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Formulating nanomedicines: Focus on carbon nanotubes as novel nanoexcipients

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Abstract

Many recently designed drug delivery systems have been constructed from nano-sized components that serve as the carrier or targeting ligand for a therapeutic agent. Even though these materials have been regarded previously as inert or non-active components of dosage forms, they are now recognized as sometimes being even more important than the drug itself. Hence, it is becoming increasingly imperative that the pharmaceutically relevant properties, including identity, physicochemical characteristics, purity, solubility and toxicity, of these functional nano-excipients be fully characterized. Carbon nanotubes (CNTs) are novel nanomaterials made of carbon atoms that have wide application potential in many areas of nanomedicine. However, because of their significant potential, CNTs, as building blocks for nanomedicines, need to be characterized more fully. Studies to date indicate that both physical and chemical properties of CNTs play an important role in their interactions with cells. Therefore, a full understanding of the physical properties of CNTs, such as identity, chirality, particle size, aspect ratio, morphology and dispersion state, as well as chemical properties such as purity, defect sites and types and functional groups, will be essential to develop a full characterization panel of these versatile nanomaterials.

Introduction

Nanomedicine is now recognized as a field within medical and pharmaceutical science that focuses on the application of nanotechnology in the treatment of patients. According to the NIH's definition, "Nanomedicine, an offshoot of nanotechnology, refers to highly specific medical intervention at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle, or nerve. A nanometer is one-billionth of a meter, too small to be seen with a conventional lab microscope. It is at

this size scale — about 100 nanometers or less — that biological molecules and structures inside living cells operate." (http://nihroadmap.nih.gov/nanomedicine/; accessed Dec 1, 2009).

When a drug substance is combined with a delivery system it produces a dosage form that becomes the basis of a new drug product. Over the past 30 to 40 years, drug delivery systems have become gradually more sophisticated and smaller. In addition to the general trend in reduction of size, other parameters, such as their specificity of interaction with cells and tissues and *in vivo* biocompatibility, have become important parameters. Many new building blocks for novel delivery systems have emerged and continue to emerge. These building blocks are nano-sized materials ranging from soft materials such as lipids, amino acids, dendrites, and polymers, to metallic nanoparticles composed of gold, silver, iron and other metals forming quantum dots, to the various nanomaterials composed of carbon.

Pharmaceutical excipients have been used typically as non-functional building blocks for drug products. The United States Pharmacopoeia (USP) defines these excipients as "any substance other than the active drug product which has been appropriately evaluated for safety and is included in a drug delivery system to either i) aid processing of the system during manufacture or ii) protect, support or enhance stability, bioavailability, or patient acceptability or iii) assist in product identification or iv) enhance any other attribute of the overall safety and effectiveness of the drug product during storage and use." The USP has described the requirements for 550 excipients in monographs; however, many more excipients currently used in pharmaceutical products are awaiting the development of such monographs.

More recently, refinement in the understanding of functionality of excipients has also become important. The functionality-related characteristics (FCRs) for a specific excipient will be those that are relevant to the dosage form or the product being developed. The new General Information Chapter on Excipient Performance <1059> will facilitate new excipient characterization and the development of additional guidelines.

2. Carbon nanotubes as pharmaceutical excipients

Carbon nanotubes (CNTs) are a novel class of carbon-based nanomaterials, with potential as future excipients for dosage form and drug delivery system development [1, 2]. CNTs are cylindrical molecules consisting of a hexagonal arrangement of sp^2-hybridized carbon atoms (C-C distance of ~1.4 Å). These hollow tubes of carbon are formed by rolling single or multiple layers of graphene sheets into seamless cylindrical structures. The most common forms of CNTs are single-walled carbon nanotubes (SWNTs) [3] and multi-walled carbon nanotubes (MWNTs) [4]. SWNTs are comprised of a single cylindrical graphene layer and MWNTs are comprised of several to tens of concentric cylinders of graphitic shells. MWNTs generally have a larger outer diameter (2.5-100 nm) than

SWNTs (0.6-2.4 nm) and consist of a number of concentric SWNT layers, with an interlayer separation of ~0.34 nm. Fig. 1 shows a variety of CNT structures, as well as other similar carbon-based nanostructures that have been employed in drug delivery studies either *in vitro* or *in vivo*.

Images of carbon nanotubes were first published by Radushkevich and Lukyanovich in the *Soviet Journal of Physical Chemistry* in 1952 (http://carbon.phys.msu.ru/publications/1952-radushkevich-lukyanovich.pdf; accessed Dec 1, 2009), but it was not until 1991, when Iijima's paper showing electron microscopic images of CNTs was published [4], that recognition of their potential piqued the interest of the scientific community. SWNTs were also independently discovered in early 1993 by scientists at IBM's Almaden Research Center and at NEC in Japan.

CNTs have attracted wide attention because of their considerable versatility and desirable physical and chemical properties [5-7]. Depending on the direction in which the graphene sheet is rolled, the resulting CNTs may have armchair, zigzag or other chiralities, resulting in different electrical properties, where the armchair types are metallic and the zigzag types are semiconducting. Nanotubes, whether single-, double-, or multi-walled, have high aspect ratios, with diameters typically in the range of 1-20 nm and lengths up to several mm. Other characteristics include ultra-light weight, high thermal conductivity, and unusual mechanical and electronic properties. Each CNT can be considered a single macromolecule formed from a number of carbon atoms in a specific structure. CNTs are produced as powders or as solid aligned tubes on a substrate from which they are scraped off to form a bulk black powder. The appearance of the CNT powder depends on the process used to synthesize them, as well as on the type of nanotubes. Table 1 shows four different types of CNT bulk materials, ranging from a light fluffy powder to a dense granular material. The physicochemical parameters shown are those provided by their respective manufacturers. The corresponding high-resolution transmission electron microscopic (TEM) images were obtained by dispersing the respective nanotubes in 1,2-dichlorobenzene and viewing them on holey 300-mesh grids using a JEOL 2010F electron microscope (detailed study in manuscript in preparation).

Individual, pristine carbon fibers can become entangled with other fibers, creating ropes that are difficult to disperse. Entanglement is particularly important to overcome, as dispersion or solubilization of CNTs in aqueous solutions is necessary for their application as drug delivery systems. Generally, pharmaceutically relevant properties of CNTs may include molecular state such as size, type, aspect ratio, and the presence and type of functional groups (Table 2). Full pharmaceutical characterization of CNTs will require a set of relevant assays that reflect the desired properties for human applications. The establishment of 'pharmaceutical grade' CNTs may require detailed and defined structural information; determination of the presence and type of defects; data on electronic properties, concentration, dispersion state; identification of the type of impurities or contaminating materials present; level of required purity and limits for impurities. Additionally, the development and

validation or modification of existing analytical methods and what constitutes pure CNT standards will be necessary.

3. Carbon nanotubes as building blocks for drug delivery systems

The versatility of CNTs can be controlled and optimized for bionanotechnological use in many ways (Fig. 2). Generally, both unfunctionalized and functionalized nanotubes may be employed to design delivery systems. Analysis of available data can be used to determine which properties of CNTs are particularly important to control in order to achieve both safety and efficacy, to what degree can general requirements be imposed and which will be specific properties that will need to be evaluated case-by-case.

3.1. CNT size, type and structure-related properties

To evaluate CNTs for drug delivery potential and biocompatibility, the first parameter of interest usually is particle size. Both length and diameter of nanotubes appear to be critical for function and avoidance of adverse effects [8]. Strano et al. [9, 10] conducted single-particle tracking experiments with NIH-3T3 cells. SWNTs (synthesized using a high-pressure CO conversion (HiPCO) process) suspended in 2% sodium cholate by probe-tip sonication (starting concentration 100 mg/L), centrifuged at 21,000g for 2 h, were separated by length using density gradient ultracentrifugation, resulting in four different average lengths: 660 nm, 430 nm, 320 nm, and 130 nm. Then, the surfactant was removed by dialysis and replaced by DNA, sonicated, ultracentrifuged at 100,000g for 4 h and the pellet discarded. The concentration used was 5 mg/L. CNT cellular uptake was by endocytosis, and an exocytotic process was also observed, where the rates of these two processes were comparable. CNT uptake was length-dependent, where 320 nm CNTs had the highest uptake. The endocytosis rate constant of SWNTs (10^{-3} min^{-1}) was nearly 1000 times that of Au nanoparticles, whereas the exocytosis rate constants were similar in magnitude (10^{-4} to 10^{-3} min^{-1}) for poly(D,L-lactide-co-glycolide), SWNT, and Au nanoparticles across several distinct cell lines.

The knowledge of *in vivo* fate, effect and clearance of CNTs from the body is critical for optimization of CNTs for therapeutic purposes. Studies in animals indicate that size, aggregation state and targeting group functionalization are major contributors to biodistribution and clearance of nanotubes.

Biodistribution studies of ^{125}I-labelled SWNTs (1.5 μg dose in 100 μL; 300 nm long and 1.4 nm diameter) in mice after intraperitoneal, intravenous, subcutaneous or oral gavage administration indicated that these nanotubes were distributed to all organs similarly as small molecules are typically distributed [11]. The brain was the only exception, where minimal uptake was seen in all cases. Excretion was mainly through urine. This biodistribution and excretion pattern was confirmed by

others [12, 13]. SWNTs and MWNTs functionalized with the chelating molecule diethylentriaminepentaacetic (DTPA) and labeled with ^{111}In was evaluated in mice after IV injection [12]. The main excretion pathway was through the kidneys in the urine and no accumulation in the liver was observed. Clearance from the blood was rapid, with a t½ =3 h. Lacerda et al. [13] also found that renal clearance of CNTs occurs 5 min after intravenous (tail vein) injection. However, while individualized MWNTs were observed crossing the renal filtration membrane, bundled MWNT aggregates were deposited in the lumen of the glomerular capillary.

Intraperitoneal injection of 50 μg doses of dispersed non-functionalized MWNTs [14] raised the possibility of long nanotubes causing mesothelioma, thus their having an asbestos-like carcinogenic effect. The four MWNTs evaluated were 1-5, 5-20, 40-50 and 20-100 μm long nanotubes with corresponding average diameters of 14.84, 10.40, 84.89, 165.02 nm. The pathogenic effects were attributable to the long fibers in the latter two groups of MWNTs, whereas the short fibers did not cause any inflammation or granuloma response. In another study, the effect of the diameter of the MWNTs on cellular toxicity in alveolar macrophages was investigated [15]. The cytotoxicity of MWNTs with diameters of 10-20, 40-60, and 60-100 nm (all with lengths of 1-5 μm) increased with increasing diameter.

Nanotube defects may also be common in CNT raw materials. During purification of CNTs, strong acid treatment oxidative reactions form carboxyl residues at the ends or at the sides of the nanotubes. Defects may vary from loss or addition of a carbon atom, creating a vacancy or a double coordinated atom and resulting in the formation of five- or seven-membered carbon rings [16, 17]. The five- or seven-membered rings in the carbon framework, instead of the normal six-membered ring, may lead to a bend in the tube, and the carbon framework damaged by oxidative conditions may create a hole lined with COOH groups. The combination of two five- and two seven-membered rings is called the Stone-Wales (7-5-5-7) defect on the sidewall of a nanotube [16] and covalent bonds between the layers of a MWNT [18] may also form. It is not clear, however, whether the presence or the extent of defects on the nanotubes has an effect on their biological function or toxicity.

3.2.Carbonaceous and non-carbonaceous impurities, solvent residues

CNT samples contain two categories of impurities: metallic catalytic impurities used for in the synthesis of CNTs, and carbonaceous impurities. Typically, CNTs purchased from vendors are accompanied by product information sheets containing quality analysis of the accompanying material. Some vendors provide very limited information in the form of a Raman spectrum and/or an electron micrograph (either by SEM or TEM) and elemental analysis for metal content. Others provide more extensive information (see Table 1). The assignment of >90% or greater purity may mean that the sample contains >90% carbonaceous content but not necessarily >90% nanotube content. Poland et al.

[14] evaluated the physicochemical properties of MWNTs from four different sources. Their analysis indicated discrepancies in nanotube dimensions and purity compared with data provided by the companies that synthesized them.

Most nanotube raw materials produced by the chemical vapour deposition (CVD) synthetic process contains metallic impurities; therefore, it is difficult to show lack of toxicity of CNTs synthesized by this method. Miyawaki et al [19] conducted a toxicity study using single-wall carbon nanohorns (SWNH), a cone-shaped type of carbon nanomaterial (see Fig. 1) produced by laser ablation, a synthesis method that does not result in contamination with undesirable metal impurities. Here, the SWNHs were shown to be non-toxic in a panel of rigorous tests, including Ames test, chromosomal aberration test, skin primary irritation and eye irritation tests, and acute peroral and intratracheal toxicity test. They also found no mutagenic, clastogenic or carcinogenic effects. The lethal oral dose in rats was greater than 2000 mg/kg, and very limited lung tissue damage was seen.

Porter et al. [20] used SWNTs (0.9-1.2 nm diameter, CVD-HiPCO process) dispersed in tetrahydrofuran (THF) for incubation with human monocyte-derived macrophages at 0-10 μg/mL. CNTs were located in the lysosomes and then in the nuclei by day 2. By day 4 apoptosis and necrosis of cells were noticeable at 5 μg/mL CNT concentration. CNT cellular uptake was by phagocytosis and by crossing the cellular and nuclear membranes by passive diffusion. The nanotube dispersion in THF was reported to contain iron catalyst particles and onion-like structures (graphitic carbon), all of which, including the solvent, could have contributed to cellular toxicity. In a subsequent study the same authors showed that with acid-purified SWNTs dispersed in aqueous media no significant changes were observed in cell viability or structure even after 4 days of exposure [21]. Additionally, purified SWNTs were less aggregated within cells compared with unpurified SWNTs, although both bundles and individual acid-treated SWNTs could be found inside lysosomes and the cytoplasm.

The presence of metals in CNTs may contribute to oxidative stress and significant toxicity. In early studies toxic effects attributed to CNTs were in fact due to the presence of metallic impurities [22, 23]. However, the extent of effect of metallic residues is still unclear. In a recent comparative study high purity MWNTs (less than 0.0005% Fe) were more toxic to human macrophages than the Fe_2O_3 catalyst itself [24].

The importance of the availability of detailed and accurate information on the CNT raw materials is key to understanding the toxic effects of nanotubes and is strongly recognized in the field as data essential for their characterization and development as nano-excipients. [25-27].

3.3. CNT surface properties

CNTs lend themselves to a range of chemical modifications [28-31]. Both covalent and non-covalent functionalizations are possible at intact CNT sidewalls, at defect sites on sidewalls or at the tip of the nanotubes. The most common modification is the formation of carboxyl residues upon strong acid treatment, followed by esterification or amidization of carboxyl groups and the 1,3-dipolar cycloaddition of azomethine ylides [7]. Some of the functionalization strategies involve the attachment of biomolecules (such as protein or DNA), and fluorescent, radioactive or electron-dense markers and targeting moieties [32-35].

Several research groups showed decreased cellular toxicity of functionalized CNTs. Kostarelos et al. [36] investigated the effect of seven different functionalized SWNTs and MWNTs in six different cell lines, as well as in yeast and *E. coli*. Both types of CNTs, regardless of the functional groups, were intracellularly localized in the perinuclear region after a non-energy dependent uptake process. Pristine nanotubes obtained from commercial sources were functionalized through an oxidative process and used at 0.5-5 μg/mL.

Cheng et al. [37] reported cell penetration of FITC-PEG-SWNTs and their nuclear accumulation in six different cell lines. SWNTs were purified in-house at 300°C using nitric acid, followed by washing, drying and functionalizing with PEG. FITC-PEG-SWNTs accumulated in the nucleus, mainly in the nucleoli of HeLa cells, U2OS cells and MEF cells. Signals from PEGylated SWNTs coincided with the nuclei. A difference in distributions of FITC-PEG-SWNTs between the nucleolus and the nucleoplasm was also noticeable. The cell uptake was by an energy-dependent process and the bidirectional uptake (i.e. a parallel exocytotic process) was also observed for these PEG-functionalized CNTs.

Specific targeting of nanotubes *in vivo* has been demonstrated [38, 39]. Liu *et al.* demonstrated effective targeting of SWNT-PEG_{5400}-RGD functionalized nanotubes (0.05 mg/mL) to integrin $\alpha_v\beta_3$-positive U87MG tumor in mice. The nanotubes were 100-300 nm long and the diameter was 1-5 nm. The presence of PEG (PEG 5400 provided longer circulation time than PEG2000; t½ = 2 h vs 0.5 h) was important for stealth function and the RGD peptide was important for targeting.

SWNTs conjugated to epidermal growth factor (EGF) and cisplatin selectively killed head and neck squamous carcinoma cells that were overexpressing EGF receptors [38]. SWNTs (0.5 mg/mL, made by HiPCO process by Carbon Nanotech) were oxidized by acid treatment and the resulting carboxyl groups were functionalized with EGF and Quantum dots using EDC chemistry. The final concentration used was 0.25 mg/mL SWNT with 1.3 μM bound cisplatin. Analysis indicated the presence of 36 EGF molecules per 100 nm length of nanotube. The nanotubes were short, 110±50 nm estimated from electron microscopic images, and the diameter was 10 nm, indicative of small bundles rather than individual SWNTs [38].

In the case of functionalized nanotubes, the nature of the functional groups on the nanotubes influences the interaction between nanotubes and cells. The presence of targeting ligands can provide selectivity for binding target cells. Interestingly, CNTs having different non-specific functional groups or different electrostatic charges (e.g., an FITC label or a small drug molecule) are internalized by cells similarly to each other [36]. There are potentially three cellular uptake mechanisms that may govern CNT internalization into cells: energy-dependent endocytotic and energy –independent diffusion and bidirectional uptake processes, however, it is not clear what combination of nanotube characteristics are the most important in each case. Further studies will be needed to better understand overall CNT structure/function versus uptake mechanism relationships

3.4. Dispersibility

The solubilization of pristine CNTs in aqueous solvents is difficult because of the hydrophobicity of the graphene sidewalls and the strong π-π interactions between individual tubes.

Although unfunctionalized CNTs are typically very hydrophobic and insoluble in aqueous media, as a solid or a dispersion in a polymer matrix without the need for solubilization they have several potential applications. Venkatesan et al. [40, 41] incorporated erythropoietin (EPO) into a CNT matrix, which was measured out by weight. Intra-jejunal administration of this EPO (100U/kg)–CNT-labrasol solid dispersion increased the bioavailability of EPO. Unfortunately, the type and purity of CNTs in this study were not available. Short CNTs were two times more efficient compared with the long tubes, and serum EPO level reached a c_{max} of 143.1+/-15.2 mIU/mL. Unfunctionalized nanotubes, in the form of CNT bundles, can also be used as a support matrix for neural cell growth. Neurons grown on CNT bundles survive for several days and increase dendrite outgrowth [42, 43]. Electrical coupling of SWNTs and neurons was demonstrated using NG108 and rat primary peripheral neurons [44].

Another example using unfunctionalized nanotubes is by Cai et al. [45] who reported the use of CNTs as spears to deliver plasmid DNA encoding green fluorescent protein (EGFP) into Bal 17 B-lymphoma and B cells. Vertically aligned nanotubes grown by plasma-enhanced CVD with ferromagnetic nickel particles embedded at the tips were used as carriers and were driven into cells membranes under the influence of a magnetic force. This technique resulted in high transduction efficiency and viability after transduction. [33]. The potential of magnetofection delivery includes a drastic lowering of vector dose since close to 100% of cells were reported to express EGFP protein [45].

CNTs can also be used to create nanocomposite materials for medical device development. Pristine CNTs mixed with Nylon-12 form a nanocomposite material that can be extruded to form microcatheters for arterial cannulation. The biocompatibility of such CNT-based microcatheters was

greater, and less cellular infiltration and no inflammatory reaction occurred, compared with a Nylon-only microcatheter [46].

Most other applications of CNTs require dispersion, dissolution or debundling of nanotubes to individual fibers. The preparation of CNT dispersions with uniform distribution of individual nanotubes is still a significant challenge. To successfully disperse CNTs in aqueous media, the medium should be capable of both wetting the hydrophobic tube surfaces and modifying the tube surfaces to decrease tube aggregation. Generally, four approaches have been used to obtain a CNT dispersion: (1) functionalization of CNT sidewalls [47-54]; (2) surfactant-assisted dispersion [55-61]; (3) solvent dispersion [62, 63]; and (4) biomolecular dispersion (e.g., single-stranded DNA wrapping around CNTs) [64-76].

CNT solubility varies depending on the source of the nanotubes (i.e. method of production), the solvent and the dispersion procedure. Typical maximum concentrations of CNTs in solvents and surfactant solutions are in the range of 0.001 – 0.2 mg/mL (Table 3). Encapsulation of SWNTs into poly(styrene)-block-polyacrylic acid copolymer increased solubility to 0.5 mg/mL [77]. A recent report showed significantly increased dissolution of SWNTs in chlorosulfonic acid at 5 mg/mL [78].

The aqueous solubility of functionalized CNTs is dramatically improved compared with the solubility of non-functionalized nanotubes. For example, PEG1500-SWNTs prepared by thermal reaction had aqueous solubility greater than 300 mg/mL [79].

However, despite the abundance of literature regarding CNT dispersion, there are no standard methods of evaluating the rate and extent of dispersion. Ultraviolet spectroscopy has been used to evaluate the dispersion efficiency of CNTs and the colloidal stability of the resultant dispersion. It has been used to monitor the exfoliation of both SWNTs [59, 80-83] and MWNTs [82, 84-86] in surfactant solutions. However, even though UV spectroscopy has been used for in-study comparison of the efficiency of exfoliation of CNTs (Table 2), the determination of absolute concentration of individually exfoliated CNTs in solution between laboratories is difficult because of the lack of reference standards. Microscopy techniques such as TEM, scanning electron microscopy (SEM) and atomic force microscopy (AFM) are suited for qualitative CNT size and purity analysis, whereas the quantitative methods to estimate dispersion in solution include UV-Vis absorbance [59, 65, 68, 74, 87-90], resonant Raman scattering [61, 74, 82, 88, 90-93], and small angle scattering [60, 88, 94-97]. These techniques are best used in combination to confirm the state of dispersion [98]. Table 2 lists the most frequently used analytical methods to characterize various physicochemical parameters of CNTs.

3.5. CNT concentration

Many studies have indicated concentration-dependent toxicity of CNTs in cell culture [99-102] However, the CNTs assessed were from different sources (with limited information on purity) and were purified and functionalized using different methods. The resulting final CNT solutions typically had no specific analysis of concentration, but probably were calculated based on the known starting amount of CNT powder. Since the starting material contains various levels of impurities and during purification significant amount of CNT aggregates may be removed, the exact concentration may be unknown. Although it is understandable that accurate concentrations may be difficult to obtain because of the lack of available CNT reference standards, this may indicate that reported values of concentrations in most papers are approximate.

UV spectroscopy is currently used by many laboratories as a simple technique to determine CNT concentration. Absorbance at selected wavelengths can be used to calculate nanotube concentration. From a standard curve the molar extinction coefficient (ε) can be measured and used to determine CNT concentration from absorbance values of unknown samples. Kam et al. [103] reported an ε value for a DNA-aided SWNT dispersion supernatant at 808 nm to be 7.9×10^6 $M^{-1}cm^{-1}$ (molecular mass about 170 kDa for the 150 nm long SWNT with diameter of about 1.2 nm). The dispersion supernatant was obtained by sonication of the original dispersion (250 mg/L SWNT; HiPCO) for 45 min and centrifugation at 22,000g for 6 h, after which the pellet was discarded. The SWNT concentration in the solution was estimated to be 25 mg/L (10% of the original).

Several authors routinely use UV absorbance at 500 nm to determine CNT concentration [67, 98, 104]. Rastogi et al. studied the effect of surfactants on the degree of dispersion of MWNTs, by monitoring absorbance at 500 nm and using $\varepsilon = 28.6$ cm^2mg^{-1} from [67], to calculate the concentration of solubilized CNTs. Ikeda et al. [67], working with aqueous solutions of SWNTs, adopted the ε_{500} from Bahr et al. [104], based on the standard curve of concentration of SWNTs in 1,2-dichlorobenzene versus optical density at 500 nm. The use of one ε value for different CNTs in different solvents without a specific calibration curve, or using calibration curves prepared from same CNT dispersion for which concentration is to be determined, introduces unreliable data, and creates confusion in interpretation of different data sets.

Therefore, even though the use of UV spectroscopy would allow routine analysis of CNT concentration, the lack of CNT standard(s) makes it difficult to conduct reliable UV analyses of different types of nanotubes in different solvents.

4. Potential methods for purification or production of CNTs with more homogeneous properties

Since the number of variables and the distribution range of parameter values in current CNT materials are too large to allow determination of the specific parameter of interest versus effect relationships, it is important to obtain CNTs with more narrow composition and characteristics profiles. For example, there is a need to separate bundled nanotubes from individual nanotubes, metallic nanotubes from semiconducting ones, populations of nanotubes with different lengths and diameters, and nanotubes with different chiralities.

The separation of nanotube bundles from individually dispersed nanotubes is relatively easy using centrifugation. Increasing centrifugal force causes smaller and smaller size aggregates to form a sediment, until only individual nanotubes remain in solution. Metallic and semiconducting nanotube separation may be achieved by density gradient ultracentrifugation [105], and certain aromatic polymers are suitable to separate semiconducting tubes by employing differential solubilization [106, 107].

Tu et al. [108] recently described the purification of CNTs with different chiralities. Twenty short DNA sequences were identified from a DNA library, each of which was capable of binding to specific (n,m) chiralities in a mixture of CNTs. For example, the sequence $(TCC)_{10}$ is highly specific for SWNT (9,1) and $(GTC)_2GT$ for SWNT (9,4). Typical yields are approximately 0.1-0.8%, thus the scale-up of the method is not economically feasible. Other methods for enrichment of specific types of nanotubes may be achieved by density gradient ultracentrifugation. Arnold et al [109] used this method to sort nanotubes by electric structure. Wei et al. [110] used cosurfactant extraction to selectively enrich (6,5) and (8,3) nanotubes. Another strategy is the growth, also termed ‘cloning’, of nanotubes, with specific chiralities from ‘templates’ or ‘seeds’ with the desired chirality [111, 112].

Selective synthesis of nanotubes with specific chiralities and narrow diameter distribution was achieved by the use of bimetallic catalysts. FeRu catalyst produced (6,5) SWNTs [113], whereas CoMo catalyst produced narrow (n,m) distribution when using different carbon precursors [114].

5. Nanoparticle classifications

Recognition of the rapidly growing number of nanomaterials and nanostructures and the lack of systematic classifications has led to recent proposals on nomenclature systems and systematic naming, as well as the development of deeper relational connections of periodic properties for nanoparticles. These recent ideas will be important for a clearer interpretation of results obtained when using nanomaterials. Gentleman and Chen [115] proposed a nomenclature system for seven categories of particles with a range of morphologies. In this system each nanoparticle system would have a code to describe its basic properties. The code would contain five typographic fields, such as the chemical

class, size and shape, core chemistry, ligand chemistry and solubility. For example, for a 7-nm diameter MWNT the code would be 1FN-7RL-0-[(Ful,Ful)]-O, where FN indicates a fullerene, 7RL indicates the diameter and the shape being elongated, 0 stand for no core, Ful, Ful indicates sheet structure and O indicates logD>1, i.e., its hydrophobic properties.

Tomalia recently proposed a systematic framework for a nanoperiodic system, in recognition of the need for predicting important risk-benefit boundaries. The system proposes extensive nanoperiodicity classifications for size, shape, surface chemistry, elemental composition, flexibility, and architecture of hard and soft nanoparticles. These so-called Critical Nanoscale Design Parameters (CNDP) [116] determine corresponding intrinsic physicochemical properties, such as viscosity, density and refractive index, leading to systematic and predictable behavior.

6. Summary

In the pharmaceutical field particle characterization has always been an integral part of formulation development for solid as well as dispersion dosage forms. However, nano-sized particles present new challenges, since small changes in their parameters can cause dramatic differences in properties, biocompatibility and pharmacokinetics [117]. These general considerations are applicable to CNTs as well. Evidence thus far supports the hypothesis that the size (i.e., length and diameter), solubility and surface characteristics including functional groups play a significant role in the biocompatibility and biological fate of CNTs. However, many other characteristics will also need to be determined and correlated with drug delivery functions and adverse effects.

Overall, the following is a list in two categories — i.e., individual particles and bulk material — of the most important parameters that are relevant for drug delivery and risk assessment of CNTs. Individual particle properties include size, surface area, shape, surface charge, surface coatings, stability and structure. Bulk properties include purity, size distribution, solubility and the medium. Full characterization of these properties will provide information toward establishing CNTs as pharmaceutical excipients.

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Table 1 Characteristics of selected SWNTs and MWNTs

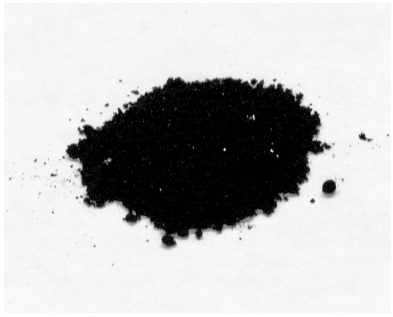
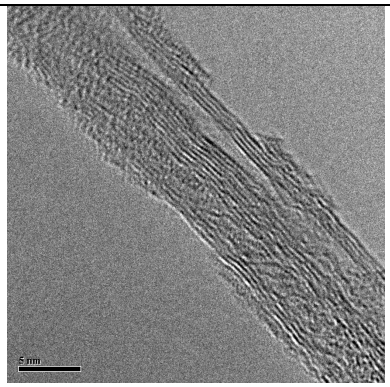
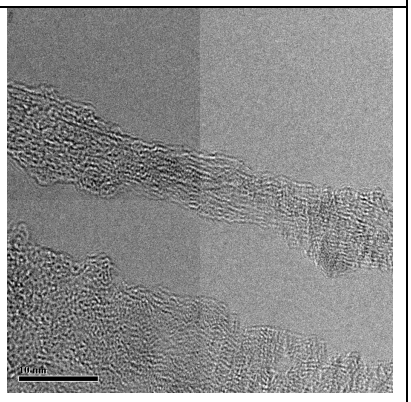
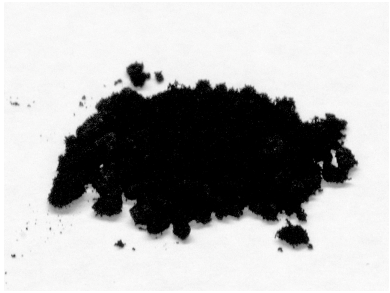
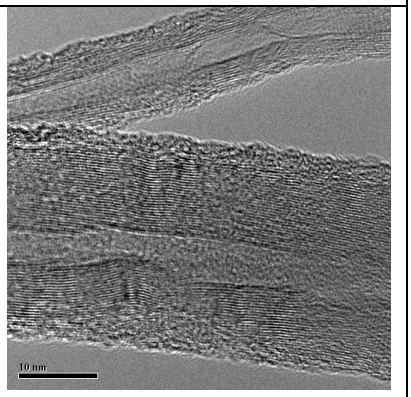
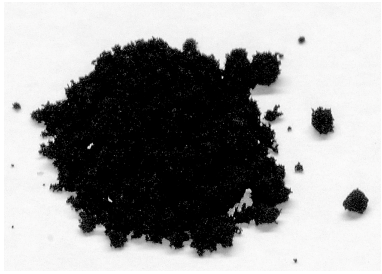
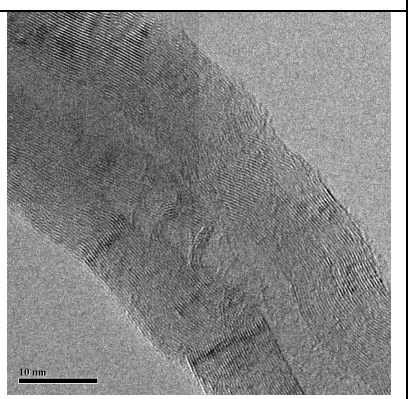
CNT type	Macroscopic photograph	TEM
SWNT Source: Cheaptubes Manufacturing method: CCVD OD: 1-2 nm; ID: 0.8-1.6 nm Length: 5-30 μm Purity: >90wt% Ash: < 1.5 wt% Additional MWNT content: >5wt% Amorphous carbon content: <3wt% Specific surface area: 407 m^2/g Electrical conductivity: >100 S/cm Bulk density: 0.14 g/cm3 True density: ~2.1 g/cm3		
SWNT-Superpure Source: Unidym Manufacturing method: HiPCO Diameter: 0.8-1.2 nm Length: 100-1000 nm % weight iron: 3.5 (certified value) Ash: < 5 wt% Maximum surface area: 1315 m^2/g BET surface area: 400-1000 m^2/g Bucky paper resistance: 0.2-2 Ω Bulk density: 0.1 g/cm^3 Maximum density: 1.6 g/cm^3		
MWNT Source: Cheaptubes Manufacturing method: CCVD OD: 8-15 nm; ID: 3-5 nm Length: 10-50 μm Purity: >95 wt% Ash: < 1.5 wt% Specific surface area: 233 m^2/g Electrical conductivity: >100 S/cm Bulk density: 0.15 g/cm^3 True density: ~2.1 g/cm^3		
MWNT Source: Cheaptubes Manufacturing method: CCVD OD: 50-80 nm; ID: 5-10 nm Length: 10-20 μm Purity: >95 wt% Ash: < 1.5 wt% Specific surface area: 60 m^2/g Electrical conductivity: >100 S/cm Bulk density: 0.18 g/cm^3 True density: ~2.1 g/cm^3		

Table 2 Pharmaceutically relevant properties of CNTs recognized as important in establishing quality requirements and corresponding analysis methods

Parameters	**Analytical Method**
Type of nanotube (single-wall, double- or multiple-wall or other morphology)	TEM, Raman spectroscopy
Size and related measurements (diameter, length, aspect ratio)	TEM, AFM, PL spectroscopy, Raman spectroscopy
Chirality (conducting, semiconducting)	PL spectroscopy, electron diffraction
Specific surface area	Brunauer-Emmett-Teller (BET) analysis
Aggregation state	TEM, AFM, analytical ultracentrifugation [118]
Purity (metallic and carbonaceous impurities)	TEM, EDX, thermogravimetric method [26]
Structural defects	TEM, electrochemical method [119]
Concentration	UV-Vis-NIR spectroscopy

Table 3 Dispersibility of CNTs in selected solvents and surfactants

CNT type	Dispersing agent	Solubility*	Reference
SWNT (gas-phase catalytic process)	1,2-dichlorobenzene Chloroform N-methylpyrrolidone Dimethylformamide Ethanol	95 μg/mL 31 μg/mL 10 μg/mL 7.2 μg/mL < 1 μg/mL	[104]
SWNT (HiPCO)	Encapsulation in poly(styrene)-block-polyacrylic acid copolymer	0.5 mg/mL	[77]
SWNT (HiPCO)	2% SDS 2% CTAB 2% Pluronic F98	9.9 μg/mL 15.3 μg/mL 28.2 μg/mL	[120]
SWNT (HiPCO)	0.1% SDS (in D_2O) 0.25% SDS (in D_2O) 0.5% SDS (in D_2O)	7 μg/mL 9 μg/mL 12 mg/L	[60]
SWNT (pulse laser vaporization)	N,N-dimethylacetamide	6.25 μg/mL	[121]
SWNT (arc discharge)	N,N-dimethylacetamide	40.97 μg/mL	[122]
SWNT (raw HiPCO)	0.1% NaDDBS (1:10 SWNT-surf ratio) 1% SDS (1:10) 0.2% CTAT (1:2) 0.5% CTAB (1:5)	0.65 mg/mL 0.45mg/mL 0.3 mg/mL 0.4 mg/mL	[123]
SWNT (HiPCO)	N-methyl-2-pyrrolidone	20 μg/mL^	[124]
SWNT (HiPCO)	γ-butyrolactone N-methyl pyrrolidone	9 μg/mL 68 μg/mL	[125]
SWNT (HiPCO)	Chlorosulphonic acid	5 mg/mL	[78]
MWNT (CVD)	0.1% Pluronic F127	180 μg/mL#	[126]
MWNT (in-house)& (shortened, acid treated)	Water	13 μg/mL	[98]

***** as determined and reported by the respective research groups
^ at 4 μg/mL 70% of all dispersed particles are individual nanotubes
# starting concentration: 0.5 mg/mL MWNT
& catalytic pyrolysis of propylene on a Fe/Mo/Al2O3 catalyst in a nano-agglomerate fluidized bed reactor

Figure legend

Figure 1. Computer-generated images of carbon nanostructures using Nanotube Modeler© 2005 from JCrystalSoft, showing the structures of (A) spherical C_{60} fullerene (Buckyball structure); (B) a single graphene sheet depicting the orientation of the graphene hexagons from which a (9,0) zig-zag, tube could be rolled out; (C) conical structure of a carbon nanohorn; (D) SWNT nanotube bundle; (E) four examples for open-ended, defect-free single-walled carbon nanotubes (SWNT): (9,0) 'zigzag' semiconducting, (9,1) and (9,4) chiral and (9,9) 'armchair' metallic; (F) double-walled carbon nanotube; (G) multi-walled carbon nanotube (MWNT) structure.

Figure 2. Potential applications of CNTs.

Figure 1

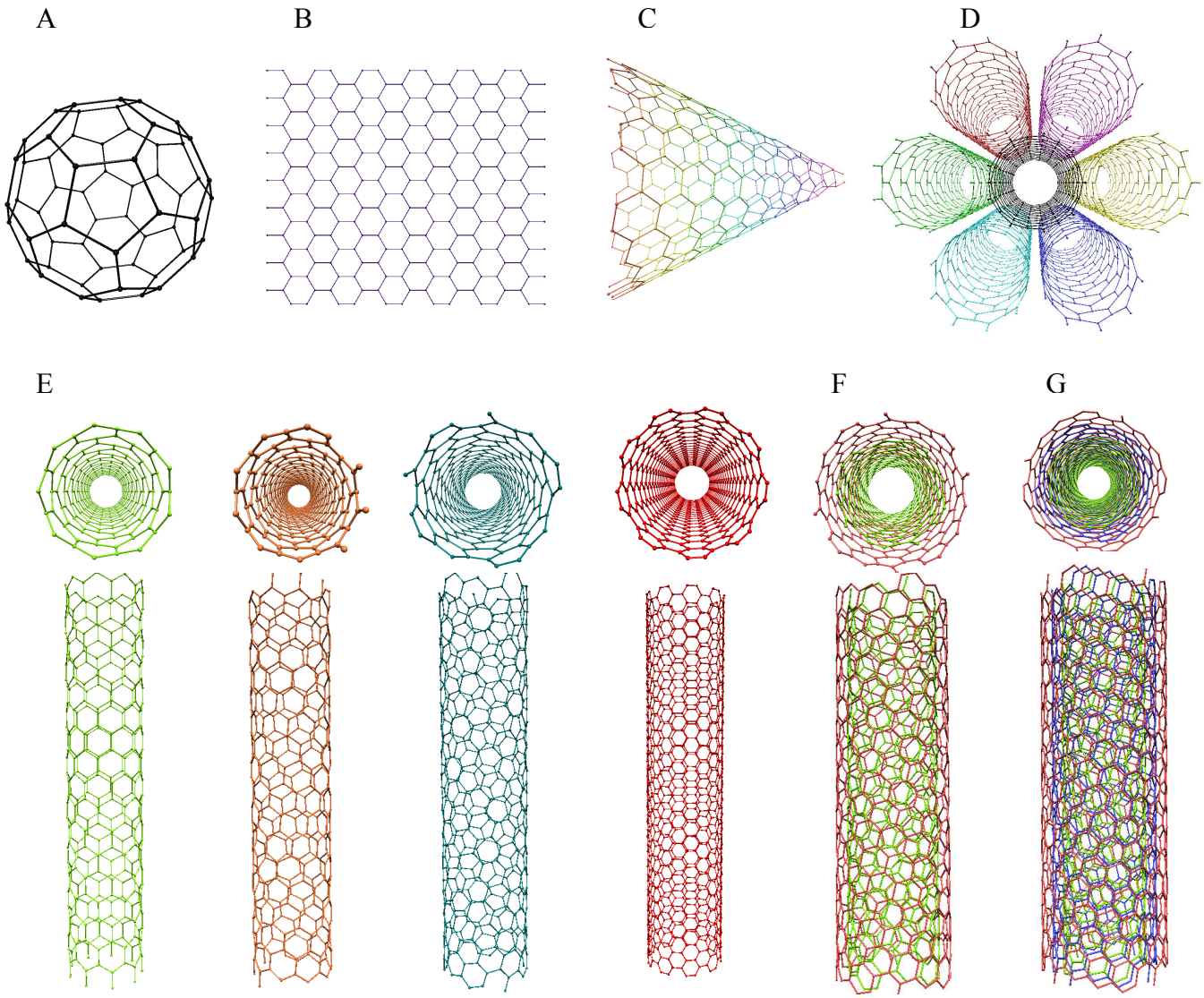
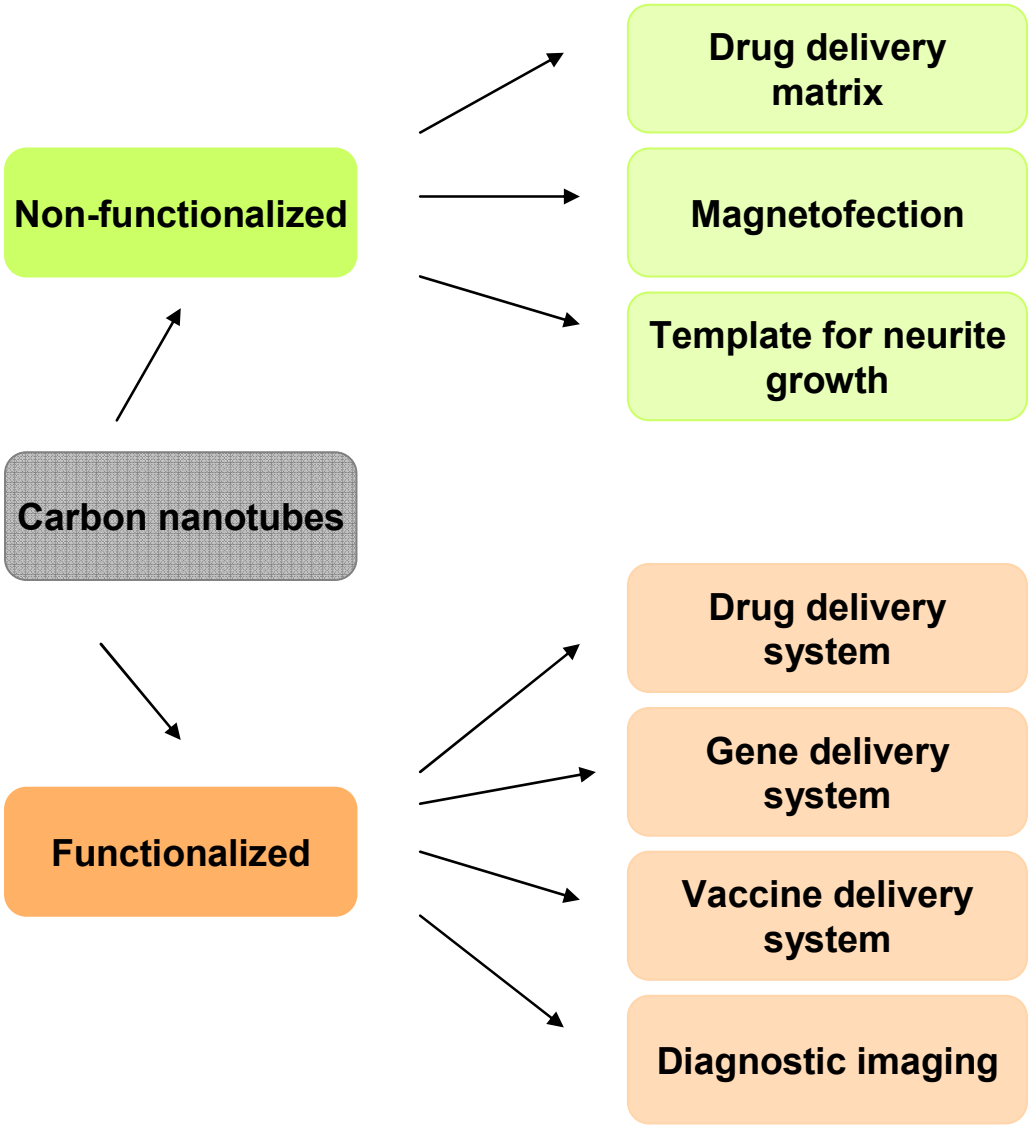


Figure 2


Drug delivery matrix
Non-functionalized
Magnetofection
Template for neurite growth
Carbon nanotubes
Drug delivery system
Gene delivery system
Functionalized
Vaccine delivery system
Diagnostic imaging

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