

THE ROLE OF CARBON NANOTUBES IN NANOBIMEDICINES

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ABSTRACT

CNTs is a fullerene molecule, described in 1991 by the Japanese Scientist “Sumio Iijima” as tube-shaped of graphitic carbon, can be obtained either single or multi-walled nanotube, having a diameter measuring on the nanometer scale, and generally known as buckytubes. Carbon nanotubes (CNTs) have established much recent interest as new entities for experimental disease diagnosis and treatment because of their unique electronic, mechanical, thermal, spectroscopic, metallic, semiconducting and superconducting electron transport properties. Carbon nanotubes can be acquired in numerous ways, the general techniques are Arc discharge, Laser ablation, and Chemical vapour deposition (CVD). Carbon nanotubes are discussed in this review in terms of characters, history, structures, properties, synthesis, purification, characterization methods, toxicity and applications. Purification of nanotubes includes many techniques: Acid treatment, oxidation, annealing, ultrasonication, cutting, magnetic purification, chromatography techniques. Further functionalization enhanced the water solubility of CNT's and completely transformed their biocompatibility profile. Carbon nanotubes, due to their large surface areas, unique surface properties, and needle-like shape, can deliver a lot of therapeutic agents, including DNA, siRNAs and proteins to the target disease sites. CNTs can be readily excreted through the renal route by means of degradation through myeloperoxidase (MOP) enzyme. As CNTs have attracted the fancy of many scientists worldwide, the work beyond our expectations and their simple mechanism with long lasting life makes it more reliable to use. The unique and unusual properties of these structures make them a unique material with a whole range of promising applications.

Keywords: Carbon Nanotube, Single-walled carbon nanotubes, Multiwalled carbon nanotubes, Cancer

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INTRODUCTION

Pharmaceutical nanotechnology focuses on the top of formulating therapeutically energetic agents in biocompatible nanoforms such as nanoparticles, nanocapsules and conjugates. These systems suggest numerous advantages in drug delivery mainly focusing on better safety and efficiency of drugs i.e., targeted delivery of drugs, improve bioavailability, extending drug or gene effect of drugs, tissue and improving the stability of therapeutic agents in opposition to chemical/enzymatic degradation [1, 2] In the current scenario, nanotechnology is rapidly expanding scientific zone that has achieved a breakthrough in molecular biology, diagnostics, imaging, bioengineering and nanomedicines etc. Carbon nanotubes (CNTs) have established much recent interest as new entities for experimental disease diagnosis and treatment because of their unique electronic, mechanical, thermal, spectroscopic, metallic, semiconducting and superconducting electron transport properties structurally they acquire a hollow core made up of graphite sheets (fig. 1) which are rolled into tubes and are closed at their ends by semi-fullerene like structure [3] (fig. 2) making them appropriate for storing guest molecules as well as their ability to traverse cellular membranes and

contain elastic or young's modulus of any recognized materials [4, 5] thus seeing much attention as a potent carrier. The sphere-shaped C_{60} molecule was discovered in 1985 by Harry Kroto and Richard Smally during experiments with laser vaporisation of graphite [6]. Carbon nanotubes were later observed through efforts in fullerene research and were discovered by Sumio Iijima in 1991 [4]. The Kroto and Smally group detected the principal generation of C_{60} and hypothesised that it may exist as a stable closed assembly. Later work by Wolfgang Krätschmer and Donald Huffman developed larger quantities of fullerenes through an arc-discharge process and established the proposed spherical structure of C_{60} (fig. 3)[7]. Through transmission electron microscopy [8], Iijima scrutinised carbon soot made by the Krätschmer and Huffman method where he found CNTs. The early discovery was of MWCNTs, the smallest being double-walled, and afterwards, discoveries in 1993 independently reported on the first SWCNTs [9]. CNTs are a fullerene molecule was described in 1991 by Sumio Iijima as tube-shaped or toroidal shape or cylindrical shells of graphitic carbon, seamless, well-ordered, flat network, high aspect ratio allotropes of carbon having a diameter measuring on the nanometer scale. These carbon-based nanomaterials carbon nanotubes are generally known as buckytubes [4, 10, 11].

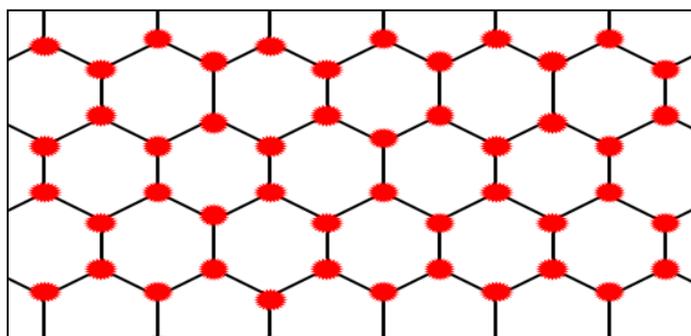


Fig. 1: A single sheet of grapheme

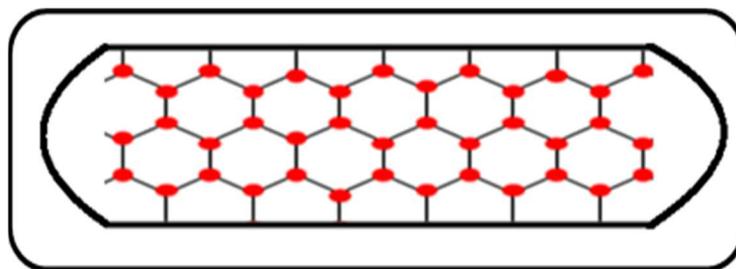


Fig. 2: Carbon nanotubes having ends close with some functional groups of hemi fullerenes

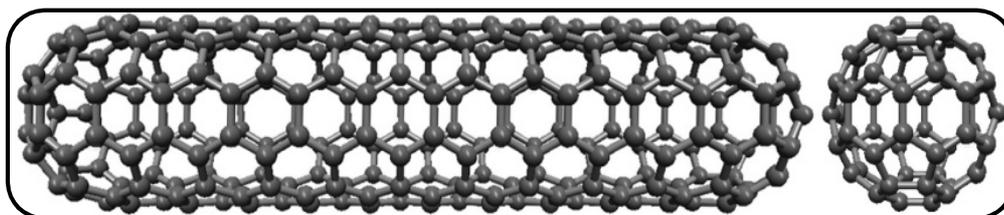


Fig. 3: Carbon nanotube structure demonstrating the resemblance of the end caps to hemispherical C₆₀ molecules shown on the right

The biocompatibility nature, non-immunogenicity, ease of size alteration, greater stability and high drug loading potential makes CNTs a famous tool over the other nanocarriers [12]. Internal and external surfaces of CNTs can be modified on an individual basis as required and a variety of functional groups can be generated on their surface in support of further conjugation with targeting ligands

as well as drug molecules[13]. There are two types of carbon nanotubes, distinguished in (table 1):-

- ✓ Single-walled carbon nanotubes (SWCNT's)
- ✓ Multi-walled carbon nanotubes (MWCNT's)

Table 1: Types and properties of CNT's

Types Structure	Single-walled carbon nanotubes <small>SWCNT</small>	Multi-walled carbon nanotubes <small>MWCNT</small>	Ref
Diameter	Ranging from 1-10 nm.	Approx 5-30 nm.	[14]
Length	20-1000 nm.	1 to several μm.	
Properties	Consist of one layer of cylinder graphene sheet	Which contain several concentric graphene sheets	

MWNTs may also be fabricated by SWNTs of distinct diameters. SWNTs are hollow cylindrical structures may be separated into three different categories, each of which is a couple of fullerene caps connected by a tube-shaped structure that is a rolled up into the seamless graphene sheets. Various important carbon nanomaterial structures (fig. 4). SWNTs are composed of a single atomic layer of

graphite, having a different chirality. They are: (A) Zigzag, (B) Chiral, (C) Armchair (table 2)[15]. Carbon nanotubes (CNT's) own high specific surface areas, electrical conductivities, chemical stability, etc. which could be achieved by the treatment of CNT with acid [16-17] (fig. 5) and named as refluxing. CNTs also enhance the electron transfer rate of many redox reactions [18].

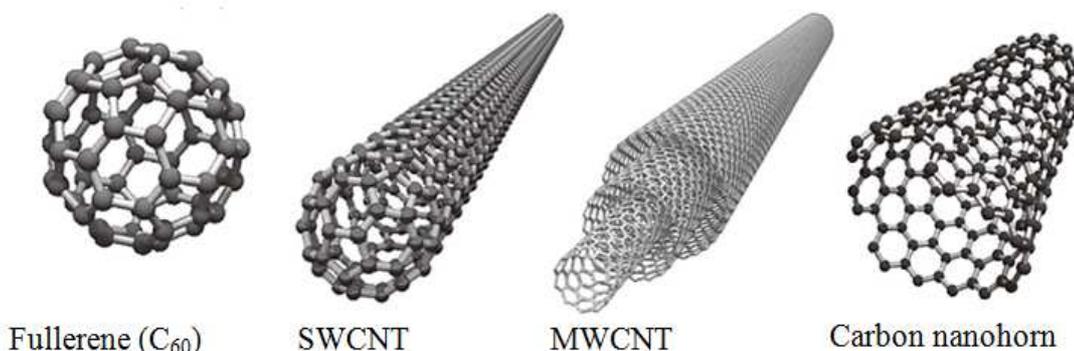


Fig. 4: Structures of various important carbon nanomaterials: Fullerene (C₆₀), Single-Walled Carbon Nanotube (SWCNT), Multi-Walled Carbon Nanotube (MWCNT) and Carbon Nanohorn

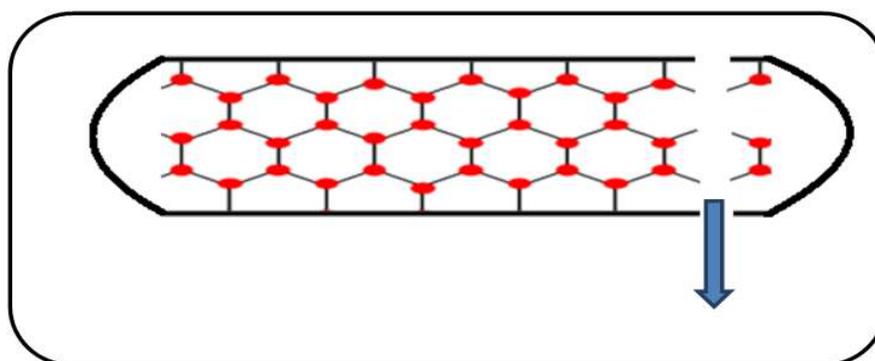


Fig. 5: Open carbon nanotube by the action of an acid

Table 2: Types of SWCNTs

Types	Categories	Properties	Structures
1.	Zigzag	Named after the pattern of the hexagon as one moves circumferentially around the body of the tubule.	
2.	Chiral	Most commonly occurring SWNT. Chiral-means handedness and indicates that the tubes may twist in either direction.	
3.	Armchair	Describe one of the two conformers of Cyclohexane, a hexagon of carbon atoms and, Describe the shape of the hexagons as one move around the body of the tubules.	

Structures

Bonding

The sp^2 hybrid orbital permit's carbon atoms to form hexagons and occasionally pentagons and Pentagon units through in-plane σ bonding and out-of-plane π bonding.

Defect-free nanotubes

These are tubular structures of a hexagonal network having a diameter as small as 0.4 nm. Tube-shaped curvature gives results in σ - π rehybridization or mixing.

Defective nanotubes

Occasionally, pentagons and heptagons are included into a hexagonal network to form bent, choroidal, branched, helical, or capped nanotubes [19].

Properties

The unique properties make CNTs very useful for supporting noble-metal nanoparticles. Metal-nanoparticle/CNT nanohybrid has many potential applications ranging from highly-developed sensors to highly efficient fuel cells [20]. CNTs are very light tubular nanomaterials with a great aspect ratio (length/diameter), huge payload capacity with rich surface chemistry for functionalization [21, 22].

Physical properties

Orientation and diameter of the nanotubes can affect the physical properties of nanotubes to a great degree. These have hexagonal carbon lattices relative to the central CNT axis [23], which can be lined up according to the purpose [10].

Electrical

Electron confinement along the tube perimeter makes defect-free nanotubes either semiconducting or metallic by quantized conductance whereas pentagons and heptagons will generate localised states.

Optical and optoelectronic

One-dimensional band structure and direct band gap make nanotubes ideal for optical applications with a wavelength ranging possibly from 300 to 3000 nm.

Mechanical and electromechanical

σ - π rehybridization gives nanotubes the highest Young's modulus of over one TPa and tensile strength of over 100 Gpa and significant electronic response to strain and metal-insulator transition.

Magnetic and electromagnetic

Electron orbits circulating around a nanotube provide rise to many attention-grabbing phenomena such as quantum oscillation and metal-insulator transition.

Chemical and electrochemical

σ - π rehybridization and high specific surface make possible molecular adsorption, doping, and charge transfer on nanotubes, which, in turn, modulates electronic properties.

Thermal and Thermoelectric

Hereditary from graphite, nanotubes to display the highest thermal conductivity while the quantum effect [19].

Growth mechanism and synthesis of carbon nanotubes

The techniques are not known exactly for the formation of the nanotube and more than one method of growth mechanism might be effective for the construction of carbon nanotubes is still a conflict. The mechanism comprises the steps shown in (fig. 6) and the simple diagrams of growth mechanism shown in (fig. 7). Firstly, C_2 acts as a precursor for the construction of nanotubes and fullerenes on the surface of the metal catalyst particle.

A rod-like carbon is producing quickly from metastable carbide particle. Secondly, CNT's wall contributes to slow graphitisation. For all technique nanotubes, actual growth mechanism seems similar [24]. On a growth mechanism of nanotubes, there are several theories one of which describes in (fig. 6), for supported metals, filaments can form either by 'extrusion/root/base growth' or by 'tip-growth'. Catalyst size plays a role in the type of nanotube obtained whether SWNTs or MWNTs.

(A) Particles of metal catalyst are supported or floating on graphite or on a different substrate, which anticipates the spherical or pear-shaped of the catalyst particles.

(B) The deposition will occur only on one-half of the surface and the carbon diffuses along the concentration gradient and precipitated on the opposite half, around and below the bisecting diameter, it does not take part from the tip of the hemisphere, which accounts for the vacant core that is distinctive of these filaments.

(C) Extrusion or root growth: nanotube grows upwards from the metal particles that remain attached to the substrate.

Tip growth: the particles detach and move at the head of the growing nanotube.

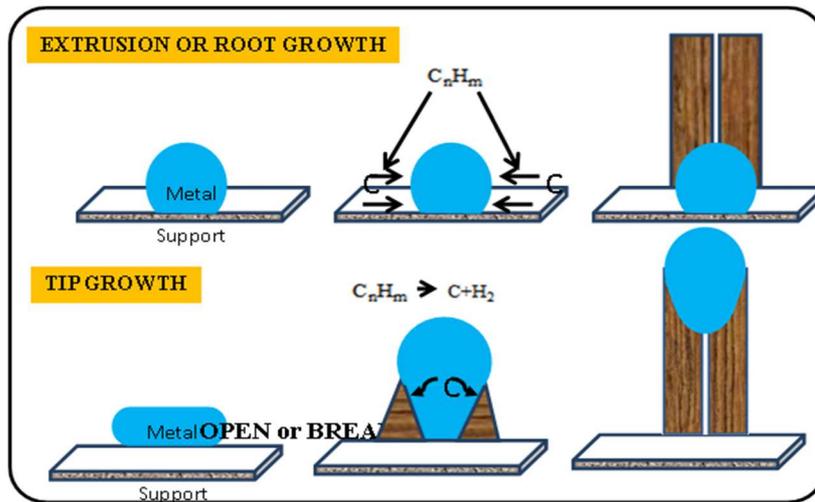


Fig. 6: The visual image of a possible carbon nanotube growth mechanism

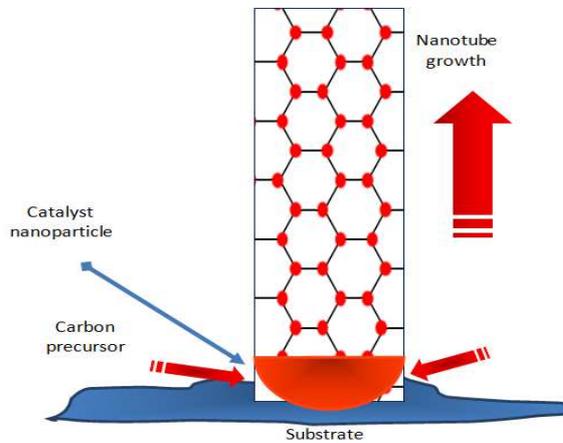


Fig. 7: Growth mechanism of nanotubes: gas based on carbon breaks apart on the surface of the catalyst by CVD, and nanotube grows outwards from the surface

Synthesis

CNT is generally produced by three main techniques:

Arc-discharge: Two electrodes of carbon in presence or absence of catalyst are taken, and an arc is discharged between them. Resulting vapours of carbon get self-assembled and give rise to nanotubes (fig. 8) [25, 9, 26].

Laser ablation: Collision occurs between the high-power laser pulse and volume of carbon, which contains the methane or carbon monoxide as a feedstock gas and produce nanotubes in small quantity (fig. 9) [23, 27, 28].

Chemical Vapor deposition (CVD): Produce MWNT's or SWNT's of poor quality and range of large diameter. CVD process is scaled up easily and favours the viable manufacture (fig. 10) [24].

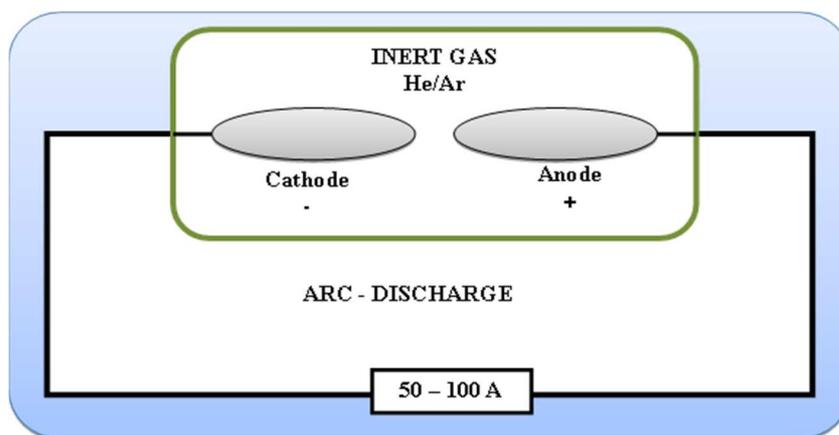


Fig. 8: Diagrammatic representation of arc discharge setup for synthesis and growth of the nanotube

From above three main methods, CVD shows the most prospective for commercial CNT production [29]. In summation to enabling higher atomic quality and percent yield than the other methods [30], CVD provides manufacturers with control over nanotube orientation, length, diameter and other parameters [31]. CVD is capable of making bulk quantities of multi-walled nanotubes for

composites or individual, aligned SWNTs on substrates for utilise in electronics [9]. A brief description of methods contributing to the mass production with their effectiveness is given in fig. 11.

A summary of the major production methods of carbon nanotubes and their efficiency described in table 3.

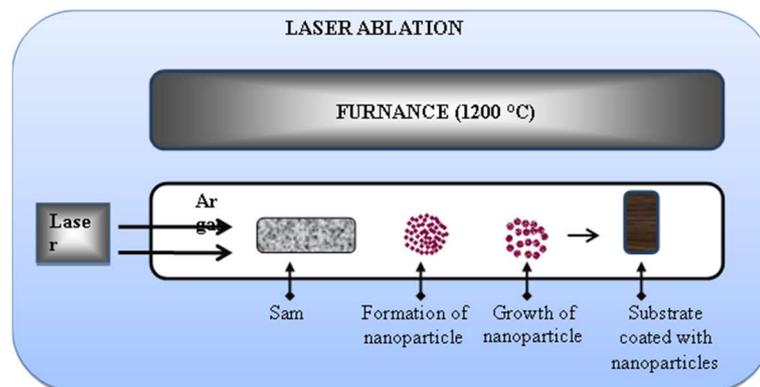


Fig. 9: Diagrammatical representation of laser ablation setup for nanotube synthesis and growth

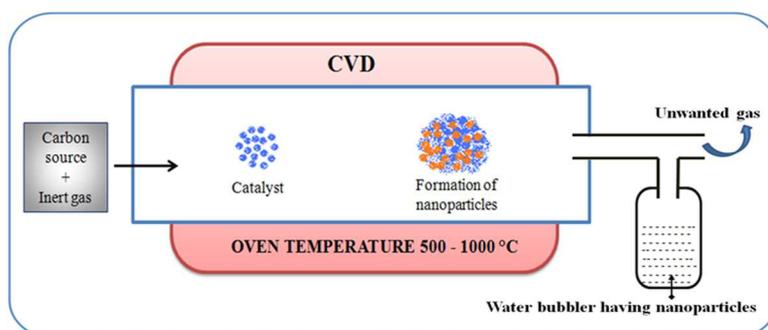


Fig. 10: Diagrammatical representation of chemical vapor deposition (CVD) setup for nanotube synthesis and growth

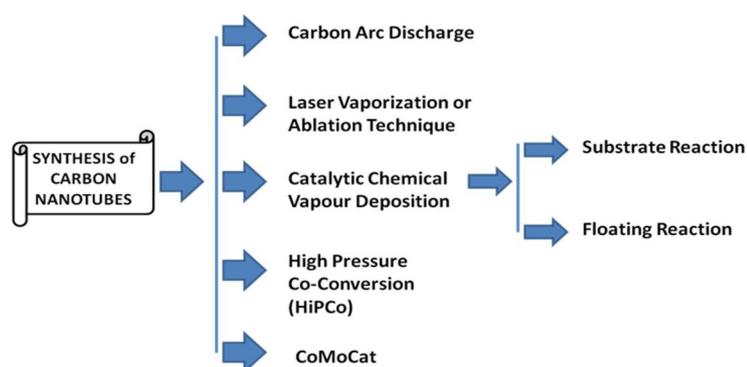


Fig. 11: Methods of synthesis for carbon nanotubes

Purification

The physicochemical properties such as surface topography, solubility, hybridization state, mechanical properties, thermal conductivity, and structural and metallic or carbonaceous impurities, of CNTs, have to be evaluated for biomedical applications [36]. Purification is the primary step and is always needed before any further use of CNTs. Prepared CNTs contain an assortment of impurities that are essential to be removed prior to their utilisation in drug, gene or vaccine delivery. Zhao *et al.* 2001 [37] reported on the numerous approaches that may be utilised for purifying the prepared CNTs that involve filtration, microfiltration, centrifugation,

chromatography, annealing, flocculation, oxidation of contaminant, ferromagnetic separation, functionalisation, ultrasonication, selective interaction with organic polymers, cutting, sedimentation and microwave irradiation. These purification techniques enhance the nanotube solubility, which is easier to separate from the insoluble impurities[38, 39]. CNT products contain substantial amounts of metal impurities and non-nanotube carbon. These are vanished by post-manufacturing treatments, and three fundamental methods have been reported for purification they are:

- Gas phase [40],
- Liquid phase [41], and

c) Intercalation techniques [42].

The occurrence of such metal impurities might lead to a variety of adverse biological end-points [43]. Wrapped up graphite sheets, small fullerenes, catalyst, etc. contributes for the major impurities which can interfere with the required properties of the CNT's, generally as the diameter decreases, impurities increases[39]. Homogenous CNT's samples are of much interest, which can be

achieved by refluxing and oxidation technique[24]. Several purification techniques of the SWNT are basically, studied under two heads viz., structure dependent and size dependent separations.

The structure dependent purification techniques will separate the impurities on the basis of the difference in the structure of the carbon nanotubes and impurities while the size dependent separation techniques yield a uniform distribution of size.

Table 3: Production methods of carbon nanotubes and their efficiency

Method	Arc discharge method	Chemical vapor deposition	Laser ablation (vaporization)	Ref
Who	Ebbesen and Ajayan, NEC, Japan 1992	Endo, Shinshu University, Nagano, Japan	Smalley, Rice, 1995	[19, 32, 33]
Process	The first carbon nanotubes were developed with the arc-discharge method with no use of metal catalysts, producing MWCNTs [7]. Arc-vaporization of two carbon rods placed end to end, separated by approximately few millimetres apart (1 mm), in an enclosure that is usually filled with inert gas at low pressure. A direct current of 50 to 100 Amps, driven by a potential difference of approximately 20 V creates a high-temperature discharge between the two electrodes. The discharge vaporises the surface of one of the carbon electrodes and forms a small rod-shaped deposit on another electrode. Primarily used to produce C ₆₀ fullerenes, is the easiest and common way to produce CNTs.	Chemical Vapor Deposition of hydrocarbons over a metal catalyst is a standard method that has been used to produce various carbon materials like carbon fibers and filaments. A lot of CNTs can be produced by catalytic CVD of acetylene over iron and cobalt catalysts supported on zeolite or silica. High yields of single-walled nanotubes have been obtained by catalytic decomposition of the H ₂ /CH ₄ mixture all over well-dispersed metal particles such as nickel, cobalt and iron on magnesium oxide at 1000 °C. The reduction produces miniature transition metal particles at a temperature of usually >800 °C. The decomposition of CH ₄ over the freshly formed nanoparticles prevents their further growth and thus results in a very high proportion of SWNTs and few MWNTs.	In Laser ablation laser vaporisation pulses were followed by a second pulse, to vaporise the target (graphite/carbon) more uniformly while an unreactive gas permeates the reaction chamber. The use of two successive laser pulses (rather than electricity) minimises the amount of carbon deposited as soot. The second laser pulse breaks up the larger particles ablated by the first one and feeds them into the growing nanotube structure. The material produced by this method appears as a mat of "ropes".	[19, 34, 35]
Typical yield	30 to 90%	20 to 100 %	Up to 70%	[28]
SWNT	Formation of Short tubes with diameters of 0.6-1.4 nm	Formation of Long tubes with diameters ranging from 0.6-4 nm	Formation of Long bundles of tubes (5-20 microns), with individual diameter from 1-2 nm.	
MWNT	Formation of Short tubes with inner diameter of 1-3 nm and outer diameter of approximately 10 nm	Formation of Long tubes with diameter ranging from 10-240 nm	Not so much interest in this technique, seeing that it is too expensive, but MWNT synthesis is possible.	
Gaseous carbon sources	Helium, argon	Methane(CH ₄), carbon monoxide and acetylene	NH ₃ gas	
Merits	Can easily produce MWNTs, SWNTs. SWNTs have fewer structural defects; MWNTs without a catalyst, not too expensive, open air synthesis possible.	Easy to scale up to industrial production; long length, simple process, SWNT diameter controllable, quite pure.	Primarily SWNTs, with good diameter control and few defects. The reaction product is quite pure.	

Methods of purification

Oxidation

Carbon based impurities and surface metal can be taken away by the oxidative treatment [44-51]. But this may lead to the oxidation of nanotubes along with the impurities. Still, the method is preferred because the damage caused to the nanotubes is comparatively less than that of the impurities and the metal catalyst also serves as the oxidising agent [45, 52, 53, 35]. Metal content, time of oxidation, an oxidising agent and environmental factor including temperature are the common process variable which affects the yield of the process. For example, when the temperature is raised to 600 °C, SWNTs will oxidise, even without a catalyst, This happens in the case with thermal [45], pure oxygen oxidations [35, 46] and fixed air [35, 51]. These can oxidise all the component without many efforts so as to have better control of time and temperature [35]. Several examples for clearing the metal surface as well as to prepare the sample for a metal removal step. Firstly the mild oxidation with soluble oxidising agents in the wet environment, such as H₂SO₄ and H₂O₂, which

oxidise the defects along with clearing the metal surface [35]. Throughout these processes the metal catalyst stays together, the outer layer of the metal will be oxidised when oxygen is used in the wet atmosphere [35]. After that the density of surface increases and deposit of surface covering carbon ruptures. Now not only the carbon impurities are oxidised but the metal is also partially oxidised and exposed.

Acid treatment

Acid treatment: It removes the metal catalyst by exposing the metal surface to oxidation or sonication. Then the metal catalyst is exposed to acid and solvated so that the nanotubes remain in the suspended form. When HNO₃ is used for the treatment, it only affects the metal catalyst but not the nanotubes or other particles of carbonaceous nature [45, 48, 49]. With HCL, less effect on single-walled carbon nanotubes and other carbon particles were observed during reflux but unlike HNO₃, metal should be exposed to acid so that lysis of graphitic carbon and short fullerenes. The metal will be melted and can be removed by using vacuum having high-temperature [50, 53, 45, 46].

Annealing

Annealing: High temperature between 873K-1873K will result in the rearrangements of nanotubes with the consumption of defects resulting in the pyrolysis of graphitic carbon and short fullerenes. The metal will be melted and can be removed by using vacuum having high temperature [54, 53, 46].

Ultrasonication

Sonication is well-known as one of the effectual processes to get rid of the amorphous impurities adhering or binding to the walls of CNTs using suitable solvents [50, 55]. During sonication, the solvent molecules are able to interact with CNTs and hence lead to solubilization, which can improve purification efficiency [56]. In ultrasonication, vibration caused by sonic waves result in separation of particles and dispersion of nanoparticles agglomerates. Separation of the particles depends on the solvent, surfactant and reagent used. Stability of the dispersed tubes is influenced by the solvent, if the solvent is poor and attached to the metal SWCNT's are more stable, but solvent like alcohol, mono-dispersed particles are comparatively stable [39, 45, 49, 52, 54]. Ultrasonication in ethanol was adopted to remove the graphite particles [57]. The purity of the nanotubes depends on the exposure time. When an acid is used and if the exposure time is less, only metal get solvates but if the time is long, it will result in cutting down of the tubes [39, 46].

Cutting

The nanotubes can be shortened or cut by three steps, chemically, mechanically and the combination of both.

a) Chemically-Chemicals are used for cutting of CNTs. After partially functionalizing the CNTs followed by the pyrolysis in the form of CF_4 or COF_2 fluorated carbon will be driven off the sidewall which leaves behind the nanotubes those are chemically cut [48].

b) Mechanically: Due to high friction between the nanoparticles and the nanotubes the bonds will break and disordered and the cutting is caused by ball-milling [55].

c) The combination of both (chemically and mechanically): In an acid solution cutting of the nanotubes is ultrasonically induced, thus the ultrasonic vibration will provide the sufficient energy for the nanotubes to leave the catalyst surface [58]. After that, the nanotubes will rupture at the defect sites in combination with an acid.

Magnetic Purification

Magnetic Purification-Removal of ferromagnetic (catalytic) particles occurs mechanically from their graphitic shells. To get rid of these ferromagnetic particles in an ultrasonic bath the SWNTs suspension and inorganic nanoparticles (ZrO_2 or $CaCO_3$) were mixed together, followed by the trapping of particles by stable magnetic poles. Since SWNT of high purity will be obtained by chemical treatment [48] (fig. 12). Large equipment is not required, and it has been observed that the production of laboratory-sized quantities of SWNTs containing no magnetic impurities [59].

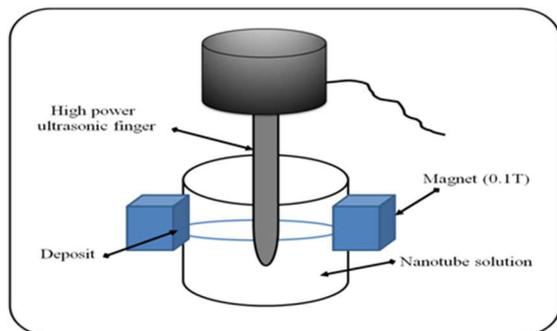


Fig. 12: Magnetic purification apparatus

Chromatography

To separate the CNTs in relation to small length and diameters the chromatography is mainly used. The carbon nanotubes with the

porous material are run through a column, through which CNTs will pass. The GPC (Gel Permeation Chromatography) and HPLC-SEC (High-Performance Liquid Chromatography-Size Exclusion Chromatography) columns are some examples which are used in the technique of separation. According to the size of molecules, they get separated with the large size of molecules eluting out first. The only requirement is that the nanotubes should be either solvated or dispersed by means like functionalization and ultrasonication [60].

Limitations and modification (functionalization)

Pristine CNT's(non-functionalized) is inherently hydrophobic, and readily aggregate in bundles due to van der Waals forces [61] and, therefore, the main barrier in the utilisation of CNT in biology and medicinal chemistry is their lack of solubility in most solvents compatible with the biological milieu (aqueous based). To defeat this problem the several ways existing, numerous strategies have been invented with different molecules is achieved by adsorption, electrostatic interaction or covalent bonding of different molecules and chemistries that make carbon nanotubes more hydrophilic and soluble [62]. The limitation in the applications of CNT's can be defeated, to some extent, by a process named functionalization [32]. Functionalization of CNTs can be achieved in two ways shown in (fig. 13).

1. Non-Covalent Functionalization-Various non-covalent interactions, for example, π stacking, hydrophobic and van der Waals interactions have allowed for the functionalization of CNTs with a wide range of molecules.

2. Covalent Functionalization-May be described as a chemical grafting of molecules onto the sp^2 carbon atoms of the π -conjugated skeleton of the CNTs. The basic reaction for CNT functionalization is oxidation [63], performed under strongly acidic conditions. There are two main strategies for covalently functionalizing nanotubes:

- End and defect modification and
- Sidewall modification.

These covalent modifications arise from the difference in reactivity at the nanotube ends and sidewalls (as well as at structurally perturbed areas) and, accordingly, each type of functionalization requires distinct chemical approaches [64-69].

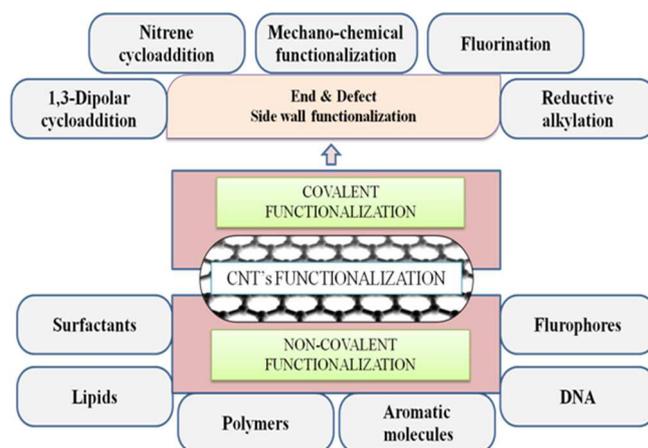


Fig. 13: Approaches for CNT functionalization

a) **Ends And Defects-** Ends of the CNT's are more reactive than the sidewalls. Treating the CNTs with oxidative agents results in introducing oxygenated groups, for instance, carboxylic acid, ketone, alcohol, and ester, at the nanotube ends and defect sites [70](fig. 14), thus leaving the ends open and possibly cutting and shortening CNTs [71]. Such treatments eliminate amorphous carbon and metal catalyst particles and can remove smaller diameter (more reactive) tubes. Techniques have been developed to probe the degree of oxidation and the type of oxygenated groups formed [72]. Oxidation also results in hole-doping of the CNTs causing disruption of its

electronic structure, [73] though the disruption is less pronounced than those produced in the case of sidewall modification, as discussed in (fig. 14).

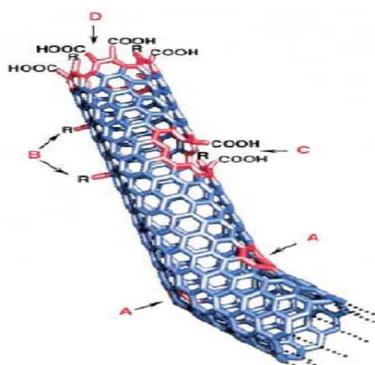


Fig. 14: Typical sites for ends and defect oxidation of MWCNT's. A) five- or seven-member rings instead of the normal hexagons, leads to a bend in the tube, B) sp³-hybridized defects such as R²H and OH, C) damage by oxidative conditions, D) open and oxidised ends

Other modifications of oxidised CNTs by non-covalent interactions have also been performed through zwitterionic interactions and

nonspecific protein binding [74], which have coupled CNTs to crown ether [75]. The common reactions employed for further covalent modification are summarised in (fig. 15).

b) Sidewall Functionalization-To introduce higher concentrations of covalently attached functional groups onto the surface of CNTs with the substitution of significant disruption in the electronic structure or the method used to break down the bonds present on the wall of CNTs which helps to attach the different functional groups. These types of reactions are summarised in (fig. 16).

Thus, functionalization heightens the scope of CNTs as raising nano vectors for the delivery of therapeutics [76]. Through surface functionalization, the water solubility of CNT is enhanced and their biocompatibility profile is completely transformed. Because CNT's are soluble in water, they were aggregate in the presence of salts, due to charge screening and therefore, cannot be directly used in biological applications due to the high salt content of most physiologic media. Hence, a further modification is needed, e. g., binding with hydrophilic polymers, such as polyethylene glycol (PEG) and forming CNT-polymer conjugates, which are stable in physiological environments [77]. The drawback of this method is that it results in the partial loss of electronic structure and optical properties of the CNTs and a loss of material, due to the oxidative process [78], [79]. On the other hand, these issues are of less importance in drug-delivery applications.

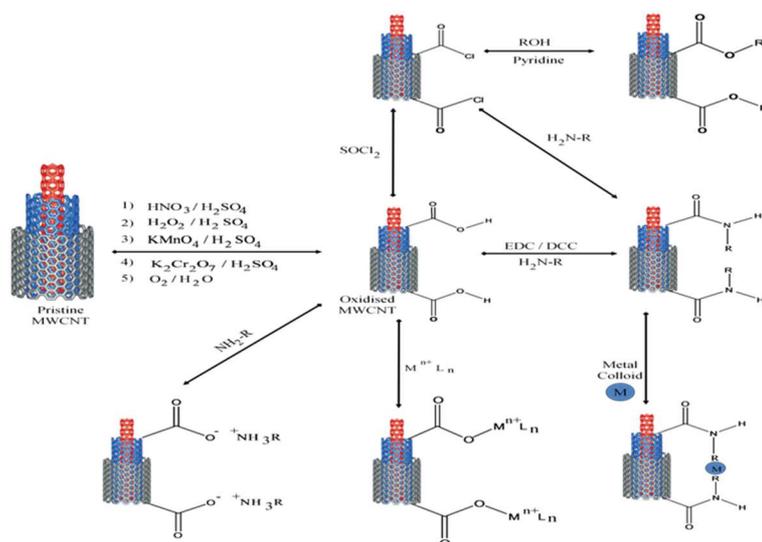


Fig. 15: Common strategies for covalent functionalization of CNT's via ends and defect site oxidation

Characterization

Carbon nanotubes are nanometric carbon particles, but it also contains many of the impurities. Characterization of CNTs headed for determination of the quantity, quality, and properties of the CNTs sample which is very important, because its applications will require certification of properties and function.

Electron microscopy (EM)

Electron microscopy (scanning and transmission electron microscopy) is an essential tool for characterising any nanomaterial it gives direct observation of size, shape, and structure [80]. Various techniques of electron microscopy, one can easily study on the CNT structures and identify their growth mechanism, which helps in modifying their structure and improves the growth process[15].

Scanning electron microscopy [81]

SEM images the sample morphology by scanning the surface with a high-energy beam of electrons. It is the first step to characterise the CNTs. Using SEM, the morphology of CNTs, their dimensions and their orientations can readily be seen [19], [27], [82]. Diameters of

CNTs also can be measured roughly with SEM [83]. Chains of single-walled nanotubes (SWNT's) in a sample, as well as highly oriented MWNT's, can be imaged by SEM using quartz or silicon substrates. Impurities like carbon-coated catalyst particles or amorphous carbon co-existing with nanotubes bundles and the tubular one-dimensional (1-D) structure of the MWNTs can also be determined more accurately using SEM as compare to other techniques [84].

Transmission electron microscopy [8]

The internal microstructure and crystal structure of samples which are thin enough to transmit electrons can be analysed with TEM. It is used to measure outer and inner radius and linear absorption coefficient for CNT studies [60], effects of nitrogen doping in MWNTs and SWNT peapod structures are a few to name, which can be investigated by TEM. It also identifies various layers of MWCNTs and separates SWNTs from the bundles. This stands for analysis of morphological structure and diameter measurement of carbon nanotubes [84]. The composition of catalyst detection responsible for nanotube nucleation enabled by TEM in conjunction with X-ray energy dispersive spectroscopy [84, 85]. Electron diffraction and electron energy loss spectroscopy (EELS) along with TEM has the

advantage that the average helicity and local variation of helicity in individual SWNT and ropes of SWNT's can be determined on the

nanoscale. EELS help researchers to find out the amount of dopant present in doped nanotube structure [86].

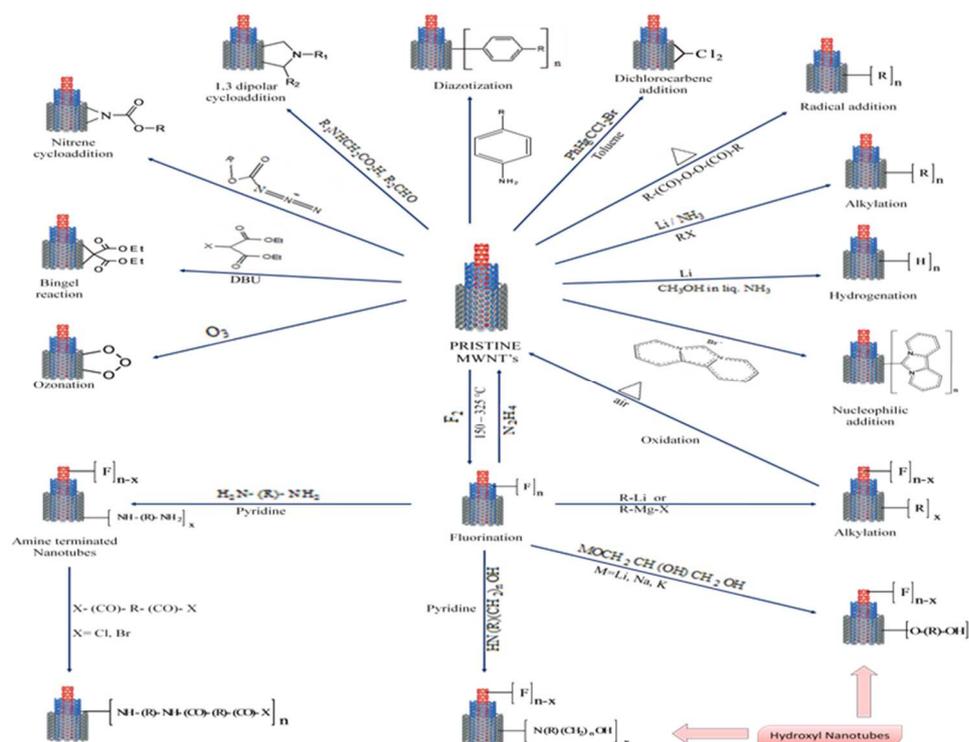


Fig. 16: Strategies for covalent sidewall functionalization of CNT's

Scanning tunnelling microscopy (STM) and atomic force microscopy (AFM)

These techniques provide morphologic information and properties (fundamental) of the nanotubes, which are vital in the research field of nanotubes. AFM is valuable in visualising isolated carbon nanotubes, which grows on the substrate of silicon by the process of either treating the nanotube bundles or chemical vapor deposition [31, 87, 88], [89]. Because of flexible nature and one-dimensional character of carbon nanotubes, they are used as AFM as well as STM tips as these properties help them to endure a crash into a sample with no/little damage to the tip, but only requirement for STM-nanotube tip is that the sample should essentially be grown on a conducting surface [90].

Raman spectroscopy

For the characterization of carbon-based materials, Raman's spectroscopy is an equally valuable method and is applied to study the properties and quality of metallic, superconducting and pristine phases of graphite intercalation compound and solids based on fullerene [60]-[91]. Inelastic light scattering from nanotubes cause's increase or decrease in an incident light energy because of emission or absorption of the photon thereby resulting in scattering known as Raman scattering [92]. This technique gives information with details about configuration of CNTs. A number of walls, the presence of crystalline and amorphous carbon and diameter of SWNT's can be determined with the Raman spectroscopy. Without sample preparation, a fast and nondestructive analysis is possible [60]. In this spectroscopy when a beam of light passes through a transparent sample of a chemical compound, a small part of the light emerges in different directions than the incoming beam. Most of these scattered lights are of unchanged wavelength, however, a small part has wavelengths different from the incident light, and its presence is a result of Raman Effect. The pattern of the Raman spectrum is characteristic for every molecular species and the intensity is proportional to the number of scattering molecules in the path of the

light. Resonance peaks are also observed in the spectrum, which symbolises the presence of a particular species type that is in abundance [15]. Basically, Raman scattering in nanotubes results from the inelastic scattering of light from the nanotubes, leading to an increase or decrease in the energy of the incident light due to an emission or absorption of a phonon present in the nanotube.

X-Ray diffraction (XRD)

This technique is used to obtain some information on the interlayer spacing, the structural strain, the impurities, SWNT bundles, several layers of MWNTs and allows morphological information for an unlimited number of crystals[84]. In X-ray diffraction system, electrons emitted from the filament (cathode) are accelerated to target (anode) and X-rays characteristics of atoms in the irradiated area are emitted. By analysing their energy, the atoms can be identified and by counting emitted X-rays number, the atom concentration in the specimen can be determined. X-ray diffraction (XRD) is mostly used for bulk structure analysis. However, if the concentration of the active component is large enough, it can be appropriate for size determination. Quantitatively, a number average size is obtained from the equation below [19, 60].

$$d = \frac{0.85\lambda}{(B^2 - b^2)^{1/2} = \cos \theta}$$

Where, b = value for a well-crystallized specimen, and λ = wavelength.

Thermal analysis (TGA/DTG)

The thermal analysis involves a dynamic phenomenological approach to the study of materials by observing the response of these materials to a change in temperature. Thermal analysis methods are useful for identifying relative changes due to processing. It is used to study the degree of CNT's purification [84]. Thermal gravimetric analysis (TGA) and derivative thermal gravimetric (DTG) analysis methods controlled oxidation process

that gives quantitative data on the weight fractions of carbon and metal catalyst in the sample, and the temperatures of bulk oxidation events [93].

Elemental analysis

The elemental analysis of raw-purified MWCNTs depicted carbon and hydrogen content in nanotubes. The purification of raw MWCNTs allowed the elimination of some catalyst impurities in oxidised form, and also raw MWCNTs could be functionalized with some oxygen-containing groups like-COOH groups and-OH groups that would be later confirmed by the elemental analysis [94].

Absorption spectroscopy (UV, Vis and IR)

Because of their unique electronic structure, CNTs and especially SWCNT have discrete optical absorptions that do not occur in other graphitic nanocarbon. Absorption Spectroscopic technique is very useful as a relative purity measurement of CNT. In the characterization of CNTs, IR spectroscopy is often used to determine impurities remaining from synthesis or molecules capped on the CNT surface. Numerous works are performed on organic molecules and CNTs: IR spectroscopy exhibits all the modification of the CNTs structure and reveals the nature of compounds added to the CNTs. The difference in catalytic activity performance between CNTs activated carbon and graphite samples in the oxidative dehydrogenation of ethylbenzene [14]. The highest catalytic activity

is obtained with MWCNTs which are oxidised before the catalytic experiments. The possibilities of the use of CNTs for catalytic removal of NO_x (NO, NO₂, NO₃) were also investigated by FT-IR [95]. For a correct characterization of CNTs, all these techniques described herein cannot be used individually but must be used in complementary ways.

Drug loading mechanism and cellular uptake of CNT's

Nanotubes are hydrophobic in nature and do not show wetting behaviour for most aqueous solvents. It is reported that various organic solvents, HNO₃, S, Cs, Rb, Se, and various oxides such as Bi₂O₃ can wet nanotubes. Nanotube provides capillary pressure proportional to (1/D). Therefore, these wetting agents can be driven to fill inside the nanotube by the capillary pressure.

It is also likely to fill non-wetting agents inside a nanotube by applying a pressure which is higher than the capillary pressure. An effective alternative is to use wetting agents such as HNO₃ to assist filling of non-wetting agents inside the nanotube [96] CNT's have very large surface area which allows multi-conjugation of various molecules on the sidewalls.

Molecules containing aromatic groups can be easily bound to CNTs non-covalently by strong π - π interactions. Thus, CNTs possess unique and excellent structural, optical and electrical properties for the development of advanced drug delivery systems [19] (fig. 17).

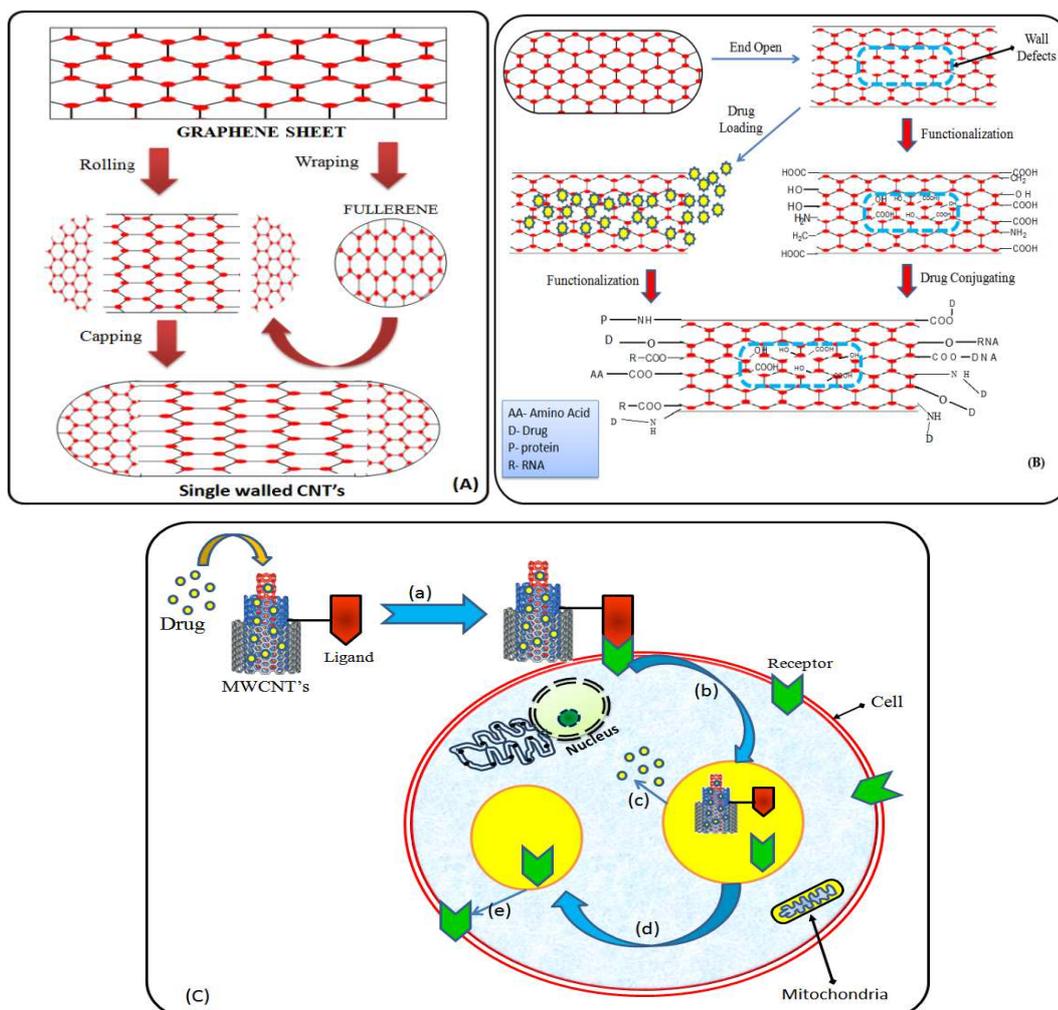


Fig. 17: Building of SWCNT's/MWCNT's and its physical and chemical treatment for use as drug carriers. (A) Demonstration of the structure formation of SWCNTs with the two ends closed. (B) The diagram of the strategy for the preparation of the CNT-based drug delivery systems. (C) Schematic representation of receptor-mediated endocytosis of CNTs. (a) Receptor association of ligand-conjugated drug-loaded CNTs, (b) endosomal uptake of conjugates, (c) release of drug at the targeted site, (d) formation of recycling vesicles and (e) receptor regeneration

Uptake OF CNT's BY cell

CNTs have been aimed and actively explored as multipurpose, innovative nano-carriers for drug-delivery systems. Thanks to their very high aspect ratio, CNTs can penetrate the cell membrane and be uptaken by cells. After entering the cell, CNTs are mainly located inside cell endosomes and lysosomes. Individualised CNTs are able to travel through various cellular barriers and even enter the nucleus [97]. The applicable cell-internalization mechanisms for CNTs are: Endocytosis-phagocytosis pathway. Endocytosis represents the engulfing of an extracellular particle by the cell (for example, viruses, ~ 100 nm in size) through the formation of a vesicle that is then integrated into the cell. Endocytosis is an energy-using process in which cells absorb molecules (proteins) by engulfing them. Endocytosis is mediated by formation of vesicles, so called endosomes, containing cell bound materials that segregate from the plasma membrane and get internalised.

Passive diffusion-Phagocytosis is similar to endocytosis, but usually involves an uptake of larger particles, such as bacteria (~1 μm). In the passive diffusion pathway, CNTs cross the lipid bilayer in a needle-like manner [98, 99]. The uptake mechanism of SWCNTs through phagocytosis can be enhanced by functionalization through conjugation with phospholipids. Raffa *et al.* reported the cellular

uptake of nanotubes within lysosomes and phagosomes of healthy and normal cells [100].

These two processes are energy-dependent and are hindered at low temperatures (fig. 18) Energy-dependent endocytosis was reported as the main internalisation mechanism of SWCNT's bound to various types of proteins [101]. In addition, the imaging of SWCNT's within the phagosomes and lysosomes of healthy cells also suggests uptake by phagocytosis [102]. Uptake by diffusion was reported for MWCNT's [103]. Here, shorter (i.e., <1 μm) MWCNT's were readily internalised by cells, while longer ones were not. Short CNTs can act as straight 'nano-needles', able to penetrate the cell membrane more efficiently than the longer CNTs, which are often arranged in a coiled or bundled shape, hindering their efficient uptake. Size, type, the chemistry of functionalization, charge and hydrophobic nature of nanotubes plays a major role in cellular uptake.[104] The f-CNTs having lengths around 100 nm are able to fit in Clathrin and caveolae vehicle, while CNTs with lengths more than 500 nm may probably be uptaken by the macropinocytosis process. Well, individualised MWCNTs with length around 300 to 400 nm can be easily cross the cell membrane and rapidly entered into the cells and freely traffic in cytoplasm within hr of post internalisation. The f-CNTs penetrate inside cells at 4 $^{\circ}\text{C}$. They may also penetrate into cells in presence of inhibitors of endocytosis i.e. sodium azide, 2, 4-dinitrophenol (DNP).

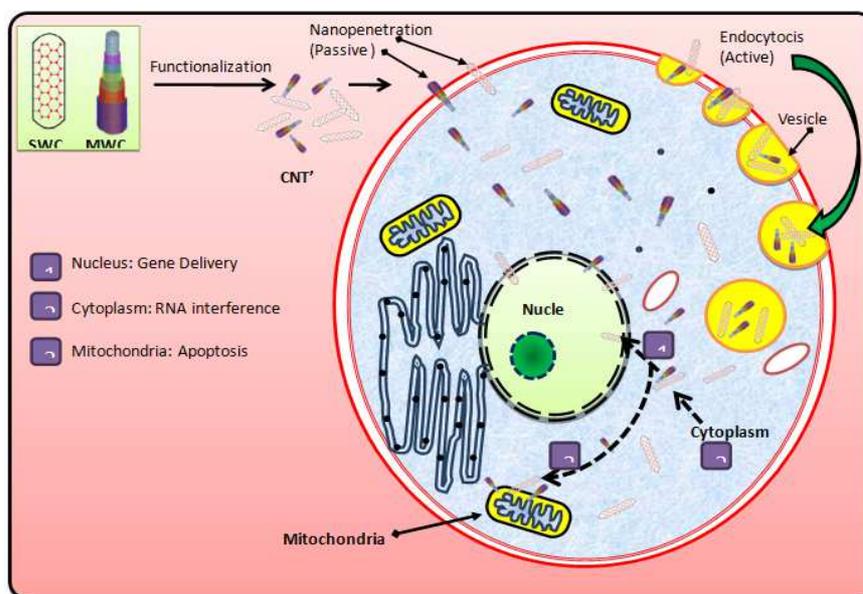


Fig. 18: Possible mechanisms of CNT and cell interactions showing receptor-mediated endocytosis or nano penetration, which is functionalization receptor dependent

Breakdown mechanism of cnts in the body

A group of Swedish and American scientists has shown for the first time that carbon nanotubes can be broken down by lysosomal enzyme-Myeloperoxidase (MPO)--an abundant enzyme of neutrophils granulocytes found in white blood cells and to a lesser extent also in certain macrophages [105]. Since, it clearly shows that endogenous MPO use to neutralise harmful bacteria and also found that the enzyme also works on carbon nanotubes, breaking them down into water and carbon dioxide. Shvedova *et al.*, (2012) [106] reported that, in simple biochemical model systems, MPO turned out to be effective in oxidative biodegradation of SWCNT via a plethora of intermediate aliphatic and aromatic products to ultimately yield carbon dioxide (CO_2) and water (H_2O) by a combination of two pathways associated with:

- Hypochlorite generated by the enzyme in the presence of chloride, and
- Reactive intermediates of the enzyme [107].

The researchers also showed that carbon nanotubes that have been broken down by MPO no longer give rise to inflammation in mice [108].

Toxicity OF CNT's

In cases where CNTs have a toxic interaction by cells, the mechanisms of toxicity are coming into focus. Results suggest CNTs may cause harm to cells by activating many pathways at once, mostly involving DNA damage [106, 108]. In one study, most cells incubated with CNTs halt at the G1 phase of the cell cycle [109]. Another study showed that mesothelial cells exposed to SWCNTs at concentrations ~ 25 $\mu\text{g}/\text{cm}^2$ activated DNA recovery along with changes in the cell cycle and generation of apoptotic signals. It was also observed that CNT/DNA interaction was the preferred route of toxicity in a 3-hour incubation study with 96 μg SWCNT/ cm^2 , which induced DNA damage (through micronucleus generation) in lung fibroblasts [110]. It should be possible, through the observation of specific toxic events that result from incubations with different types of f-CNTs, to test for functional groups that reduce the severity of such events.

The harmful effect of nanoparticles arises because of high surface area and intrinsic toxicity of the surface [111]. CNT, in the context of toxicology, can be classified as 'nanoparticles' due to their nanoscale

dimensions, therefore unexpected toxicological effects upon contact with biological systems may be induced.

The study of the adverse effects of nano-sized particles and fibres and their interaction in the living organism has been termed as "nanotoxicology". A nanosized particle could have the potential to cause the toxicity.

Certain properties determine the toxicity of nano-sized particles:

- The surface area/mass ratio of the particles; if the particle is having the larger surface area, it provides the greater contact with the cellular membrane, and as well as provides greater capacity for adsorption and transport of toxic substance.

- Retention of particles within a physiological environment; retention time determines the cellular contact and therefore causes the greater chances for damage. Retention time also determines its mobility either through clearance or migration to nearby tissue.

- Inherent toxicity of the contaminants present in nanomaterials.

The basic idea of nanomaterials' toxicity can be revealed by its lung deposition. The lung deposition of a nanosized material depends upon its surface area/mass ratio. C₆₀ fullerenes do not prove significant toxicity, it shows the speedy distribution in rates and deposition in many tissues like brain, liver and spleen [112, 113]. Studies showed the toxicity profile of CNTs are summarised in (table 4).

Table 4: Toxicity of CNT's

S. No.	CNTs description	Mode of administration/exposure condition	Toxicity/Comments	Ref
Lung toxicity				
1	Unrefined CNT's (Synthesised via Arc-Discharge Sublimation Method)	Intrathecal installation (dispersion in small amount of surfactants)	No measurable pulmonary dysfunction (as seen by either non-invasive procedure or BAL examination). Comments- Working with soot containing carbon nanotubes is unlikely to be associated with any health risk.	[35]
2	MWCNTs (produced by arc-discharge and CVD methods)	Intratracheal instillation (suspension in sterile saline with SDS)	A significant evidence of pulmonary toxicity. Alveolar Macrophage infiltration in BAL of all non-control animals. Multiple lesion was observed in CNT's exposed animals. Comments- Alveolar macrophage infiltration was absent in pyrograph MWCNTs. Exposure time was critical for induction of pathology.	[114]
4	Pristine laser SWCNTs	Intratracheal installation (in phosphate buffer saline solution with 1% Tween 80)	15% initial mortality was seen, the occurrence of only transient inflammation of SWCNTs and non-dose dependent tissue multimodal granulomas. Comments- Lung asphyxiation was attributed to the initial mortality. Multimodal granuloma was considered inconsistent with the lack of lung toxicity.	[115]
Skin toxicity				
5	CNT's synthesised via the arc discharge process	-h	No irritation in comparison to a CNT-free soot control Comments- 'No special precautions have to be taken while handling these carbon nanostructures'	[116]
Cytotoxicity				
6	Water dispersible SWCNTs	In human dermal fibroblast cell cultures	With an increase in the degree of sidewall functionalization, the SWCNT's became less cytotoxic. Cytotoxicity Low (on the basis of <i>in vitro</i> test)	[114]
7	Pristine-laser SWNT	Intratracheal instillation (Suspension in PBS With 1% Tween 80)	Exposure to the high dose produced mortality within 24 h post-instillation. Pulmonary inflammation with non-dose-dependent granulomas. Comments- Mechanical blockage of upper airways. Foreign tissue body reaction.	[63]
8	SWCNT cytotoxicity	SWCNTs inhibited human embryonic kidney (HEK 293) cells	By inducing cell apoptosis and decreasing cellular-adhesion ability.	
9	Utilising f-CNTs for gene therapy	On the delivery of Plasmid DNA	Low cytotoxicity and also suggested the use of the f-SWCNTs for intracellular delivery using the mammalian HeLa cell. Comments- The ammonium functionalized-CNT's was bound to plasmid DNA via electrostatic interaction and was internalised within the mammalian cell.	[116]
10	MWNT	Intratracheal instillation (Suspension in sterile 0.9% saline with 1% Tween 80)	Not ground MWNT accumulate in the airways, ground MWNT were cleared more rapidly. Both MWNT have induced inflammation (more marked for ground MWNT) and fibrotic reactions. Also, both have caused pulmonary lesions at 2 mo. Comments- Length appears to modulate clearance kinetics. Biopersistence, intrinsically toxic to the lung.	[117]
11	Carboxylated CNT's	Intraperitoneal administration	CNT accumulation in bone. Comments- Organ-specific accumulation of I. V. Administered nanotubes could be visualised. No signs of renal or other severe acute toxicity responses were observed.	[118]

Applications of CNT's

Treatment of central nervous system disorders

Central nervous system (CNS) disorders consist of neurodegenerative diseases and brain tumours. It is more difficult to diagnose and treat CNS disorders than any other diseases because of the unique and complex environment and the restricted anatomical access (blood brain barrier) of the CNS. Nanotechnology is promising to modernise the status quo in this field. Because of their tiny dimensions and accessible external or exterior modifications, nanomaterials are able to cross the blood-brain barrier by various targeting mechanisms and, thus, they can act as effective delivery carriers for targeting the brain. Both pristine and chemically functionalized CNTs have positive effects on neuronal growth, charge on the nanotubes could be manipulated to control neurite outgrowth [119], and CNTs functionalized with nerve growth factor or brain-derived neurotrophic factor could stimulate growth of neurons on the nanotube scaffold [120] proved that conventional tungsten and stainless steel wire electrodes coated with CNTs could enhance both recording and electrical stimulation of neurons in culture, rats and monkeys. CNT-coated electrodes are expected to improve current electrophysiological techniques and to promote the development of long-lasting brain-machine interfacing devices, both of which will aid the diagnosis and treatment of CNS disorders. The nanoparticle-based formulations of some chemotherapeutic agents (e. g. doxorubicin [121, 122] have the potential for the systemic chemotherapy of brain tumours with higher efficacy than the free agents. MWCNTs (p-MWCNTs or MWCNTs functionalized with DNA and siRNA) were found to be internalised by brain microglia (macrophage derived from migratory monocytes in the brain that are believed to be an effective target for brain cancer treatment) *in*

vitro and *in vivo* without inducing proliferative and cytokine changes [123]. It indicated that MWCNTs may possibly act as a safe nano-vector delivery system for immune therapies of brain cancers (e. g. gliomas) [124, 125].

CNT'S in delivery of bioactive

Bioactives are chemicals or chemical molecules that have some biological effect on our bodies. For example, Hydroxyapatite is a form of calcium phosphate having chemical resemblance with the mineral component of bones and teeth tissues. Tissue adhesion and bone growth get promoted and formed bone-like apatite layer which is biologically active. Thus, Hydroxyapatite classified as biocompatible and bioactive materials having biological applications, such as skeletal implants or dental and bone repair scaffolds [126]. Amphotericin B-loaded MWCNT employed as efficient nano-carrier for antileishmanial therapy [127], Gemcitabine-loaded smart CNTs for effective targeting to the cancer cell. Materials containing CNTs may, however, be strong enough to build space elevators, spacecraft, artificial muscles, sea and land vehicles. SWCNTs can conduct twice the electricity of copper, making these materials excellent electrical conductors, and may also be used to improve rechargeable batteries and fuel cell production, for instance. CNT's also have a distinctive electron-transport property and commonly in a manufactured material bulk sample 30% of the SWCNTs are conductors and 70% are semiconductors [128].

CNT's in cancer therapies

Cancer is a disease characterised by out-of-control cell growth. Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumours. CNTs are hopeful drug carriers in the target drug delivery systems for cancer therapies.

Table 5: Potential applications of CNT's; *in vitro* and *in vivo* evaluation

Functionalized CNT's	Cell lines	Bioactives	Evaluation parameters	Outcomes of the study	Ref
PL-PEG-FA and PL-PEG-FITC-SWCNT's.	Hela cells.	Cy3-labeled DNA	<ul style="list-style-type: none"> Atomic force microscopy. Confocal laser scanning microscopy. 	SWCNTs internalised in living cells can act as tiny NIR "heaters" or "antennas".	[131]
PL-PEG-SWCNT's	CXCR4 cell-surface receptors in human T cells and PBMC cells	siRNA	<ul style="list-style-type: none"> Proliferation and Cytotoxicity assay. Fluorescence analysis 	SWNTs can be used as molecular transporters for human T cells and primary cells, with superior silencing effects over conventional liposome-based non-viral agents.	[132]
MWCNT's-g-PCA and MWCNT's-g-PCA-PTX	A549 and SKOV3 cell lines	Paclitaxel	<ul style="list-style-type: none"> Cell line studies Electron microscopy Atomic force microscopy 	Conjugation of paclitaxel to MWNT-g-PCA is an effective delivery system for cancer chemotherapy.	[133]
Carboxylated MWCNT's (C-MWCNT's).	--	Epirubicin HCl via π - π stacking.	<ul style="list-style-type: none"> Adsorption efficiency <i>In vitro</i> release 	The amount of EPI released from the C-MWCNT was 30.65% after 6 h in the acidic solution, which is almost 1.5 times larger than that in the neutral solution.	[134]
DOX-SWCNT-CHI-FA and DOX-SWCNT	<i>In vitro</i> release studies	Doxorubicin	<ul style="list-style-type: none"> Electron microscopy FTIR spectroscopy 	High drug loading capacity ~ 91% and showed in controlled and sustained release pattern of the developed formulations	[135]
Amb/Mannose-MWCNT's	J774 cells	Amphotericin B (Amb)	<ul style="list-style-type: none"> CLSM <i>In vitro</i> and <i>in vivo</i> studies. Macrophage uptake study. 	Amb tubes showed better targeting efficiency to macrophages i.e. J 774 cell lines with reduced toxic effects associated with Amb.	[136]
DOX/FA/CHI/SWNT's	SMMC-772 Hepatocellular carcinoma	Doxorubicin	<ul style="list-style-type: none"> Microplate reader (Model 680, Bio-Rad) <i>In vitro</i> cell culture study <i>In vivo</i> studies Light microscopy Flow cytometry 	The therapeutic efficiency of DOX/FA/CHI/SWNT's was time-dependent and dosage-dependent. Tumour volumes reduced.	[137]
Biotin-SWNT's or MWNT's	Hela cells and MCF-7 cancer cell lines	Paclitaxel	<ul style="list-style-type: none"> Flow cytometry 	Formation of a stable microtubule-taxoid complex and finally caused apoptosis and cell death	[138]
PAMAM dendrimersFA-treated MWNT's	Hela cell	Doxorubicin	<ul style="list-style-type: none"> Flow cytometry Confocal microscopy 	Targeting of FA receptors overexpress cancer cells	[139]
SWCNT's Polysaccharide [sodium alginate (ALG) and Chitosan (CHI)]	Hela cells	Doxorubicin	<ul style="list-style-type: none"> Magnetic field 	Modified CNTs reduced side-effects as well as increase therapeutic amount of the drug in patient	[139]

In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice for cancer therapy [77]. Tumor-targeted drug delivery system is also possible via covalent conjugation of the specific ligand to oxidised SWCNT. Studies performed *in vitro* demonstrated a rapid decrease of tumour size in comparison to non-targeted SWCNT thus ensuring maximum drug efficiency with minimum side effects [10, 129]. The potential application of CNT's in drug delivery has been compiled in table 5 with special reference to *in vitro* and *in vivo* evaluation.

Many functionalized carbon nanotubes may determine useful applications in the area of materials science and technology, including photovoltaics. Also in medicinal chemistry carbon nanotubes are set to act an important role. They utilise as drug delivery scaffolds and substrates for vaccines. CNT functionalized through bioactive moieties are particularly fitted for targeted drug delivery. In fact, not only they get less toxic but also display a high tendency to cross cell membranes [130]. Some of the examples of active moieties are doxorubicin-loaded SWNTs, paclitaxel-CNT[28], dexamethasone mesylate-loaded MWCNTs [62]. Molecular and ionic migration takes place by carbon nanotubes, thus offering novel opportunities designed for molecular sensors and electronic nucleic acid sequencing. The variation of a carbon nanotube on a molecular level via biological molecules is effectively an example of the 'bottom-up' fabrication principle of bio-nanotechnology.

Medical applications

CNT's bound to an antibody, which is developed by chickens, have been shown to be useful in laboratory tests to demolish breast cancer tumours. The antibody containing nanotubes is attracted to proteins produced by one type of breast cancer cell. Then the nanotubes absorb light from an infrared laser, incinerating the nanotube and attached to the tumour. Using nanotubes as a cellular scale needle to convey quantum dots and proteins in cancer cells- Improve the healing process for broken bones by putting up a carbon nanotube scaffold for new bone material to grow on [140].

An additional application of CNT's in medicine is for sensing the molecules or species. Many studies on the electrochemical reactivity of carbon nanotubes showed that carbon nanotubes can enhance the biomolecules and promote the transfer of electron in proteins (heam containing proteins). In heam containing proteins, carbon nanotubes are able to access the heamcenter of biomolecules that is generally not sensed by the glass electrodes. CNT's can also be utilised as blood vessels in order to deliver drugs to their target. When the drug delivery is done that way, the drug dosage can be lowered (and it's cheaper for the pharmaceutical companies). There are two methods, both equally effective-

- a) The drug can be attached to the side or behind, or
- b) The drug can actually be placed inside the nanotube.

Synthetic muscles—due to their high contraction/extension ratio given an electric current CNT's are ideal for synthetic muscle.

Artificial muscles-CNT's have enough contractility to make them candidates to replace muscle tissue [14].

Nanotubes function like a needle at the cellular level. This property is used in attaching molecules that are attracted to cancer cells to nanotubes to deliver drugs directly to diseased cells. The attachment of ethylene glycol molecules to nanoparticles of nanotubes stops WBCs from recognising the nanoparticles as foreign materials, allowing them to circulate in the blood streams long enough to attach in cancer tumour therapy. Magnetic fields drive drug-loaded nanoparticles to reduce blood vessel blockages in an animal study. Functionalized CNT's used as emerging nano vectors for the delivery of therapeutics. Functionalization of SWCNT's enhances solubility and allow for efficient tumor-targeting drug delivery. It prevents SWCNT's from being cytotoxic and altering the function of immune cells [141].

CONCLUSION

CNTs are discussed and described in this review from their discovery to present day in the requisites of historical background,

structure, properties, growth mechanism, synthesis, purification, characterization methods, cellular uptake of CNT's, breakdown mechanism, toxicity and its biomedical applications. Carbon nanotubes may have only recently caught the attention of the world but many modernizations have been made since their discovery. They are unique nanostructures that display the desirable properties of any other known material. Production methods include many approaches which change types, yields, and structural surfaces of CNTs, which results in the change in the electrical and mechanical properties, and the actual structure of CNTs. Useful techniques for CNTs characterization are used are Electron Microscope, XRD etc. But the most powerful technique to characterise CNT's remains the Raman spectroscopy in which without sample preparation, a fast and nondestructive analysis is possible. Significant advances have been made in the delivery of anticancer and anti-inflammatory drugs, and bioactive molecules i.e. DNA, RNA and proteins. Drugs and biomolecules can be loaded in CNTs, which can then be utilised as targeted molecules. The toxicity of pristine CNT's is still a major concern based on the highly conflicting results obtained by various researchers. However, functionalized CNTs have been considered biocompatible and safe for drug and biomolecule delivery applications as they are soluble in physiological media and non-toxic. They have shown no accumulation in the tissues; conversely, once functionalized, they can be readily excreted through the renal route by means of degradation through myeloperoxidase (MOP) enzyme. However, CNT-based delivery systems are undoubtedly very promising in terms of their numerous advantages over the existing technologies.

CONFLICT OF INTERESTS

Authors hereby declare that there are no conflicts

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