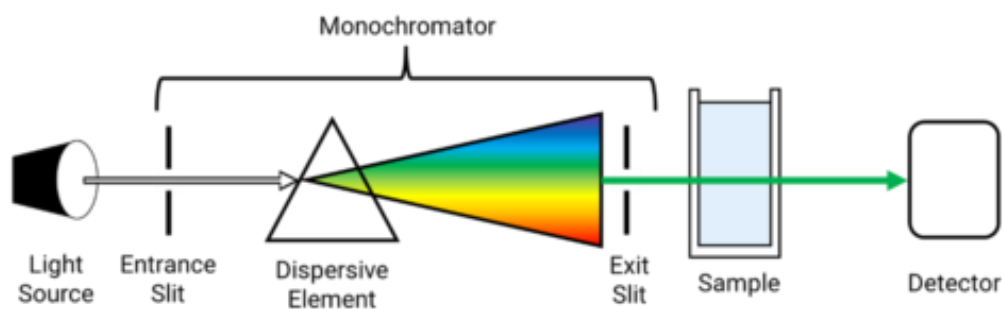


Figure 4.3: Schematic diagram of UV-vis spectroscopy

(source: Jasco)

4.2.3.2 FTIR Spectroscopy

The functional groups that are present on the sample's surface are analyzed using FTIR. High-spectral-resolution data throughout a broad spectral range is concurrently collected by an FTIR spectrometer. For the same information, Fourier-transform spectroscopy is a less natural method. With this approach, a multi-frequency laser beam is simultaneously shone at the sample in place of a monochromatic one, and the amount of the beam that is absorbed by the sample is measured. Subsequently, an alternative frequency combination is added to the beam, providing a second data point. This procedure is carried out repeatedly. After that, a computer uses all of this information to calculate the absorption at each wavelength by going backwards.

FTIR analysis of nanoparticles can be used to determine which chemical groups contributed to their creation and stability (Amini N, *et al.*, 2021). Fourier FTIR spectrum analyses of the pure C₆₀ were done to verify any possible structural features in the samples. It is possible to analyze the surface of C₆₀S chemical functionalization by oxidation using Fourier transform infrared spectroscopy. Using an Agilent Cary 630 FTIR, FTIR analysis was performed in the mid-infrared band of 7000–350 cm⁻¹. 200 scans were averaged at a resolution of 1 cm⁻¹ to get the FTIR spectra of powdered C₆₀ ground in KBr pellet.

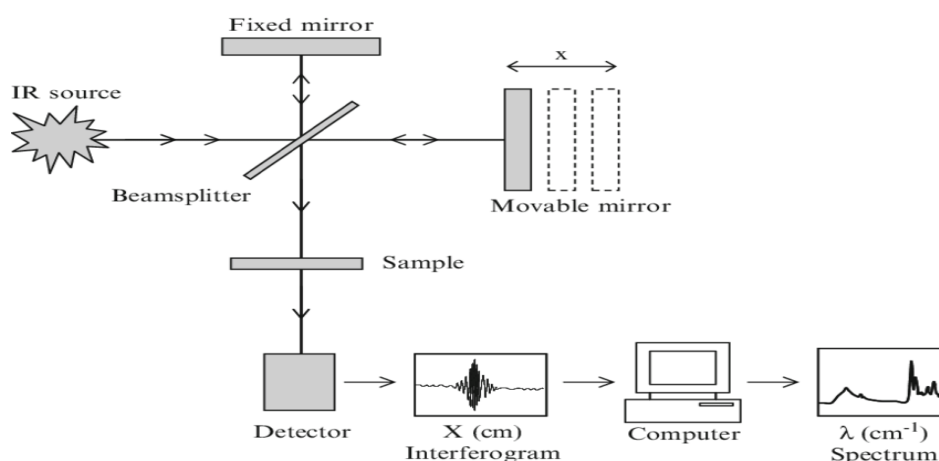


Figure 4.4: Schematic diagram of FTIR

(Source: Areef Billah, A.H.M., 2016)

4.2.3.3 Raman spectroscopy

The investigation of surfaces and heterointerfaces between the component layers of low-dimensional structures has also benefited from the application of Raman spectroscopy. Raman spectroscopy may identify surface-specific vibrational states that result from surface reconstructions. For the fullerenes, Raman spectroscopy has been an essential experimental method. The high Raman response resulting from relatively substantial resonance enhancement, the spectra's simplicity, the presence of fullerene species with at least some symmetry, and their accessibility are the reasons for this. C₆₀ has the greatest molecular symmetry, icosahedral I_h symmetry, which results in a rather simple Raman spectrum with only eight strong Raman lines. the most common is the pentagonal pinch mode (PPM), also known as Ag, which has been used frequently as an analytical probe for C₆₀'s structural and electrical characteristics. (Kuzmany, H. *et al.*, 2004)

Prepared powder samples of C₆₀ were subjected to Raman spectra and defect intensities of the nanomaterial were evaluated using a 785 nm Micro-Raman Spectrophotometer (Renishaw, UK, Invia0719-17) in spectral frequency 100 to 3,500 cm⁻¹ with spectral resolution of 0.75 cm⁻¹ (FWHM)

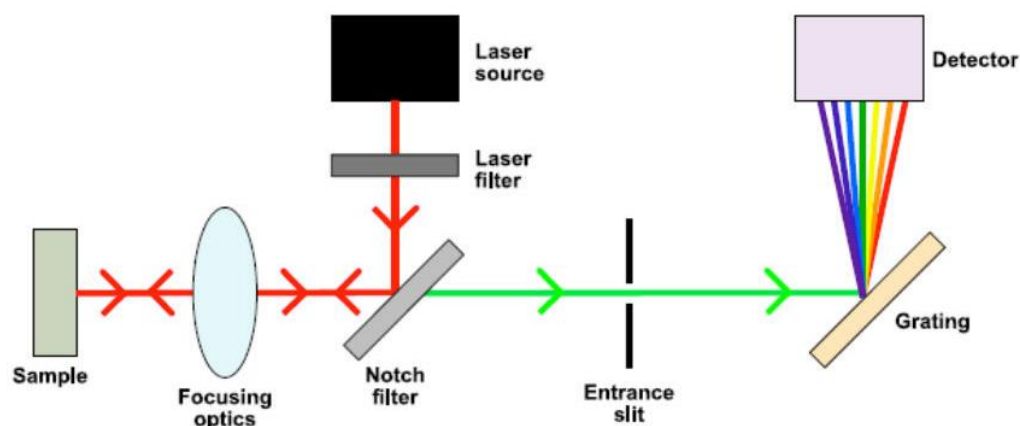


Figure 4.5: Schematic diagram of Raman spectroscopy

(The Duffy research group)

4.2.3.4 Field Emission Scanning Electron Spectroscopy

Using a FE-SEM [EVO-60, Zeiss business], the surface topography of the membrane top-layer and the pore morphology of the powdered C₆₀ were studied. Additionally, it is employed to analyze the dispersion or distribution of C₆₀'s. The C₆₀ powder samples were dropped onto a glass coverslip and allowed to dry for the whole night. The resultant samples were examined at a 20kV operating voltage using a FE-SEM. Using a method for calculating the layer thickness and particle diameter based on the usage of a raster scanning mode, the particle size of the C₆₀ was computed. The Zeiss company's FE-SEM EVO-60 conducted the analysis. FESEM microphotographs were used to track variations in the top-layer thickness of the membranes at several random positions throughout the membrane cross section and the average measurements was reported.

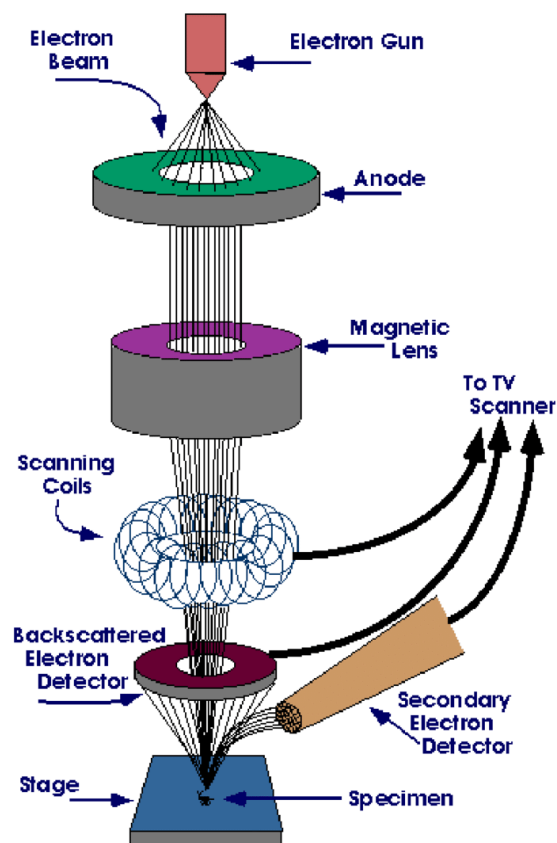


Figure 4.6: Schematic diagram of FESEM

(Source: Areef Billah, A.H.M., 2016)

4.2.3.5 X-Ray diffraction analysis

The X-Ray diffraction (XRD) was used to ascertain the crystallographic properties of the C₆₀'s. The most important method for describing crystal structures is X-ray diffraction (XRD). Analysing crystal structure is crucial, even for materials at the nanoscale. The arrangement of atoms spaced apart by around 0.1 nm determines a material's crystal structures, even though structures on the nanoscale scale may have an impact on its characteristics. Powder XRD operates on the basis of the widely recognized Bragg's law, which states that $\lambda = 2d \sin \theta$. The Joint Committee on Powder Diffraction Standards International Centre for Diffraction Data (JCPDS-ICDD) system is the most widely used database for crystal structure identification. Information on the space group and structural properties of a specific material may be obtained by Rietveld profile analysis of experimental XRD patterns [Rao, C.N.R. and Biswas, K., 2009]

An XRD study using an ARL EQUINOX 3000 was performed. Based on the diffractogram, several lattice planes versus certain diffraction angles were computed and utilized to ascertain the inherent features of the structure.

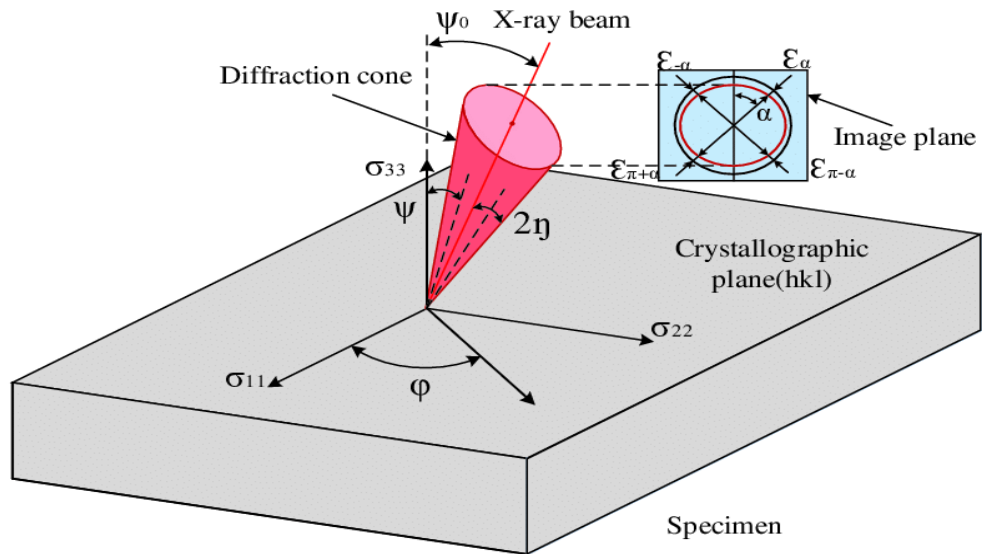


Figure 4.7: Schematic diagram of XRD

(Source: Shen, Q et al., 2015)

4.2.4 Cytotoxicity analysis

4.2.4.1 MTT assay

To evaluate the cytotoxicity of virgin C₆₀'s, the MTT test was employed. In summary, DMEM with glutamax and high-glucose media supplemented with 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% FBS was used to cultivate HepG2 cells (NCCS, Pune, India). The cells were cultivated to near confluence at 37 °C and 5% CO₂ in an incubator with 95% air humidity. They were then stimulated with agonists for 18 hours while being denied serum. Following an overnight subculture in 0.25% trypsin and 0.53 mM EDTA, the collected cells were planted at a density of around 1×10⁶ cells/well onto 96-well plates with a fat bottom. A C₆₀ solution dilution series of 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml in medium was added. The media was withdrawn after 24 hours of incubation, and 20 µl of MTT (5 mg/ml in PBS) was added to treat the cells in fresh medium. The cells were then incubated for an additional 2 hours in the dark. Following a 2-hour incubation period, formazan crystals developed in every well and were dissolved in 100 microliters of DMSO each well. The absorbance was measured at 570 nm using a Spectramax M5, Sunnyvale, CA. IC₅₀, or the concentration of C₆₀'s required to cause 50% cell death, was used to represent the percentage of cytotoxicity (Sharma *et al.*, 2020; Vijayalakshmi *et al.*, 2023).

The percentage of cell viability was determined, and the outcome was given as follows: percent mean of viability ± standard error of the mean.

Viability (%) = (Mean absorbance of treated cells / Mean absorbance of control) × 100

Cytotoxicity (%) = 100 – Viability (%).

Cell relative survival rate (RSR) and relative growth rate (RGR) were calculated according to the following equations:

RSR = {absorbance value (experimental group) / absorbance value (control group)} × 100%

$$\text{RGR} = \left\{ \frac{\text{average absorbance value (experimental group)}}{\text{average absorbance value (control group)}} \right\} \times 100\%$$

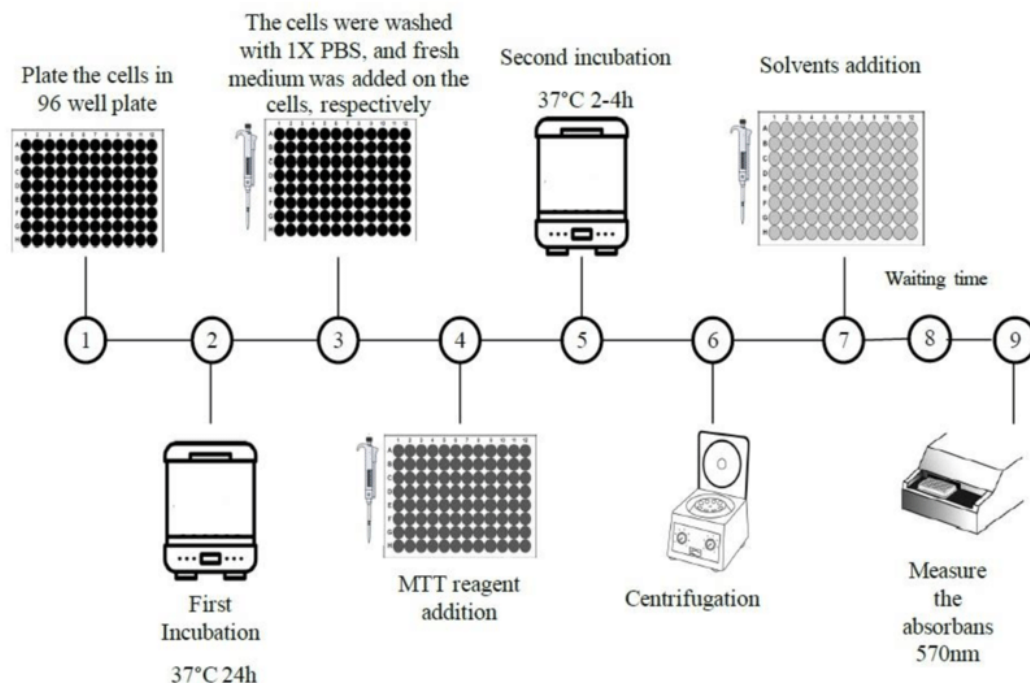


Figure 4.8: Workflow of MTT procedure

(Source: Babacan, Ü., et al., 2021)

4.2.4.2 Intracellular ROS analysis

Using the oxidation-sensitive fluorescent dye 2',7'-dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich), intracellular ROS generation was quantified. A nonfluorescent substance called DCFH-DA 32 is readily absorbed by cells and hydrolyzed by esterase to remove the DA group. Cellular oxidants subsequently convert DCFH to fluorescent dichlorofluorescein (DCF), which shows the amount of ROS inside cells. Particle suspensions were added to the 96-well plate wells after the DMEM medium was withdrawn, at a final concentration of either 20 or 40 $\mu\text{g}/\text{cm}^2$ (40 or 80 $\mu\text{g}/\text{mL}$). In a nutshell, 96-well black plates were seeded with 1×10^6 MCF7 human epithelial adenocarcinoma cells per well, and the cells

proliferated for two days. After that, the cells were exposed to the particles for four hours at 37 °C with 5% CO₂ in a humidified environment. Following exposure, the cells were cleaned using DMEM media and the particle suspensions were discarded. Subsequently, each well was filled with 40 µM DCFH-DA diluted in 37 °C DMEM, and the cells were incubated for 30 minutes. After the DCFHDA was taken out, 400 µL of PBS (37 °C) was used to wash each well. A computerized micro plate fluorometer was used to measure the fluorescence after adding two hundred microliters of PBS. To reduce the fluoroprobe's fading, the plate was kept out of the light for the duration of the DCFH-DA process.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SYNTHESIS OF C₆₀

After subjecting the procured Graphite into the muffle furnace at 1200°C for more than 2 hours. This resulted in the generation of soot, which is a powdery condensate called C₆₀ fullerene. Which is then made into a fine substance through sonochemical method. The soot is dissolved in a solvent called toluene and subjected to ultra sonication for 2 hours and it is evaporated and grind as a fine powder texture as shown in figure 5.1 using mortar and pestle.



Figure 5.1: Powdered pristine C₆₀

This technique is easier and more reliable when compared to other synthesis methods such as Chemical vapour discharge, electric arc-discharge and laser ablation (Porto, L.S., *et al.*, 2009). We have got a pure and fine fullerene particle which are very useful for dissolving, characterization and other experimental studies.

5.2 CHARACTERIZATION STUDIES

5.2.1 UV-visible spectroscopy

After dissolving 0.003 g of C_{60} in 3 mL of double distilled water, the concentration of the C_{60} fullerene solution was brought to 0.1 (w/v) %. An absorption spectra scan was then conducted on the C_{60} solution using double distilled water. UV-vis absorption spectra of C_{60} were recorded using a double-beam spectrophotometer JascoV-630 (China). The solution was poured in a quartz cuvette having an optical path length of 1 cm, which enabled to perform the measurements in the range of 200–800 nm. The temperature was maintained constant at $T=298$ K. In order to exclude background scattering from C_{60} fullerene clusters all experiments were carried out by recording spectra of the C_{60} molecules against the solution of pure fullerene at a similar concentration in the reference cuvette.

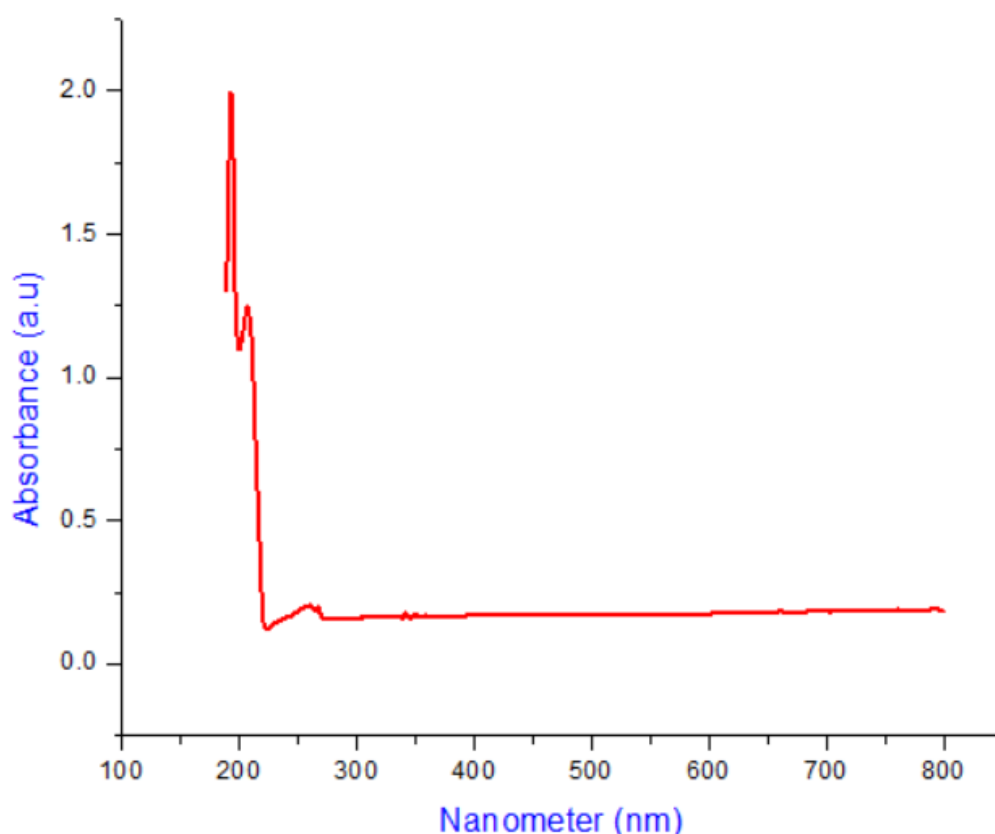


Figure 5.2: UV-visible spectroscopy of pristine C_{60}

The C₆₀ showed a maximum of absorbance (OD) at 1.9. Fig. 5.2 represents the UV-Vis absorption spectra of the pristine C₆₀ nanoparticle. The nanoparticle gave a sharp absorption peak at 150-200 nm. This indicates the presence of nanoparticle in the solution. Intensity of the peak in spectrum could be used to quantify the amount of individual C₆₀s in the solution.

According to Scharff, P *et al.*, 2004 studies, at room temperature UV–VIS absorption spectra of C₆₀ fullerene in water across the wavelength range of 200-800nm are studied. The spectrum is characterized by two strong wide absorption bands, with maximums at 265. These peaks are in close proximity to the C₆₀ fullerene UV absorption spectra in n-hexane (270 and 335 nm) and toluene (269 and 335 nm). We link the hydration of C₆₀ fullerene in water to the slight shift of these bands in the C₆₀ spectra (up to 10 nm). It is evident that a rise in the concentration of C₆₀ fullerene in water causes an increase in absorption, a little shift of the spectral bands into the longwave region, and the emergence of an extra thin spectral line at 450 nm and weak band at 622nm. C₆₀ fullerene in water testifies to the solid character of absorption.

5.2.2 FTIR spectroscopy

In nano particle FTIR analysis will help to find out whichever chemical groups helped in the fabrication and stabilization of the nanoparticles. For the analysis the sample is given in the powder form. The instrument used is Agilent Cary 630 FTIR in mid-infrared range of 4000–500 cm⁻¹. Fig. 5.3 indicates the FTIR analysis of the pristine C₆₀ Powdered form of samples was used for analysis.

A sharp peak at approximately 3747.01 cm⁻¹ suggests the presence of hydroxyl (OH) groups, which are characteristic of alcohols or phenols. The peak around 2356.59 cm⁻¹ could indicate the presence of a carbonyl group (C=O), possibly from a ketone or aldehyde. Peaks at 1793.47 cm⁻¹ and 1519.63 cm⁻¹ may correspond to C=C stretching vibrations in aromatic compounds. The peak at 1228.43 cm⁻¹ suggests the presence of C-O stretching vibrations, often found in ethers, esters, or carboxylic acids.

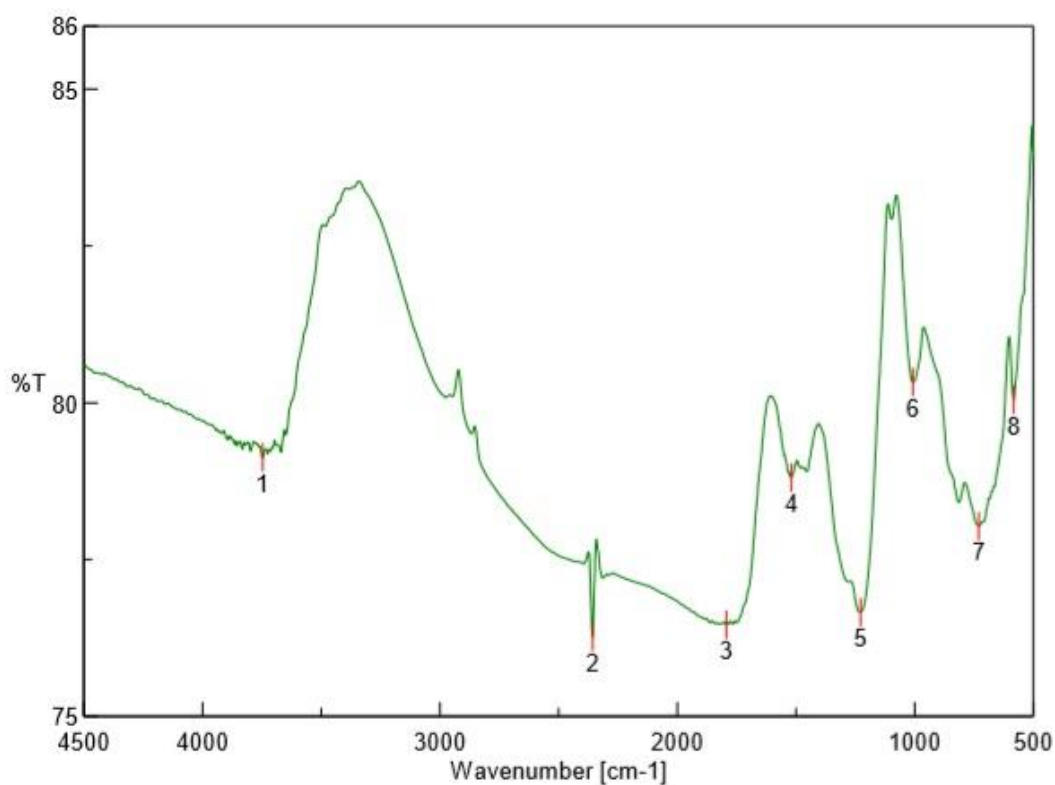


Figure 5.3: FTIR analysis of pristine C₆₀

Table 4.2 Peak position and intensity of FTIR

S.NO	Position	Intensity
1.	3747.01	79.1289
2.	2356.59	76.2671
3.	1793.47	76.4621
4.	1519.63	78.8048
5.	1228.43	76.662
6.	1007.62	80.33
7.	729.925	78.0309
8.	582.397	80.0449

A peak at 1007.62 cm^{-1} is indicative of C-H bending vibrations, which are commonly observed in alkanes or alkyl groups. Peaks around 729.925 cm^{-1} and 582.397 cm^{-1} could correspond to fingerprint regions, providing additional structural information.

The identification of aromatic compounds is further supported by the presence of aromatic C=C bonds. The existence of ethers, esters, or carboxylic acids, which contribute to the sample's overall chemical makeup, is indicated by the C-O stretching vibrations. Greater intensities at specific locations, as 1007.62 cm^{-1} and 582.397 cm^{-1} , imply that comparable functional groups are more prevalent there. The sample looks to include a combination of organic chemicals, perhaps including alcohols, ketones, aromatic compounds, ethers, or esters, based on the functional groups that have been discovered.

Studies have similar results in which two primary peaks of the C_{60} are located at 1725 and 1636 cm^{-1} . The overlapping of C_{60} 's remaining carboxyl group may be the cause of the large peak at 1725 cm^{-1} . Additionally, a novel peak in C_{60} has been identified at 1636 cm^{-1} , which is associated with the amide carbonyl stretching mode. This suggests that C_{60} and the graphene sheet are chemically bonded (Zhang, X *et al.*, 2009)

5.2.3 Raman spectroscopy

Raman spectroscopy is an important analytical tool that provides both qualitative and quantitative data on nanomaterials based on its chemical composition and structure. The Raman plot in Fig. 5.4 contains the ID/IG ratio of ~ 1.15 , which is characteristic of powdered pristine C_{60} . The carbon sp^2 bond-derived G band peak served as the standard for all profiles. The graphite imperfections, such as edge and grain boundary flaws, are shown by the D band.

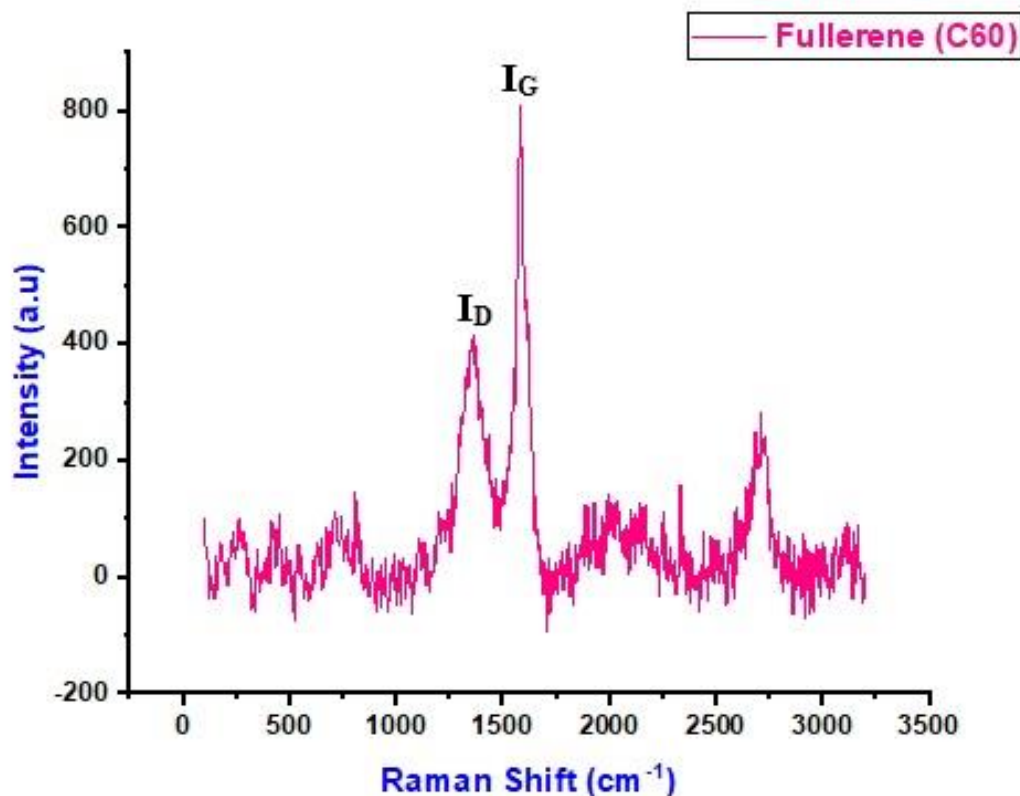


Figure 5.4: Raman spectroscopy of pristine C_{60}

It could be seen from Figure 5.4 that, A particular vibrational mode, or Raman-active phonon mode, in C_{60} fullerene is shown by the peak at 1.15 cm^{-1} . This peak in the case of C_{60} is frequently linked to the radial breathing mode (RBM), which is the result of collective carbon atom vibrations along the fullerene cage's radial direction. Usually seen in the lower frequency range of the Raman spectrum, the RBM peak is indicative of C_{60} . A clean C_{60} is anticipated to have a relatively low I_D/I_G ratio, which denotes a high level of structural integrity and little to no disorder or fault.

The defect proportions in the as synthesized C_{60} nanomaterials shows I_D/I_G ratio of 1.15. The result signifies the presence of less dangling bands present in the synthesized C_{60} nanomaterial. Such vibration and structural properties of C_{60} clearly indication the Bio-compatibility attributes of the nanomaterial when introduced into the biosystem.

5.2.4 Field Emission Scanning Electron Microscope

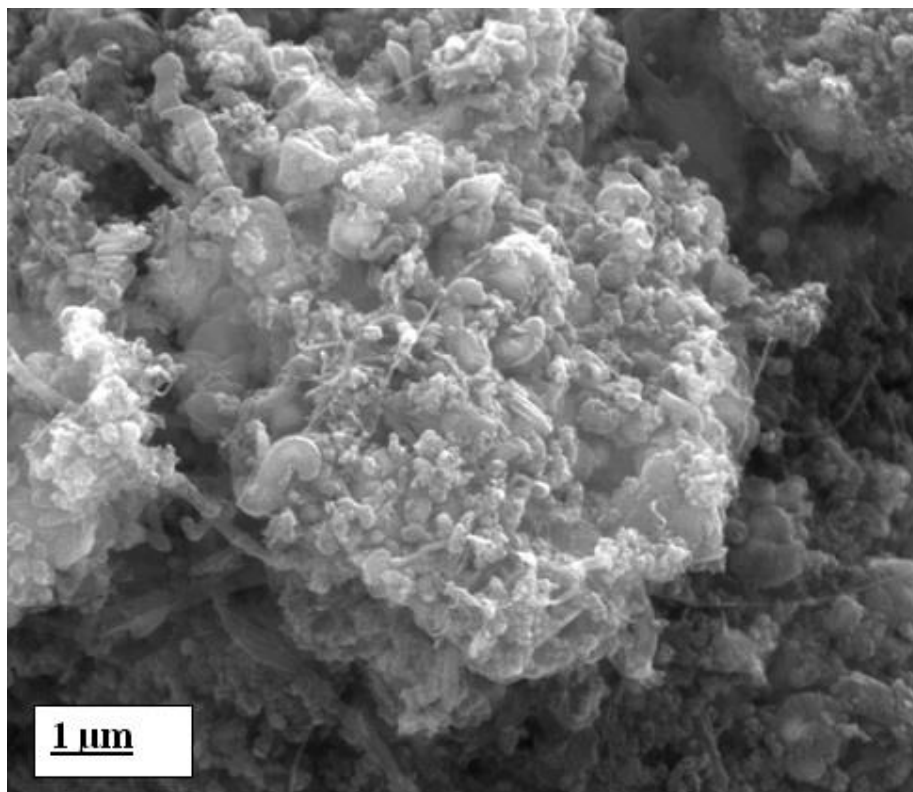


Figure 5.5: FE-SEM image of pristine C₆₀ at 1 μm

To determine the size, shape, and morphology of the produced nanoparticle, a scanning electron microscope is utilized. The nanoparticle's shape and approximate size are depicted in the figure.

The FESEM images of C₆₀-fullerene are shown in Figure 5.5. The fullerene particles have tiny facets and an uneven form. There were a lot of macropores in the agglomerates. The range of particle sizes, from 20 to 400 nm, was non-uniform. It was hard to determine the average particle size and to locate single particles due to the high particle aggregation. It is probable that bulky crystals or quasi-crystals with modulated surfaces and regular forms are formed by particles with comparable or near crystallographic orientations (Zhu, W *et al.*, 2004)

5.2.5 X-Ray diffraction analysis

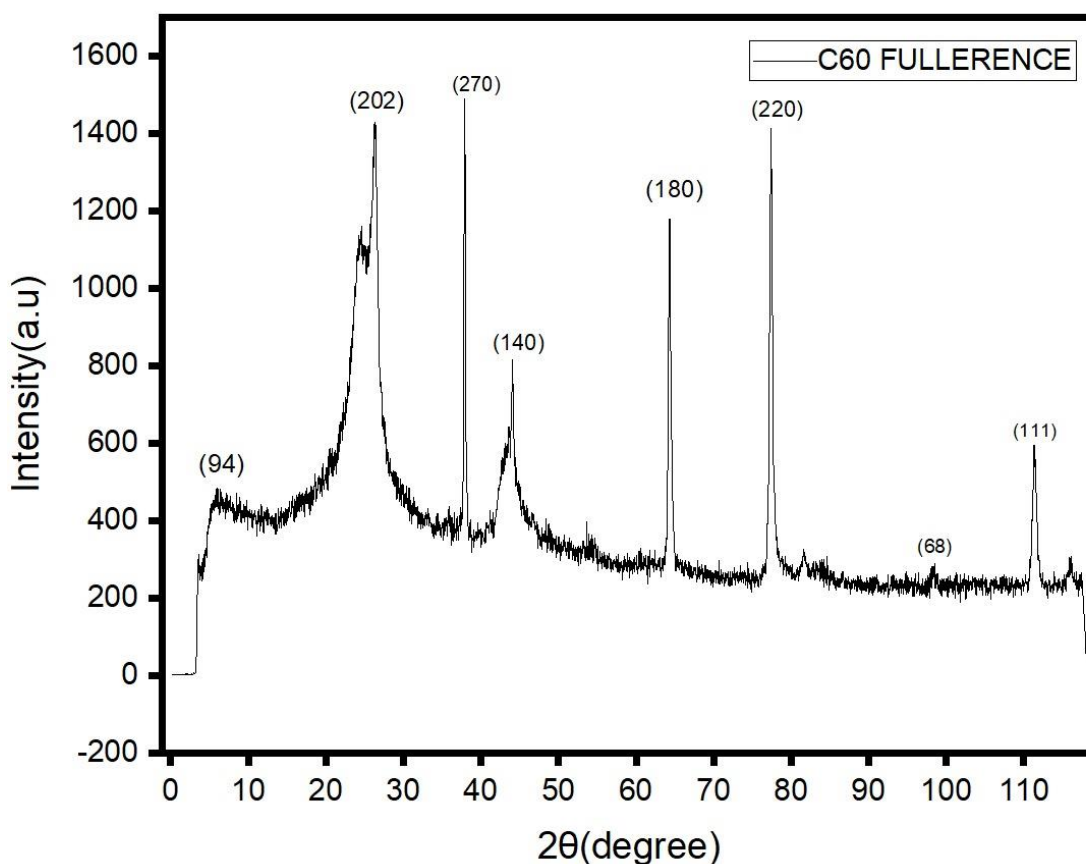


Figure 5.6: XRD pattern of pristine C₆₀

It could be seen from figure 5.6, that the crystalline lattice observed at the Bragg's diffraction angle at around 25° is predominantly noticed. Some shoulder peak at different diffraction angle around 45°, 65°, 75° are also been noticed. Each of the seven peaks in the data is identified by its 2θ angle, d-spacing (d), peak area (counts), peak intensity (I/I₀ or peak height), and full width at half maximum (FWHM). The greatest intensities are seen in peaks 4, 6, and 7, which may indicate greater crystallinity or bigger crystallite sizes. In comparison to other peaks, peaks 3, 5, and 6 have wider widths, which may indicate lower crystallite sizes or lattice strain. We may infer the phases that are present in the sample by comparing the d-spacing values to patterns that are known to exist in databases. Peak 4, for example, is associated with a d-spacing of around 2.3724 Å, indicating a specific crystallographic plane

in a given phase. Lattice parameters and crystal symmetry may be deduced from the d-spacing values, offering insight into the crystal structure of the material.

When compared to previously reported data by Keypour, H, *et al.*, 2013 Pure fullerene C₆₀ exhibits a distinct XRD pattern that can be seen at $2\theta = 10.8, 17.6,$ and 20.7 that shows that our as synthesized Fullerene nanomaterials have crystallographic plane. Such poly-crystallinity nature of the as synthesized C₆₀ nanomaterial is evident from the X-Ray diffraction studies. Understanding the crystalline blue print of the C₆₀ nanomaterial would enable for further explore the nano-biological interaction studies in cancer cell lines.

5.3 CYTOTOXICITY ANALYSIS

5.3.1 MTT assay

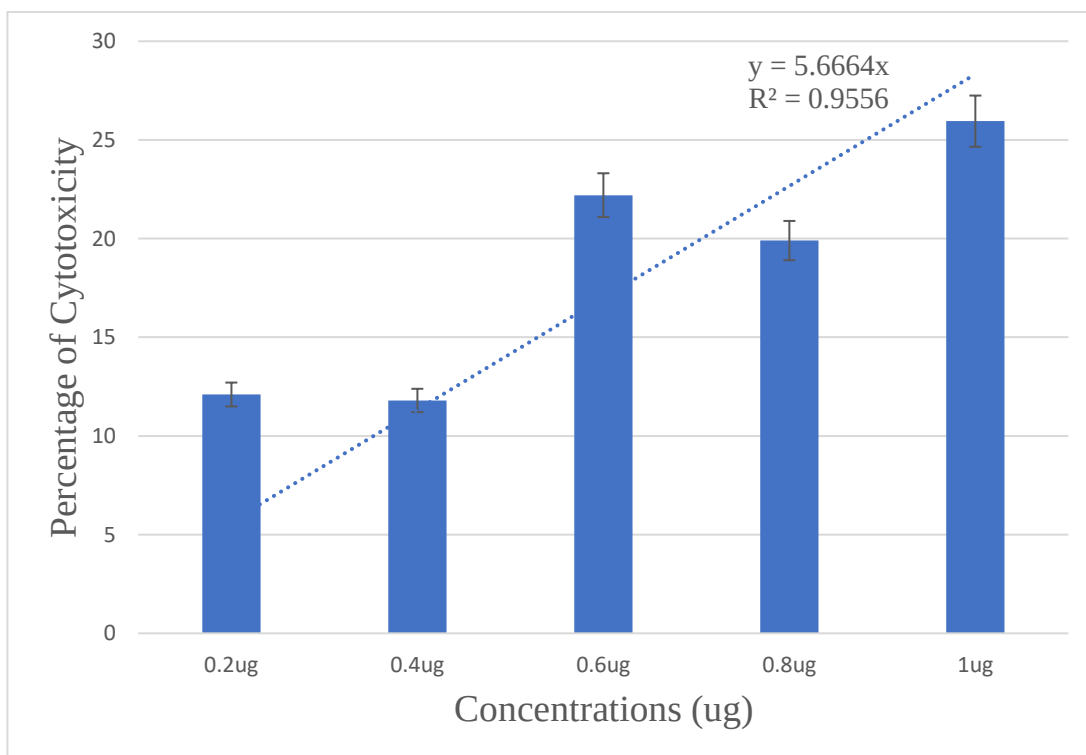


Figure 5.7: % of Cell Viability calculated based on MTT absorbance value of MCF-7 cell line cultures.

After successful characterization of pristine C₆₀, cytotoxicity has performed in vitro MTT assay study in MCF7 cell line by spectrophotometric absorption at 570 nm. The results showed that the viability of the HepG2 cells line was influenced by various concentrations (0.2, 0.4, 0.6, 0.8, 1.0 µg/ml) of pristine C₆₀s drug solution. After incubation, cell viability was evaluated by applying the 2-(4-Iodophenyl)-3-(4- nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium.

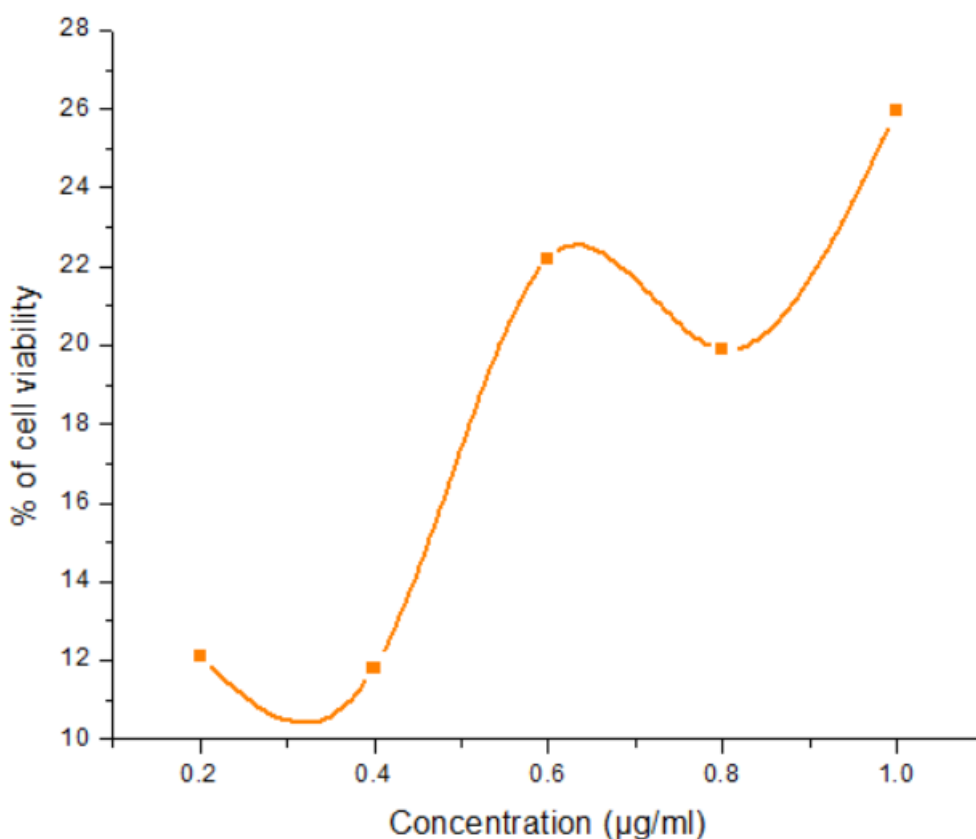
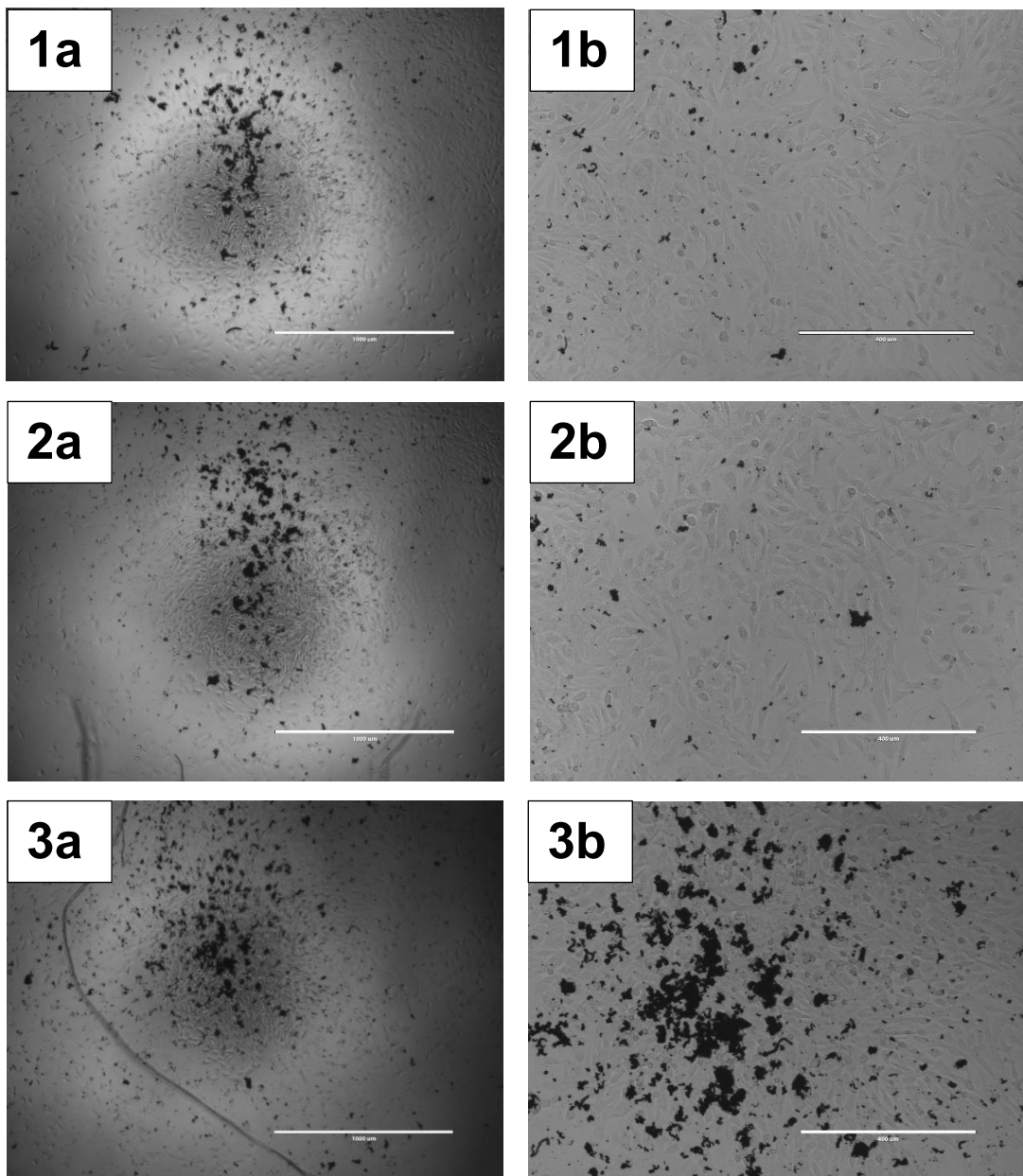


Figure 5.8: % of Cell Viability Dose-Response curve of C₆₀ treated MCF-7 cells.

As seen in Fig. 5.8, the data collected indicated that there was a statistically significant difference in cell viability at dosages of 0.2, 0.4, 0.6, 0.8, and 1.0µg/ml when compared to the control group. The microscopic pictures of MCF7 cells treated with various doses of C₆₀ are shown in Figure 5.11. Additionally, preparation showed an IC₅₀ value of 8.823945µg/ml for 24 hours. 8.82 ug/ml is the estimated IC₅₀ value, which indicates the

concentration of the substance needed to 50% inhibit cell viability. This is a crucial metric that shows how well the substance affects cell survival or proliferation. The complicated nature of the compound's interaction with the cells may be reflected in the non-monotonic response seen in the data, which shows a drop in cell viability at 0.4 ug, an increase at 0.6 ug, and a subsequent decrease at higher doses. The greater cell survival at 0.6 ug in comparison to 0.4 ug points to a possible hermetic response, in which the substance stimulates cell growth at low dosages while inhibiting it at higher ones. All things considered, the computed IC50 value offers crucial information about the compound's potency and impact on cell survival.



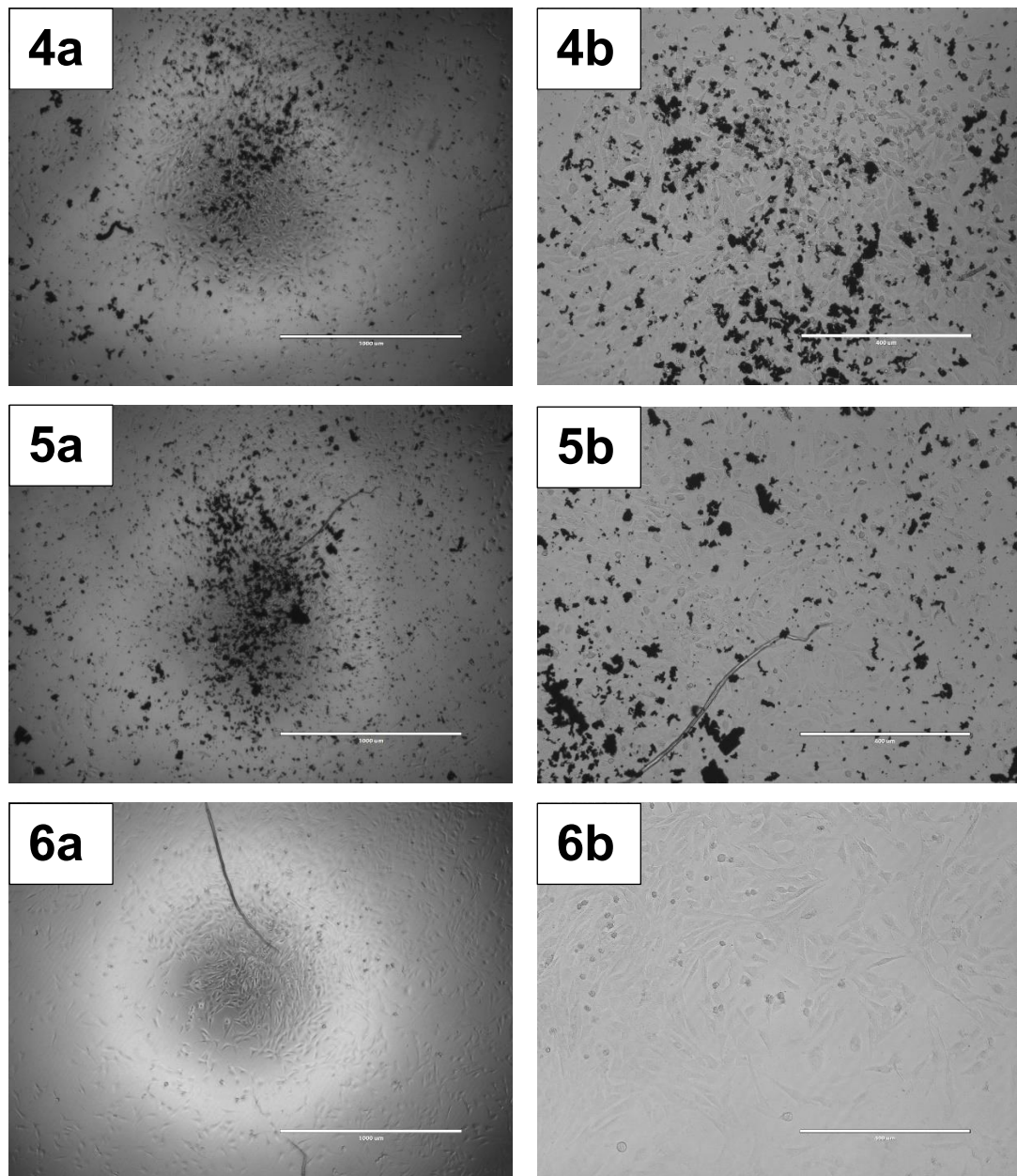


Fig 5.9: Morphology of MCF-7 cells under phase contrast; after 24 h incubation with 1a) 0.2 $\mu\text{g/ml}$ at 4x, 1b) 0.2 $\mu\text{g/ml}$ at 10x, 2a) 0.4 $\mu\text{g/ml}$ at 4x, 2b) 0.4 $\mu\text{g/ml}$ at 10x, 3a) 0.6 $\mu\text{g/ml}$ at 4x, 3b) 0.6 $\mu\text{g/ml}$ at 10x, 4a) 0.8 $\mu\text{g/ml}$ at 4x, 4b) 0.8 $\mu\text{g/ml}$ at 10x, 5a) 1 $\mu\text{g/ml}$ at 4x, 5b) 1 $\mu\text{g/ml}$ at 10x, 6a) and 6b) Represents control cells, with normal morphology at 4x and 10x respectively.

5.3.2 Intracellular ROS

The imbalance between reactive oxygen species (ROS) and the body's natural antioxidant defences can be upset, which can lead to oxidative damage of macromolecules inside the cell and eventually result in cell death. A dye molecule called DCFH-DA may cross lipid membranes and is converted to DCFH by esterase. When DCFH comes into contact with ROS like OH⁻ or O₂⁻, it transforms into the fluorescent molecule DCF. Using flow cytometry, the fluorescence intensity of each cell is correlated with the quantity of reactive oxygen species (ROS) produced within the cell (Requardt et al., 2015; Shen et al., n.d.; Wan et al., 2015).

Using DCDFDA dye as a fluorescent probe, we examined the possibility of C₆₀ as a medication to induce the generation of Reactive Oxygen Species (ROS) in breast cancer cells. MCF7 breast cancer cells were used in the experiment, and they were exposed to C₆₀ concentration with respect to the obtained the IC₅₀ of 8.82 µg/ml for varied amounts of time. Following C₆₀ therapy, we saw a dose-dependent rise in ROS levels. The generation of ROS was shown by the considerable rise in DCDFDA fluorescence intensity, which increased with increasing C₆₀ concentrations, as Figure 5.10 illustrates. It is now known that one of the main mechanisms driving the lethal effects of several chemotherapeutic drugs on cancer cells is the formation of ROS. Extremely reactive chemicals known as ROS, such as hydrogen peroxide (H₂O₂), hydroxyl radical (•OH), and superoxide anion (O₂•⁻), can cause oxidative damage to cellular components, leading to apoptosis or cell death. The observation indicates the interplay of free radicals generated inside the treated breast cancer cell lines, resulting into the mobility of cancer cells. Such analysis helps in understanding the molecular mechanism taking place for eliciting the desired anti-cancerous activity.

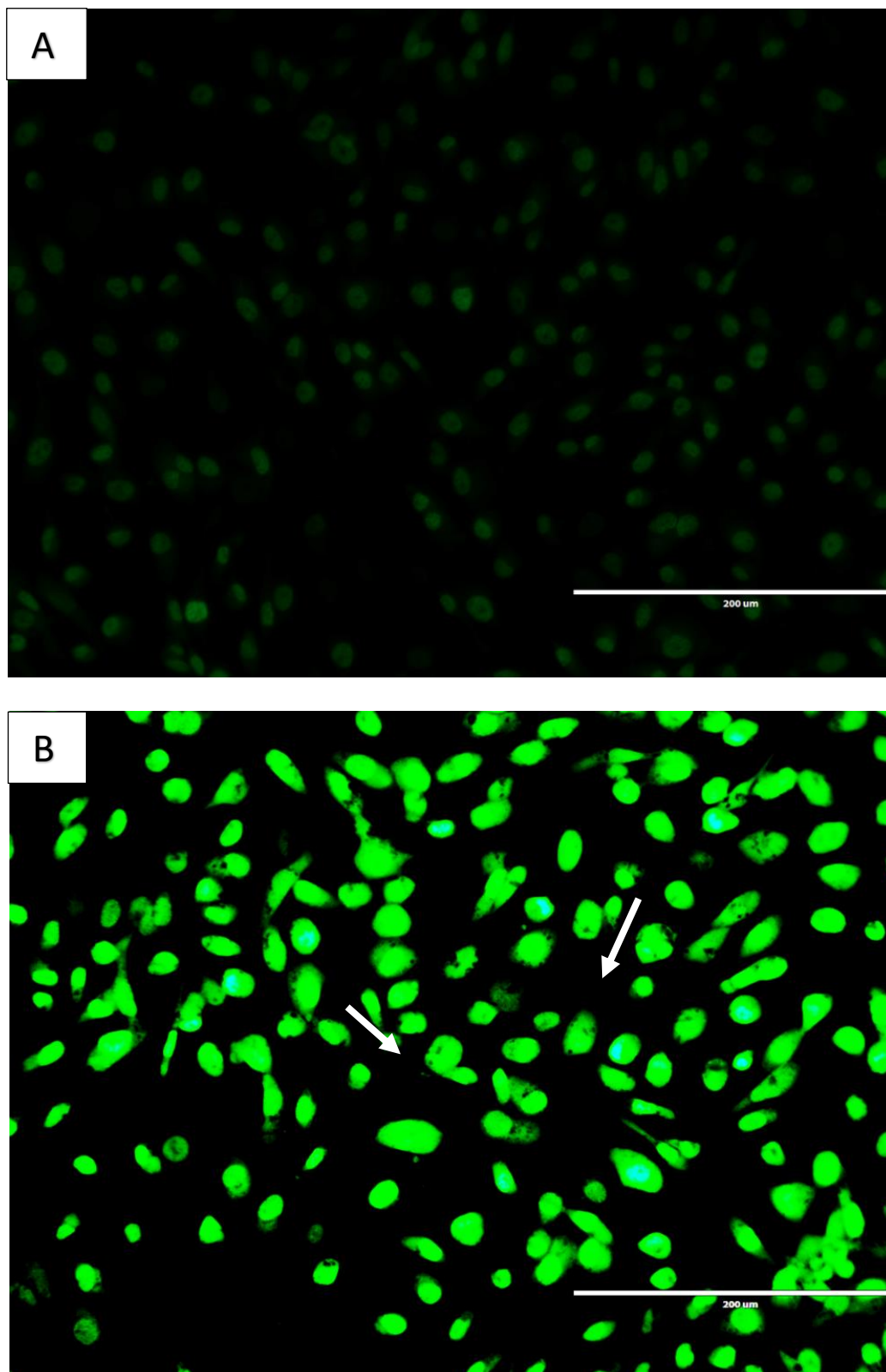


Fig 5.10: Confocal images of ROS generation in MCF-7 cells; at 24 hrs after C₆₀ treatments; Control group (A), C₆₀ treated MCF-7 cells (B); Arrows indicate DCFH stained cells; Scale bar: 200 μm

CHAPTER 6

SUMMARY AND CONCLUSION

The findings from this study provide valuable insights into the physical properties of synthesised C₆₀, essential for understanding its potential applications in various fields, including nanotechnology and biomedicine. UV-vis spectroscopy, supported by FESEM imaging, confirms the successful dispersion of C₆₀ in water, a crucial aspect for its use in aqueous environments. FTIR analysis elucidated the presence of covalent linkages between carbon and functional groups, contributing to the understanding of surface chemistry. XRD results indicating the crystalline nature of C₆₀ further enhance our knowledge of its structural characteristics.

Previous research has demonstrated that C₆₀ can reduce human keratinocyte and ocular cell viability. This study discovered that the C₆₀ reduced MCF-7 breast cancer cell viability in a concentration-dependent way, which is similar to previous research. Several studies have indicated that the alteration may make C₆₀s more cytotoxic. Since C₆₀ was one of the C₆₀s used in this investigation, FE-SEM could look into the potential impact of surface modification. Images revealed that C₆₀s were internalized by endocytic pathways and were found inside of single-layer membrane vesicles within cells. MTT results from the cytotoxicity tests revealed a little but significant reduction in cellular viability following exposure to 40 µg/ml C₆₀s. Additionally, we discovered in this investigation that C₆₀ markedly increased intracellular ROS, indicating the possibility of oxidative stress induction in hepatocytes exposed to C₆₀. The assessment of the cytotoxic effects of C₆₀s was also frequently done through interference on the plasma membrane. C₆₀s interact with cell surfaces easily due to their size and shape. Therefore, it's possible that the damage to the plasma membrane caused by C₆₀s is more sensitive than the formation of intracellular ROS and cell viability.

It can be inferred from the research that is currently available that C₆₀s Nanoparticles may function as drug molecule nanocarriers for targeted drug

delivery. Nevertheless, there is currently a lack of a scientific paradigm about the potential (adverse) reactivity of nanoparticles, and our knowledge of the fundamentals of how nanoparticles interact with living things is limited. In order to create and use safe nanoparticles in medication delivery in the future, a conceptual understanding of biological responses to nanomaterials is required. To advance this issue, close cooperation between individuals involved in particle production and drug administration is also required for the sharing of ideas, strategies, and expertise.

In conclusion, the investigation of C₆₀'s nano-caged structure has yielded useful insights into its molecular toxicity and biocompatibility in a variety of biosystems. The study emphasizes the diverse responses of different biological entities to C₆₀ exposure, underlining the need of taking context into account. While noting C₆₀'s potential applications, particularly in medicine and industry, obstacles such as long-term impacts and sophisticated molecular pathways necessitate further research. The findings highlight the importance of ongoing research in informing risk assessment and guiding the responsible development of C₆₀-based technologies, providing a balance between innovation and the safety of biological systems and the environment.

REFERENCES

Amini, N., Latifi, H. and Hayati, P., 2021. Effects of Nano-CuO, MWCNT and SBS on the Aging of Asphalt Binder: FTIR and XRD Analyses. *Jordan Journal of Civil Engineering*, 15(2).

Arbogast, J.W., Darmany, A.P., Foote, C.S., Diederich, F.N., Whetten, R.L., Rubin, Y., Alvarez, M.M. and Anz, S.J., 1991. Photophysical properties of sixty atom carbon molecule (C₆₀). *The Journal of Physical Chemistry*, 95(1), pp.11-12.

Areef Billah, A.H.M., 2016. Investigation of multiferroic and photocatalytic properties of Li doped BiFeO₃ nanoparticles prepared by ultrasonication.

Awan, F., Bulger, E., Berry, R.M. and Tam, K.C., 2016. Enhanced radical scavenging activity of polyhydroxylated C₆₀ functionalized cellulose nanocrystals. *Cellulose*, 23, pp.3589-3599.

Babacan, Ü., Kaba, A., Akçakale, F., Cengiz, M.F. and Akıncı, E., 2021. Optimization of some parametric values of MTT for the determination of human melanoma (SK-Mel-30) cell viability. *International Journal of Life Sciences and Biotechnology*, 5(1), pp.9-20.

Baei, M.T., Taghartapeh, M.R., Soltani, A., Amirabadi, K.H. and Gholami, N., 2017. Interaction of pure and metal atom substituted carbon nanocages with CNCl: a DFT study. *Russian Journal of Physical Chemistry B*, 11, pp.354-360.

Behrend, L., Henderson, G. and Zwacka, R.M., 2003. Reactive oxygen species in oncogenic transformation. *Biochemical society transactions*, 31(6), pp.1441-1444.

Belgorodsky, B., Fadeev, L., Ittah, V., Benyamini, H., Zelner, S., Huppert, D., Kotlyar, A.B. and Gozin, M., 2005. Formation and characterization of stable human serum albumin– tris-malonic acid [C60] fullerene complex. *Bioconjugate chemistry*, 16(5), pp.1058-1062.

Benyamini, H., Shulman-Peleg, A., Wolfson, H.J., Belgorodsky, B., Fadeev, L. and Gozin, M., 2006. Interaction of C60-fullerene and 51mmunosuppression with proteins: docking and binding site alignment. *Bioconjugate Chemistry*, 17(2), pp.378-386.

Borm, P.J. and Kreyling, W., 2004. Toxicological hazards of inhaled nanoparticles—potential implications for drug delivery. *Journal of nanoscience and nanotechnology*, 4(5), pp.521-531.

Bozzini, G., Colin, P., Betrouni, N., Nevoux, P., Ouzzane, A., Puech, P., Villers, A. and Mordon, S., 2012. Photodynamic therapy in urology: what can we do now and where are we heading?. *Photodiagnosis and photodynamic therapy*, 9(3), pp.261-273.

Brieger, K., Schiavone, S., Miller Jr, F.J. and Krause, K.H., 2012. Reactive oxygen species: from health to disease. *Swiss medical weekly*, 142(3334), pp. w13659-w13659.

Briviba, K., Klotz, L.O. and Sies, H., 1997. Toxic and signaling effects of photochemically or chemically generated singlet oxygen in biological systems. *Biological chemistry*, 378(11), pp.1259-1265.

Castro, E., Garcia, A.H., Zavala, G. and Echegoyen, L., 2017. Fullerenes in biology and medicine. *Journal of Materials Chemistry B*, 5(32), pp.6523-6535.

Chen, K.L. and Elimelech, M., 2006. Aggregation and deposition kinetics of fullerene (C60) nanoparticles. *Langmuir*, 22(26), pp.10994-11001.

Chung, Y.M., Bae, Y.S. and Lee, S.Y., 2003. Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. *Free Radical Biology and Medicine*, 34(4), pp.434-442.

Corrie, P.G., 2008. Cytotoxic chemotherapy: clinical aspects. *Medicine*, 36(1), pp.24-28.

Curtin, J.F., Donovan, M. and Cotter, T.G., 2002. Regulation and measurement of oxidative stress in apoptosis. *Journal of immunological methods*, 265(1-2), pp.49-72.

Dröge, W., 2002. Free radicals in the physiological control of cell function. *Physiological reviews*.

Du, Z., Gao, N., Wang, X., Ren, J. and Qu, X., 2018. Near-Infrared Switchable Fullerene-Based Synergy Therapy for Alzheimer's Disease. *Small*, 14(33), p.1801852

Dugan, L.L., Turetsky, D.M., Du, C., Lobner, D., Wheeler, M., Almli, C.R., Shen, C.K.F., Luh, T.Y., Choi, D.W. and Lin, T.S., 1997. Carboxyfullerenes as neuroprotective agents. *Proceedings of the National Academy of Sciences*, 94(17), pp.9434-9439

Elshater, A.E.A., Salman, M. and Mohamed, A.S., 2017. Effect of Fullerene C60 (NPs) on Cyclophosphamide-Induced Hepato and Cardiac Toxicity in Male Albino Rats. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, 9(1), pp.27-36.

Fernandes, N.B., Shenoy, R.U.K., Kajampady, M.K., Dcruz, C.E., Shirodkar, R.K., Kumar, L. and Verma, R., 2022. Fullerenes for the treatment of cancer: an emerging tool. *Environmental Science and Pollution Research*, 29(39), pp.58607-58627.

Florea, A.M. and Büsselberg, D., 2011. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. *Cancers*, 3(1), pp.1351-1371.

Freixa, A., Acuña, V., Sanchís, J., Farré, M., Barceló, D. and Sabater, S., 2018. Ecotoxicological effects of carbon based nanomaterials in aquatic organisms. *Science of the Total Environment*, 619, pp.328-337.

Friedman, S.H., DeCamp, D.L., Sijbesma, R.P., Srdanov, G., Wudl, F. and Kenyon, G.L., 1993. Inhibition of the HIV-1 protease by fullerene derivatives: model building studies and experimental verification. *Journal of the American Chemical Society*, 115(15), pp.6506-6509.

Fujita, K., Morimoto, Y., Ogami, A., Myojyo, T., Tanaka, I., Shimada, M., Wang, W.N., Endoh, S., Uchida, K., Nakazato, T. and Yamamoto, K., 2009. Gene expression profiles in rat lung after inhalation exposure to C60 fullerene particles. *Toxicology*, 258(1), pp.47-55

Funakoshi-Tago, M., Miyagawa, Y., Ueda, F., Mashino, T., Moriwaki, Y., Tago, K., Kasahara, T. and Tamura, H., 2016. A bis-malonic acid fullerene derivative significantly suppressed IL-33-induced IL-6 expression by inhibiting NF-Kb activation. *International Immunopharmacology*, 40, pp.254-264.

Gharbi, N., Pressac, M., Hadchouel, M., Szwarc, H., Wilson, S.R. and Moussa, F., 2005. [60] fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Letters*, 5(12), pp.2578-2585.

Ghasemi, A.S., Ashrafi, F., Babanejad, S.A. and Elyasi, A., 2019. Study of the Physicochemical Properties of Anti-Cancer Drug Gemcitabine on the Surface of Al Doped C 60 and C 70 Fullerenes: A DFT Computation. *Journal of Structural Chemistry*, 60, pp.13-19.

Giacalone, F. and Martin, N., 2006. Fullerene polymers: synthesis and properties. *Chemical reviews*, 106(12), pp.5136-5190.

Gordon, R., Podolski, I., Makarova, E., Deev, A., Mugantseva, E., Khutsyan, S., Sengpiel, F., Murashev, A. and Vorobyov, V., 2017. Intrahippocampal Pathways Involved in Learning/Memory Mechanisms are Affected by

Intracerebral Infusions of Amyloid- β 25-35 Peptide and Hydrated Fullerene C 60 in Rats. *Journal of Alzheimer's Disease*, 58(3), pp.711-724.

Guldi, D.M. and Prato, M., 2000. Excited-state properties of C60 fullerene derivatives. *Accounts of chemical research*, 33(10), pp.695-703.

Halenova, T., Raksha, N., Savchuk, O., Ostapchenko, L., Prylutsky, Y., Ritter, U. and Scharff, P., 2020. Evaluation of the biocompatibility of water-soluble pristine C60 fullerenes in rabbit. *BioNanoScience*, 10(3), pp.721-730.

Hazrati, M.K. and Hadipour, N.L., 2016. Adsorption behavior of 5-fluorouracil on pristine, B-, Si-, and Al-doped C60 fullerenes: A first-principles study. *Physics Letters A*, 380(7-8), pp.937-941.

Hekimi, S., Lapointe, J. and Wen, Y., 2011. Taking a “good” look at free radicals in the aging process. *Trends in cell biology*, 21(10), pp.569-576.

Holmannova, D., Borsky, P., Svadlakova, T., Borska, L. and Fiala, Z., 2022. Reproductive and Developmental Nanotoxicity of Carbon Nanoparticles. *Nanomaterials*, 12(10), p.1716.

Hu, Z., Guan, W., Wang, W., Huang, L., Xing, H. and Zhu, Z., 2007. Protective effect of a novel cystine C60 derivative on hydrogen peroxide-induced apoptosis in rat pheochromocytoma PC12 cells. *Chemico-biological interactions*, 167(2), pp.135-144.

Hynds, R.E., Vladimirou, E. and Janes, S.M., 2018. The secret lives of cancer cell lines. *Disease models & mechanisms*, 11(11), p.dmm037366.

Isakovic, A., Markovic, Z., Todorovic-Markovic, B., Nikolic, N., Vranjes-Djuric, S., Mirkovic, M., Dramicanin, M., Harhaji, L., Raicevic, N., Nikolic, Z. and Trajkovic, V., 2006. Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene. *Toxicological Sciences*, 91(1), pp.173-183.

Kapun, A.P., Salobir, J., Levart, A., Kotnik, T. and Svete, A.N., 2012. Oxidative stress markers in canine atopic dermatitis. *Research in veterinary science*, 92(3), pp.469-470.

Kato, S., Aoshima, H., Saitoh, Y. and Miwa, N., 2009. Highly hydroxylated or γ -cyclodextrin-bicapped water-soluble derivative of fullerene: The antioxidant ability assessed by electron spin resonance method and β -carotene bleaching assay. *Bioorganic & Medicinal Chemistry Letters*, 19(18), pp.5293-5296.

Keypour, H., Noroozi, M. and Rashidi, A., 2013. An improved method for the purification of fullerene from fullerene soot with activated carbon, celite, and silica gel stationary phases. *Journal of nanostructure in chemistry*, 3, pp.1-9.

Koren Carmi, I., Haj, R., Yehuda, H., Tamir, S. and Reznick, A.Z., 2015. The role of oxidation in FSL-1 induced signaling pathways of an atopic dermatitis model in HaCaT keratinocytes. *Environmental Biomedicine*, pp.1-10.

Krätschmer, W., Lamb, L.D., Fostiropoulos, K.H.D.R. and Huffman, D.R., 1990. Solid C₆₀: a new form of carbon. *Nature*, 347(6291), pp.354-358.

Kroto, H.W., 1987. The stability of the fullerenes C_n, with n= 24, 28, 32, 36, 50, 60 and 70. *Nature*, 329(6139), pp.529-531.

Kroto, H.W., Heath, J.R., O'Brien, S.C., Curl, R.F. and Smalley, R.E., 1985. C₆₀: Buckminsterfullerene. *Nature*, 318(6042), pp.162-163.

Krusic, P.J., Wasserman, E., Keizer, P.N., Morton, J.R. and Preston, K.F., 1991. Radical reactions of C₆₀. *Science*, 254(5035), pp.1183-1185.

Kumar, C.S. ed., 2013. *UV-VIS and photoluminescence spectroscopy for nanomaterials characterization* (Vol. 111). Berlin: Springer.

Kuzmany, H., Pfeiffer, R., Hulman, M. and Kramberger, C., 2004. Raman spectroscopy of fullerenes and fullerene–nanotube composites. *Philosophical Transactions of the Royal Society of London*.

Series A: Mathematical, Physical and Engineering Sciences, 362(1824), pp.2375-2406.

Langenstroer, P.E.T.E.R. and Pieper, G.M., 1992. Regulation of spontaneous EDRF release in diabetic rat aorta by oxygen free radicals. *American Journal of Physiology-Heart and Circulatory Physiology*, 263(1), pp.H257-H265.

Lee, W.K. and Thévenod, F., 2006. A role for mitochondrial aquaporins in cellular life-and-death decisions?. *American Journal of Physiology-Cell Physiology*, 291(2), pp.C195-C202

Li, M., Xu, C., Wu, L., Ren, J., Wang, E. and Qu, X., 2013. Self-Assembled Peptide–Polyoxometalate Hybrid Nanospheres: Two in One Enhances Targeted Inhibition of Amyloid β -Peptide Aggregation Associated with Alzheimer's Disease. *Small*, 9(20), pp.3455-3461.

Lin, M.S., Chen, R.T., Yu, N.Y., Sun, L.C., Liu, Y., Cui, C.H., Xie, S.Y., Huang, R.B. and Zheng, L.S., 2017. Fullerene-based amino acid ester chlorides self-assembled as spherical nano-vesicles for drug delayed release. *Colloids and Surfaces B: Biointerfaces*, 159, pp.613-619.

Macip, S., Igarashi, M., Fang, L., Chen, A., Pan, Z.Q., Lee, S.W. and Aaronson, S.A., 2002. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. *The EMBO journal*, 21(9), pp.2180-2188.

Marcorin, G.L., Da Ros, T., Castellano, S., Stefancich, G., Bonin, I., Miertus, S. and Prato, M., 2000. Design and synthesis of novel [60] fullerene derivatives as potential HIV aspartic protease inhibitors. *Organic letters*, 2(25), pp.3955-3958..

Markovic, Z. and Trajkovic, V., 2008. Biomedical potential of the reactive oxygen species generation and quenching by fullerenes (C60). *Biomaterials*, 29(26), pp.3561-3573.

Mirkov, S.M., Djordjevic, A.N., Andric, N.L., Andric, S.A., Kostic, T.S., Bogdanovic, G.M., Vojinovic-Miloradov, M.B. and Kovacevic, R.Z., 2004. Nitric oxide-scavenging activity of polyhydroxylated fulleranol, C₆₀ (OH)₂₄. *Nitric oxide*, 11(2), pp.201-207.

Mitschker, J. and Klüner, T., 2012. New insight into CO photodesorption from C₆₀. *The Journal of Physical Chemistry A*, 116(46), pp.11211-11218.

Mohan Gokhale, M. and Ravindra Somani, R., 2015. Fullerenes: chemistry and its applications. *Mini-Reviews in Organic Chemistry*, 12(4), pp.355-366.

Montellano, A., Da Ros, T., Bianco, A. and Prato, M., 2011. Fullerene C₆₀ as a multifunctional system for drug and gene delivery. *Nanoscale*, 3(10), pp.4035-4041.

Mroz, P., Pawlak, A., Satti, M., Lee, H., Wharton, T., Gali, H., Sarna, T. and Hamblin, M.R., 2007. Functionalized fullerenes mediate photodynamic killing of cancer cells: Type I versus Type II photochemical mechanism. *Free Radical Biology and Medicine*, 43(5), pp.711-719

Nakai, K., Yoneda, K., Maeda, R., Munehiro, A., Fujita, N., Yokoi, I., Moriue, J., Moriue, T., Kosaka, H. and Kubota, Y., 2009. Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis. *Journal of the European Academy of Dermatology and Venereology*, 23(12), pp.1405-1408.

Nakai, K., Yoneda, K., Maeda, R., Munehiro, A., Fujita, N., Yokoi, I., Moriue, J., Moriue, T., Kosaka, H. and Kubota, Y., 2009. Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis. *Journal of the European Academy of Dermatology and Venereology*, 23(12), pp.1405-1408

Naz, S., Shamoan, M., Wang, R., Zhang, L., Zhou, J. and Chen, J., 2019. Advances in therapeutic implications of inorganic drug delivery nano-

platforms for cancer. *International Journal of Molecular Sciences*, 20(4), p.965.

Nimibofa, A., Newton, E.A., Cyprain, A.Y. and Donbebe, W., 2018. Fullerenes: synthesis and applications. *J Mater Sci*, 7, pp.22-33.

Pacor, S., Grillo, A., Đorđević, L., Zorzet, S., Lucafò, M., Da Ros, T., Prato, M. and Sava, G., 2015. Effects of two fullerene derivatives on monocytes and macrophages. *BioMed Research International*, 2015.

Nishizawa, C., Hashimoto, N., Yokoo, S., Funakoshi-Tago, M., Kasahara, T., Takahashi, K., Nakamura, S. and Mashino, T., 2009. Pyrrolidinium-type fullerene derivative-induced apoptosis by the generation of reactive oxygen species in HL-60 cells. *Free radical research*, 43(12), pp.1240-1247.

Panchuk, R.R., Prylutska, S.V., Chumak, V.V., Skorokhyd, N.R., Lehka, L.V., Evstigneev, M.P., Prylutsky, Y.I., Berger, W., Heffeter, P., Scharff, P. and Ritter, U., 2015. Application of C60 fullerene-doxorubicin complex for tumor cell treatment in vitro and in vivo. *Journal of biomedical nanotechnology*, 11(7), pp.1139-1152.

Pinheiro, H.M., Touraud, E. and Thomas, O., 2004. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and pigments*, 61(2), pp.121-139.

Porto, L.S., Silva, D.N., de Oliveira, A.E.F., Pereira, A.C. and Borges, K.B., 2019. Carbon nanomaterials: Synthesis and applications to development of electrochemical sensors in determination of drugs and compounds of clinical interest. *Reviews in analytical chemistry*, 38(3), p.20190017.

Prylutska, S., Bilyy, R., Overchuk, M., Bychko, A., Andreichenko, K., Stoika, R., Rybalchenko, V., Prylutsky, Y., Tsierkezos, N.G. and Ritter, U., 2012. Water-soluble pristine fullerenes C60 increase the specific conductivity and capacity of lipid model membrane and form the channels in cellular plasma membrane. *Journal of Biomedical Nanotechnology*, 8(3), pp.522-527.

Prylutska, S., Grynyuk, I., Matyshevskaya, O., Prylutsky, Y., Evstigneev, M., Scharff, P. and Ritter, U., 2014. C 60 fullerene as synergistic agent in tumor-inhibitory doxorubicin treatment. *Drugs in R&D*, 14, pp.333-340.

Prylutska, S., Panchuk, R., Gołuski, G., Skivka, L., Prylutsky, Y., Hurmach, V., Skorohyd, N., Borowik, A., Woziwodzka, A., Piosik, J. and Kyzyma, O., 2017. C 60 fullerene enhances cisplatin anticancer activity and overcomes tumor cell drug resistance. *Nano Research*, 10, pp.652-671.

Prylutska, S.V., Burlaka, A.P., Prylutsky, Y.I., Ritter, U. and Scharff, P., 2011. Pristine C60 fullerenes inhibit the rate of tumor growth and metastasis. *Experimental Oncology*.

Prylutska, S.V., Grynyuk, I.I., Matyshevskaya, O.P., Prylutsky, Y.I., Ritter, U. and Scharff, P., 2008. Anti-oxidant properties of C60 fullerenes in vitro. *Fullerenes, Nanotubes and Carbon Nanostructures*, 16(5-6), pp.698-705.

Prylutska, S.V., Matyshevskaya, O.P., Golub, A.A., Prylutsky, Y.I., Potebnya, G.P., Ritter, U. and Scharff, P., 2007. Study of C60 fullerenes and C60-containing composites cytotoxicity in vitro. *Materials Science and Engineering: C*, 27(5-8), pp.1121-1124.

Prylutska, S.V., Matyshevskaya, O.P., Grynyuk, I.I., Prylutsky, Y.I., Ritter, U. and Scharff, P., 2007. Biological effects of C60 fullerenes in vitro and in a model system. *Molecular Crystals and Liquid Crystals*, 468(1), pp.265-617.

Rancan, F., Rosan, S., Boehm, F., Cantrell, A., Brellreich, M., Schoenberger, H., Hirsch, A. and Moussa, F., 2002. Cytotoxicity and photocytotoxicity of a dendritic C60 mono-adduct and a malonic acid C60 tris-adduct on Jurkat cells. *Journal of photochemistry and Photobiology B: Biology*, 67(3), pp.157-162.

Rao, C.N.R. and Biswas, K., 2009. Characterization of nanomaterials by physical methods. *Annual Review of Analytical Chemistry*, 2, pp.435-462.

Rozhkov, S.P., Goryunov, A.S., Sukhanova, G.A., Borisova, A.G., Rozhkova, N.N. and Andrievsky, G.V., 2003. Protein interaction with hydrated C60 fullerene in aqueous solutions. *Biochemical and biophysical research communications*, 303(2), pp.562-566.

Satoh, M. and Takayanagi, I., 2006. Pharmacological studies on fullerene (C60), a novel carbon allotrope, and its derivatives. *Journal of pharmacological sciences*, 100(5), pp.513-518.

Sayers, B.C., Germolec, D.R., Walker, N.J., Shipkowski, K.A., Stout, M.D., Cesta, M.F., Roycroft, J.H., White, K.L., Baker, G.L., Dill, J.A. and Smith, M.J., 2016. Respiratory toxicity and immunotoxicity evaluations of microparticle and nanoparticle C60 fullerene aggregates in mice and rats following nose-only inhalation for 13 weeks. *Nanotoxicology*, 10(10), pp.1458-1468.

Scharff, P., Risch, K., Carta-Abelmann, L., Dmytruk, I.M., Bilyi, M.M., Golub, O.A., Khavryuchenko, A.V., Buzaneva, E.V., Aksenov, V.L., Avdeev, M.V. and Prylutsky, Y.I., 2004. Structure of C60 fullerene in water: spectroscopic data. *Carbon*, 42(5-6), pp.1203-1206.

Schuetze, C., Ritter, U., Scharff, P., Fernekorn, U., Prylutska, S., Bychko, A., Rybalchenko, V. and Prylutsky, Y., 2011. Interaction of N-fluorescein-5-isothiocyanate pyrrolidine-C60 with a bimolecular lipid model membrane. *Materials Science and Engineering: C*, 31(5), pp.1148-1150.

Scott, L.T., 2004. Methods for the chemical synthesis of fullerenes. *Angewandte Chemie International Edition*, 43(38), pp.4994-5007.

Sharma, P., Mehta, M., Dhanjal, D.S., Kaur, S., Gupta, G., Singh, H., Thangavelu, L., Rajeshkumar, S., Tambuwala, M., Bakshi, H.A. and Chellappan, D.K., 2019. Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. *Chemico-biological interactions*, 309, p.108720.

Sharma, S., Naskar, S. and Kuotsu, K., 2020. Metronomic chemotherapy of carboplatin-loaded PEGylated MWCNTs: Synthesis, characterization and in vitro toxicity in human breast cancer. *Carbon Letters*, 30, pp.435-447.

Shen, Q., Liu, Z., Hua, Y., Zhao, J., Lv, W. and Mohsan, A.U.H., 2018. Effects of cutting edge microgeometry on residual stress in orthogonal cutting of Inconel 718 by FEM. *Materials*, 11(6), p.1015.

Shershakova, N., Baraboshkina, E., Andreev, S., Purgina, D., Struchkova, I., Kamysnikov, O., Nikonova, A. and Khaitov, M., 2016. Anti-inflammatory effect of fullerene C 60 in a mice model of atopic dermatitis. *Journal of nanobiotechnology*, 14, pp.1-11.

Shytikov, D., Shytikova, I., Rohila, D., Kulaga, A., Dubiley, T. and Pishel, I., 2021. Effect of long-term treatment with C60 fullerenes on the lifespan and health status of CBA/Ca mice. *Rejuvenation Research*, 24(5), pp.345-353.

Simon, H.U., Yousefi, S., Schranz, C., Schapowal, A., Bachert, C. and Blaser, K., 1997. Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *Journal of immunology (Baltimore, Md.: 1950)*, 158(8), pp.3902-3908

Skivka, L.M., Prylutska, S.V., Rudyk, M.P., Khranovska, N.M., Opeida, I.V., Hurmach, V.V., Prylutsky, Y.I., Sukhodub, L.F. and Ritter, U., 2018. C60 fullerene and its nanocomplexes with anticancer drugs modulate circulating phagocyte functions and dramatically increase ROS generation in transformed monocytes. *Cancer nanotechnology*, 9(1), pp.1-22.

Skivka, L.M., Prylutska, S.V., Rudyk, M.P., Khranovska, N.M., Opeida, I.V., Hurmach, V.V., Prylutsky, Y.I., Sukhodub, L.F. and Ritter, U., 2018. C60 fullerene and its nanocomplexes with anticancer drugs modulate circulating phagocyte functions and dramatically increase ROS generation in transformed monocytes. *Cancer nanotechnology*, 9(1), pp.1-22.

Sorce, S. and Krause, K.H., 2009. NOX enzymes in the central nervous system: from signaling to disease. *Antioxidants & redox signaling*, 11(10), pp.2481-2504.

Srđenović, B.U., Slavic, M.N., Stankov, K.M., Kladar, N.V., Jović, D.S., Seke, M. and Bogdanović, V.V., 2015. Size distribution of fulleranol nanoparticles in cell culture medium and their influence on antioxidative enzymes in Chinese hamster ovary cells. *Hemijska industrija*, 69(4), pp.425-431.

Sumi, N. and Chitra, K.C., 2019. Cytogenotoxic effects of fullerene C60 in the freshwater teleostean fish, *Anabas testudineus* (Bloch, 1792). *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 847, p.503104.

Taylor, R. and Walton, D.R., 1993. The chemistry of fullerenes. *Nature*, 363(6431), pp.685-693.

Taylor, R., 1991. A valence bond approach to explaining fullerene stabilities. *Tetrahedron letters*, 32(30), pp.3731-3734.

Taylor, R., 1999. *Lecture notes on fullerene chemistry: a handbook for chemists*. World Scientific.

Taylor, R., Hare, J.P., Abdulsada, A.K. and Kroto, H.W., 1990. Characterization of the Fullerenes C-60 And C-70-The 3rd Form of Carbon. *Journal of the Chemical Society-Chemical Communications*, 1423.

Thomas, M.P., Chartrand, K., Reynolds, A., Vitvitsky, V., Banerjee, R. and Gendelman, H.E., 2007. Ion channel blockade attenuates aggregated alpha synuclein induction of microglial reactive oxygen species: relevance for the pathogenesis of Parkinson's disease. *Journal of neurochemistry*, 100(2), pp.503-519.

Trpkovic, A., Todorovic-Markovic, B. and Trajkovic, V., 2012. Toxicity of pristine versus functionalized fullerenes: mechanisms of cell damage and the role of oxidative stress. *Archives of toxicology*, 86, pp.1809-1827.

Ueno, H., Yamakura, S., Arastoo, R.S., Oshima, T. and Kokubo, K., 2014. Systematic evaluation and mechanistic investigation of antioxidant activity of fullerenols using β -carotene bleaching assay. *Journal of Nanomaterials*, 2014, pp.7-7.

Vachier, I., Chanez, P., Le Doucen, C., Damon, M., Descomps, B. and Godard, P., 1994. Enhancement of reactive oxygen species formation in stable and unstable asthmatic patients. *European Respiratory Journal*, 7(9), pp.1585-1592.

Vijayalakshmi, V., Sadanandan, B. and Anjanapura, R.V., 2023. In vitro comparative cytotoxic assessment of pristine and carboxylic functionalized multiwalled carbon nanotubes on LN18 cells. *Journal of Biochemical and Molecular Toxicology*, 37(3), p.e23283.

Wang, S., Ding, L., Liu, S.S., Wang, C., Leng, R.X., Chen, G.M., Fan, Y.G., Pan, H.F. and Ye, D.Q., 2012. IL-33: a potential therapeutic target in autoimmune diseases. *Journal of Investigative Medicine*, 60(8), pp.1151-1156.

Wang, Z., Chang, X., Lu, Z., Gu, M., Zhao, Y. and Gao, X., 2014. A precision structural model for fullerenolsChem.

Williams, D., 2008. The relationship between biomaterials and nanotechnology. *Biomaterials*, 29(12), pp.1737-1738.

Xiao, L., Takada, H., Maeda, K., Haramoto, M. and Miwa, N., 2005. Antioxidant effects of water-soluble fullerene derivatives against ultraviolet ray or peroxy lipid through their action of scavenging the reactive oxygen species in human skin keratinocytes. *Biomedicine & pharmacotherapy*, 59(7), pp.351-358.

Yamakoshi, Y., Umezawa, N., Ryu, A., Arakane, K., Miyata, N., Goda, Y., Masumizu, T. and Nagano, T., 2003. Active oxygen species generated from photoexcited fullerene (C60) as potential medicines: O₂-• versus 1O₂. *Journal of the American Chemical Society*, 125(42), pp.12803-12809.

Yamawaki, H. and Iwai, N., 2006. Cytotoxicity of water-soluble fullerene in vascular endothelial cells. *American Journal of Physiology-Cell Physiology*, 290(6), pp.C1495-C1502.

Ye, L., Kollie, L., Liu, X., Guo, W., Ying, X., Zhu, J., Yang, S. and Yu, M., 2021. Antitumor activity and potential mechanism of novel fullerene derivative nanoparticles. *Molecules*, 26(11), p.3252.

Zhang, X., Huang, Y., Wang, Y., Ma, Y., Liu, Z. and Chen, Y., 2009. Synthesis and characterization of a graphene–C60 hybrid material. *Carbon*, 47(1), pp.334-337.

Zhu, W., Miser, D.E., Chan, W.G. and Hajaligol, M.R., 2004. Characterization of combustion fullerene soot, C60, and mixed fullerene. *Carbon*, 42(8-9), pp.1463-1471.

Zogovic, N.S., Nikolic, N.S., Vranjes-Djuric, S.D., Harhaji, L.M., Vucicevic, L.M., Janjetovic, K.D., Misirkic, M.S., Todorovic-Markovic, B.M., Markovic, Z.M., Milonjic, S.K. and Trajkovic, V.S., 2009. Opposite effects of nanocrystalline fullerene (C60) on tumour cell growth in vitro and in vivo and a possible role of Immunosuppression in the cancer-promoting activity of C60. *Biomaterials*, 30(36), pp.6940-6946.

ANNEXURE

Presented the article “Unravelling the potentiality of C₆₀ fullerene molecule in targeted delivery of agricultural fertilisers in crops: A review” on 2nd February, 2024 in ICEISA'24 conducted by VIT, Vellore and the abstract has been published in the ICEISA proceedings.

nutritional status, disease resistance, and nutrient regulation in medicinal plants.

Keywords: Organic fertilizer, Liquid fertilizers, Precision farming and medicinal plants.

07

Systematic Literature Review on Adoption of Precision Farming

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Precision farming plays a role in optimization of resources use, increase productivity, promote sustainability it often involves use of automated machinery and robots. Precision farming has been the astonishing phrases of agriculture research around the global in recent times. Investigation studies how precision farming technologies are utilized in agriculture through a systematic literature review (SLR). We investigate current trends, hurdles, and emerging possibilities in the integration of precision farming practices through analyzing an extensive amount of academic literature. The assessment emphasizes how essential it's to utilize technologies like sensors, GPS, and data analytics to enhance farm management practices. It also examines potential solutions and eliminates hurdles to wider adoption. With the objective of providing scholars, decision-makers, and practitioners a thorough grasp of the present state of affairs, the findings aim to encourage the successful implementation of precision

farming for efficient and sustainable agricultural practices.

Key words: Precision farming, GPS, potential solution, sustainable agriculture.

08

Unravelling the Potentiality of C₆₀ Fullerene Molecule in Targeted Delivery of Agricultural Fertilisers in Crops: A Review

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Nanotechnology is one of the most important tools in modern agriculture in such a way that Precision farming techniques could be utilized to increase crop yields, while minimizing soil and water damage, reduce nitrogen loss owing to leaching and emissions, and improve nutrient long-term assimilation by soil microorganisms. C₆₀ fullerene, A promising nano-sized material that attracted interest with its distinct molecular structure, provides a viable platform for precisely encapsulating and distributing fertilizers. It is often known as buckyball, which has distinct physicochemical features that make it an attractive choice for agricultural uses. Because of its spherical form, excellent stability, and biocompatibility, it may be customized for fertilizer functionalization allowing for controlled mechanisms and targeted fertilizer release to enhance nitrogen



Presented the article “Exploring the molecular mechanism of Reactive Oxygen Species imparted by Fullerene (C₆₀) nanosystems over lung cancer cell lines: A Review” on 27th February, 2024 in AFMD’24 conducted by SRM University, Chennai and the abstract has been published in the AFMD proceedings.

Exploring the molecular mechanism of Reactive Oxygen Species imparted by Fullerene (C₆₀) nanosystems over lung cancer cell lines: A Review

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ABSTRACT

Lung cancer remains the top cause of cancer-related death worldwide, underscoring the crucial need for novel therapeutic approaches. Fullerene (C₆₀) nanosystems have attracted interest for their achievable application in cancer therapy, owing to their unique and outstanding physicochemical features and innate capacity to either create or quench cell damaging Reactive Oxygen Species (ROS). This study elevates the molecular processes driving ROS-mediated cytotoxicity in lung cancer cell lines produced by Fullerene C₆₀ [1]. Implementation of various lung cancer cell lines in in-vitro tests to determine the Fullerene C₆₀'s effect of on cell viability and ROS generation. Many other researches projected that; C₆₀ Fullerene are potent to increase ROS levels when treated on a dose-dependent mode. There are more and impactful pathways that is associated with ROS-mediated cell death including oxidative stress, DNA damage, mitochondrial malfunction, and apoptosis. This contribution of signaling pathways and independent genes in ROS generation or quenching was investigated, offering adequate guidance on attainable and feasible therapeutic targets [2]. The effect of Fullerene nanosystems changes from cancer cells to normal lung cells, this paved a way considering uncomplicated access to their potential for targeted cancer therapy. Here, this study sheds light and valuable insights on the molecular pathways by which Fullerene (C₆₀) nanosystems induce ROS-mediated cytotoxicity in lung cancer cell lines [3]. Understanding these pathways is crucial for the development of robust nanoscale-based cancer therapeutics, which could pave the way for targeted and effective lung cancer treatments.

References:

1. S. Beyaz, A. Aslan, O.Gok, I.H. Ozercan and C.A Agca, Free Radical Research, Vol: 57, Issue: 5, 2023, pp: 373-383
2. Z. Markovic and V. Trajkovic, Biomaterials, Vol: 29, Issue: 26,2008, pp: 3561-3573
3. V. Bilobrov, V. Sokolova, S. Prylutska, R. Panchuk, O. Litsis, V. Osetskyi, M. Evstigneev, Y. Prylutsky, M. Eppe, U. Ritter and J. Rohr, Cellular and Molecular Bioengineering Vol: 12, Issue: 2019, pp: 41-51

