From Functionalization to Characterization: Investigating Tryptophan-Functionalized Carbon Nanotubes and Unveiling its Properties

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Abstract

Nanobiotechnology is a combination of nanotechnology and biotechnology that suggests new methods to produce lighter and stronger materials widely used in biology and medicine. Carbon-based nanomaterials are one of the tools of nanobiotechnology, and we can mention carbon nanotubes (CNTs) as one of the most important of them. They are classified into multi-walled (MWCNTs) and single-walled (SWCNTs) CNTs categories. As CNTs are insoluble in water and are toxic for cells, we can functionalize them with various agents to improve their performance. In this study we functionalized MWCNTs with tryptophan an essential amino acid with unique properties and potential applications in biomedicine and nanotechnology. Then we use scanning electron microscope (SEM) and FT-IR analyses to ensure in functionalization of MWCNTs. Results from these analyses show that functionalization of MWCNTs have be done correctly. Functionalization of MWCNTs makes lower their insolubility and toxicity and improve their performance in cell environment. Now they can be used in biological and medical applications like drug delivery, biosensing, tissue engineering and so on.

Keywords: carbon nanotubes, functionalization, nanobiotechnoiogy, multi-walled carbon nanotubes

1. Introduction

The prefix "nano" originates from the Greek word for "dwarf," with one nanometer (nm) representing one-billionth of a meter—in other words, the width of six carbon atoms or ten water molecules. For context, a human hair measures about 7000 nm in width. Although individual atoms are smaller than 1 nm, many molecules, such as proteins, have sizes from 1 nm and larger [1]. The National Nanotechnology Initiative (NNI) describes nanotechnology as research that covers atomic and molecular scales below 100 nm, leading to createon of new structures and devices with special properties. This technology can help to create stronger, lighter materials and has a significant effect on biology and led to the emergence of a new science named "nanobiotechnology" [2].

Nanobiotechnology combines the fields of biotechnology and nanotechnology, merging molecular biological techniques with traditional nanotechnology. Biotechnology uses biological knowledge and methods to manipulate genetic, molecular, and cellular processes to develop products and services, influencing various sectors from agriculture to medicine [3]. The potential of this combination is considerable; the junction of nanotechnology, biotechnology, and information technology exposed many significant applications in life sciences. It is predicted that this combination will yield a new generation of multifunctional devices and systems for biological and chemical analysis, defer by increasing sensitivity, specificity, and faster recognition speed compared to existing solutions [4].

Techniques in nanobiotechnology may improve our understanding of disease mechanisms, leading to better biomarkers, drug delivery systems and so on. Nanobiotechnology will profoundly impact on disease prevention, diagnosis, and treatment [5]. To achieve the goals of nanobiotechnology, such as genetics and molecular manipulations in living cells, tools at the nano level must be used. Among these nanotools that play a central role in analytical applications, we can mention nanotubes, nanochannels, nanoparticles, nanopores, nanocapacitors, and nanofibers [4]. These Nanomaterials can also be categorized into three primary groups: metallic particles, organic particles, and hybrids of both [6].

Organic nanoparticles are nanosized materials ranging from 1 to 100 nanometers in diameter and are composed of organic materials, such as polymers, proteins, lipids, and other carbon-associated compounds. Organic nanoparticles are used in various applications, like biomedical applications, environmental applications, electronics, catalysis and so on [7]. The study of carbon-based nanomaterials (CBNs) attracted much interest in biomedical applications because of their unique properties [8]. CBNs typically include fullerenes, graphene, carbon nanofibers, carbon quantum dots (CQDs) and carbon nanotubes (CNTs) [9]. CBNs are a new class of materials with unique properties such as small size, a high ratio of surface to volume, and special strengths, which are widely used in purification, analytical chemistry and sensing, microbial fuel cell, renewable energies, energy storage, antimicrobial and other biomedical applications [10-12].

Since the discovery of CBNs, carbon nanotubes (CNTs) have been studied and it has been found that they have huge potential applications in biology and medicine [13]. Carbon nanotubes are hollow nanostructures composed only of carbon, known as all-carbon nanomaterials. The two types of CNTs are single-walled carbon nanotubes (SWCNTs), diameters ranging from 0.4–2 nm, and multi-walled carbon nanotubes (MWCNTs), in the range of 2–100 nm. The structure of a carbon nanotube could be imagined by rolling up the graphene sheet, where the central axis of the rolled-up graphene has a planar-hexagonal arrangement of carbon atoms distributed in a honeycomb lattice [14]. Further, the bond between the adjacent hexagonal rings is sp^2, which enables CNTs to possess the so-called delocalized pi-electron system in a direction. Owing to the sp^2 hybridization crystallographic structure in the axial and radial directions, CNTs manifest remarkable mechanical, thermal, electrical and chemical stability. CNTs are known to exhibit ultra-lightweight and high-strength behavior due to these unique structural and chemical properties which make them useful in biomedical and environmental applications [15].

In addition, CNTs can be functionalized with biological molecules, such as proteins and nucleic acids to form bioconjugate carbon nanotubes. These bioconjugation methods have allowed for creating complex molecules from simple ones to create precisely designed molecules. This has led to the development of inorganic nanoparticle-bioconjugates that have special properties and improved functions. [16]. CNTs can also be used to deliver a variety of therapeutic agents, including biomolecules, to the target disease sites. In addition, their unparalleled optical and electrical properties make them excellent candidates for bioimaging and other biomedical applications [17]. CNTs are relatively flexible and interact with the cell membranes and penetrate various biological tissues due to a "snaking effect". Hence both the pharmacological and toxicological profiles of CNTs have gathered much attention recently [17]

Despite CNTs potential in biological and biomedical applications, their poor solubility in water and high toxicity of them has been a significant hurdle. Moreover, the outer wall of pristine CNTs is, in principle, conceived as chemically inert. One of the most promising routes to overcome this difficulty is to functionalize CNTs [16-18]. The functionalization of CNTs improves their solubility and biocompatibility and alters their cellular interaction pathways, resulting in much reduced cytotoxicity in biological systems for biomedical applications [19]. The ability to customize their surface properties and modify them with specific molecules enhances their utility in various applications [16,18].

There are wide variety of noncovalent and covalent methods for CNT functionalization, which can increase their efficiency in biomedicine and other applications [20]. A range of cargo molecules [21], including fluorescently labeled streptavidin, DNA, small interfering RNA (siRNA) [22], an anticancer drug [23], bioactive peptides [24] and amino acids [25], have been loaded onto carbon nanotubes (CNTs) using either covalent or noncovalent bonds and successfully delivered into living cells [20].

Functionalization techniques are categorized into two main types: direct attachment and use of Nanotube-Bound carboxylic acids [26]. The first method involves attaching functional groups directly to the graphitic surface of CNTs. Methods that we can mention in this category include fluorination [27,28], hydrogenation via Birch reduction [29], and various reactions with anilines [30], nitrenes, and radicals [31]. The second method utilizes carboxylic acids that arise from intrinsic or induced defects in the nanotubes. These acids can facilitate the attachment of long alkyl chains through amide linkages [32], ionic interactions [33] or esterification [34]. Esterification of these carboxylic acids is also a means to functionalize and solubilize CNTs, allowing for easy recovery through hydrolysis [34].

In vitro and in vivo studies indicate that functionalized carbon nanotubes (f-CNTs) hold great potential for creating innovative delivery systems for anticancer drugs [35]. Lay et al. (2011) showed that CNTs functionalization with polyethylene glycol can increase the dispersity in aqueous solution and biocompatibility of CNTs. They loaded several types of anticancer drugs, such as paclitaxel and doxorubicin, onto f-CNTs and investigated their treatment efficacy *in vitro* and *in vivo*. They concluded that f-CNTs can be platform materials for anticancer drug delivery with improved efficacy. [35].

It has also been shown in some studies that the functionalization of carbon nanotubes reduces their toxic effect on cells [36]. Zhang et al. (2011) investigate and compare the cytotoxic effects of single-walled carbon nanotubes (SWCNTs) and polyethylene glycol-functionalized SWCNTs (SWCNT-PEGs) on neuronal PC12 cells, examining biochemical, cellular, and gene expression levels in different concentration of these nanoparticles. Their results showed that SWCNTs induced cytotoxicity in a concentration-dependent manner, while SWCNT-PEGs demonstrated lower cytotoxicity compared to pristine SWCNTs. They suggested that the functionalization of SWCNTs with PEG reduces ROS-mediated toxicological responses in vitro [37].

The toxic effect of carbon nanotubes is not always undesirable and we can sometimes take advantage of it. Holghoomi et al. (2021) also observed that the use of pristine and functionalized carbon nanotubes in different concentrations in the culture medium of *Ocimum basilicum* L., increases the production of antioxidant enzymes in the plant and leads to an increase in the production of secondary metabolites in it [25].

Scientists also predict that carbon nanotubes can be used as gene delivery device. Pantarotto et al. (2004) showed that carbon nanotubes that have been functionalized and have a positive electric charge, are soluble in water and easily pass into the cell. They suggested that these nanotubes can transport plasmid DNA by the formation of noncovalent DNA–nanotube complexes. Such nanotubes, according to their suggestion, can be used as novel nonviral delivery systems for gene transfer [38].

The aim of this study is to explore the functionalization and characterization of carbon nanotubes (CNTs) with tryptophan, an essential amino acid known for its unique properties and potential applications in biomedicine and nanotechnology. This research is necessary to enhance the functional capabilities of CNTs, which are widely recognized for their exceptional mechanical, electrical, and thermal properties. By functionalizing CNTs with tryptophan, we can improve their biocompatibility and facilitate their integration into biological systems, paving the way for innovative applications in drug delivery, biosensing, and tissue engineering. Understanding the interactions between CNTs and tryptophan will provide valuable insights into the development of advanced materials that can meet the growing demands of various scientific and industrial fields.

2. Material and Methods

Multi-walled carbon nanotube functionalized with carboxylic acid with an outer diameter of 10-20 nm and an inner diameter of 5-10 nm and a length of 10-30 micrometers were prepared from Pisgaman Nano Materials Iranian Company (Mashhad - Iran) with the specifications listed in Table 1 and content 99.8% carbon and 2% chlorine shown in EDS analysis. To prepare the colloidal suspension of nanoparticles, 400 mg of MWCNT multi-walled carbon nanotube powder was directly distilled in double distilled water and suspended using 35 kHz ultrasonication for 20 minutes.

Table 1- Carbon nanotube characteristicsType

of particle	COOH Functionalized MWCNTs
Purity (%)	> 95 % carbon nanotube (from TGA[1] and TEM[2]) > 97% wt % cabon content
Outside diameter(nm)	10-20 (from HRTEM, Raman)
Inside diameter (nm)	5-10
Length (µm)	10-30 (TEM)
SSA[3]	>200 m^2/g (BET)
Color	Black
Ash	< 1.5 wt% (TGA)
Electrical conductivity	>100 s/cm
Tap density	0.22 g/cm^3
True density	~ 2.1 g/cm^3
Multi-walled carbon nanotubes manufacturing method	CVD[4]

2.1. Functionalization of multi-walled carbon nanotubes

In order to functionalize the multi-walled carbon nanotubes, first, 400 mg of MWCNT was dissolved in 50 ml of distilled water and 50 ml of DMSO. Next, 400 mg of tryptophan amino acid was added to it and it was mixed completely for 24 hours on a magnetic stirrer so that tryptophan amino acid factors were non-covalently attached to the carbon nanotube [42].

2.2. Caracterization of functionalized MVCNTs

MWCNTs were characterized morphologically using scanning electron microscope (SEM) (Figure1) and Functionalized MWCNTs were characterized using FT-IR analyzers to ensure in case of attachment of –OH and –NH groups, carboxylic acid C=O and amidic C=O functional groups on the surface of CNTs (Figure 2).

3. Results and discussion

Nowadays, nanomaterials are widely utilized in various scientific fields, particularly in biology. Many studies have explored the effects of these materials on plants, suggesting that the interaction between plant cells and nanoparticles can modify gene expression and associated

[1] *Thermogravimetric* Analysis
[2] Transmission Electron Microscopy
[3] Specific Surface Area
[4] *Chemical Vapor Deposition*

biological pathways. Among these nanoparticles, carbon nanotubes attracted a great deal of attention due to their distinctive chemical, thermal, mechanical, and electrical properties [43].

Like other nanomaterials, carbon nanotubes can have both positive and negative effects due to their dual nature. Moore (2006) highlighted that the use of nanomaterials may lead to the generation of reactive oxygen species (ROS). Although ROS serve as signaling molecules in various growth and developmental processes, they can lead to cellular damage and ultimately cell death when their production surpasses the antioxidant capacity of the cell. The balance between ROS production and the activation of the antioxidant system is crucial for maintaining oxidative signaling and preventing damage within plant cells [44].

Functionalization of carbonnanotubes can decrease negative effect of them on cells and improve their chemical and physical properties. Functional groups in agents that attached to CNTs like -OH or -NH in aminoacids increase their solubility in aquatic environments and decrease their toxicity in cells and make them more suitable for biological applications [20].

Tryptophan is an essential aminoacid with unique properties in biomedicine and nanotechnology. We use this aminoacid to functionalizing of MWCNTs to make them more suitable for biological applications. We also use some methids such scanning electron microscope (SEM) and FT-IR analyses to ensure in functionalization of MWCNTs.

SEM images,were obtained from MWCNT are shown in figure 1. The outside diameter was calculated in the range of 20–30 nm, and the length of these nanotubes was approximated as 10–30 µm. As shown in the obtained images, the tubular morphology of MWCNTs could be seen clearly. The SEM images show a notable difference between COOH-functionalized carbon nanotubes (CNTs) and tryptophan-functionalized CNTs, primarily due to the unique functional groups present on their surfaces, which influence their morphology, structure, and integrity. In the case of COOH-functionalized CNTs, the carboxyl groups (COOH) create polar sites on the surface, resulting in a more uniform and smooth appearance in the SEM images. Although the functionalization process can cause the CNTs to appear slightly thicker due to the addition of these functional groups, their overall tubular structure remains distinctly recognizable.

The FTIR spectra of pristine MWCNT and functionalized MWCNT is shown in Figure 2. Based on the peak around 3400 cm^{-1} presented in the pure MWCNT spectrum, it may be related to the N–H bonds of carbon nanotube itself . Conversely, this spectrum in functionalized carbon nanotube has been appeared as a sharp peak, indicating the stretching vibration of -NH_2 present in the tryptophan structure. Besides, around 1500 to 1632 cm^{-1}, there are sharp peaks in functionalized MWCNT, which also indicates the presence of carbonyl and carboxylic acid functional groups in the structure of tryptophan.

The FT-IR spectrum was taken from the synthesized samples without agent, and the functionalization was confirmed according to the obtained results. The spectrum of tryptophan-functionalized carbon nanotubes shows peaks in the range of 3457 to 3475 nm, indicating the presence of type II amines. A peak at 3132 nm suggests the existence of carbon-carbon double bonds. The fingerprint region peak at 1923 nm further confirms the functionalization of the carbon nanotubes with tryptophan, indicating the presence of cyclic compounds. Additionally, small peaks around 1661 nm confirm the existence of carboxylic acid groups.

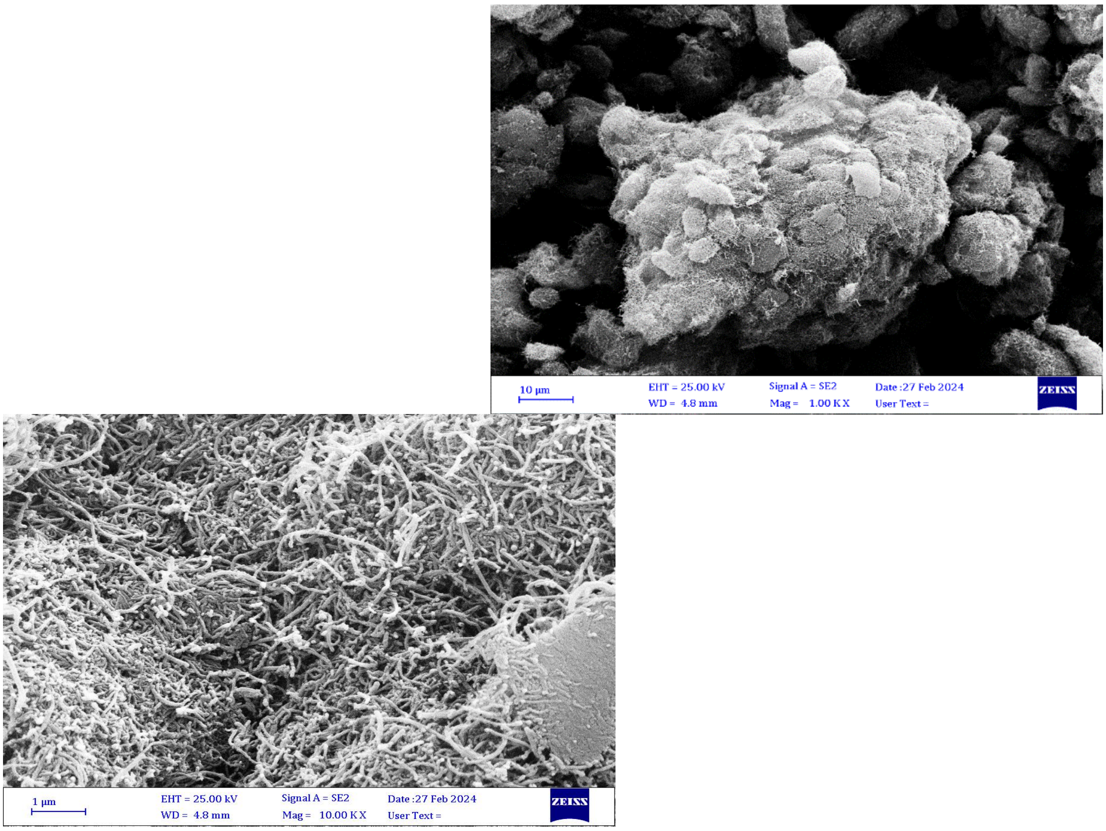

Figure 1- Transmission electron microscope images with 10 and 1 micrometer magnification of multi-walled carbon nanotubes with carboxylic acid agents

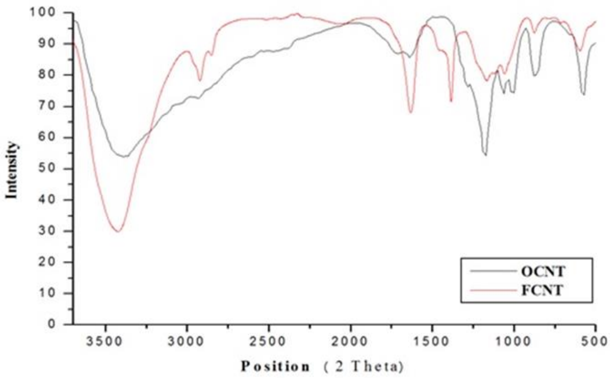

Figure 2- Graph related to FT-IR spectrum of carbon nanotube raw and functionalized with tryptophan

4. Conclusion

In conclusion, this study highlights the promising role of functionalized multi-walled carbon nanotubes (MWCNTs) in enhancing the compatibility of carbon-based nanomaterials with biological systems. Functionalizing MWCNTs with tryptophan, a significant reduction in the nanotubes' toxicity and insolubility, paving the way for broader application in biomedicine. SEM and FT-IR analyses confirmed successful functionalization, indicating improved interaction potential within cellular environments. These tryptophan-functionalized MWCNTs present a versatile tool for advanced biomedical applications, such as drug delivery, biosensing, and tissue engineering, suggesting substantial future potential in nanobiotechnology.

5. References

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