Defence Science Journal, Vol. 58, No. 5, September 2008, pp. 617-625

REVIEW PAPER

Carbon Nanotubes: Detection of Chemical and Biological Warfare Agents

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ABSTRACT

Discovery of carbon nanotubes has great impact on the development of newer methodologies and devices useful for the analysis of various types of chemicals. The functionalisation of CNTs with biomolecules related to chemical and biological warfare agents makes these useful for the detection of these agents. The detection sensitivity can be increased manyfold. Various types of chemical and biological sensors were developed using various type of carbon nanotubes as well as nano particles of different metals.

Keywords: CNTs, SWCNT, MWCNT, arc discharge, laser ablation, chemical vapour deposition, functionalisation, chemical and biological warfare agents, DNA, protein and antibodies

1. INTRODUCTION

Carbon nanotubes (CNTs) have received much attention as a new class of nanomaterials since their discovery[1,2]. These show unique mechanical, chemical, and electrical properties[3,4] which led to a variety of applications, such as scanning probes[5], nanoelectronics, and memory storage devices[6-8]. Distinctive properties of CNTs, such as surface area, ability to accumulate analyte, minimisation of surface fouling and electrocatalytic activity are very attractive for electrochemical sensing[9-11]. Different type of carbon nanotubes are presented in Figure 1.

Depending on the sheet direction and diameters, CNT may be classified either metallic or semiconducting. Relatively to all kinds of natural materials, CNTs have the highest strength, which is more than 100-times that of steel of same diameter, although their specific gravity is one sixth of steel. CNTs nanotubes are seamless tubes of graphite sheet with nanosized diameter. Single-walled carbon nano tubes (SWNTs) are graphene sheet rolled up into a tube form with nanodimensions[11]. SWNT is a strikingly inert material in the form of bundles due to van der Waals attractive interactions. SWNT with additional garphene tubes around the core of an SWNT are called multi-walled carbon nanotubes (MWNTs)[12]. MWNT are always electrically conductive and found to have an electrical conductivity[13] of approximately 1.85X103 S/cm. Compared to the use of composites filled with carbon black or carbon fibre as conductive reinforcement, polymeric composite with MWCNTs can form the conductive path at a relatively low content, owing to their high aspect ratio of 100-1000 and high specific area. Therefore, their use in various applications, such as antistatic film, electromagnetic shielding materials, etc, has long been anticipated[14].

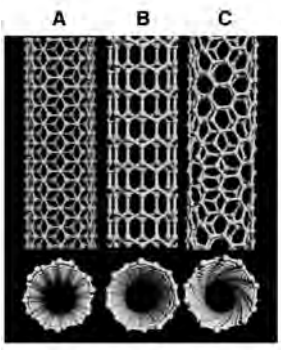


Figure 1. Different type of carbon nanotubes. A: Armchair single-walled carbon nanotube; B: Zigzag single-walled carbon nanotube and C: Chiral single-walled carbon nanotube. (Reprinted with permission from Shenzhen, Nanotech Port Co Ltd, China).

CNTs can be prepared using arc-discharge, laser ablation, and chemical vapour deposition (CVD) methods. The first two methods employ solid state carbon precursors and lead to a near-perfect nanotube structure, with large amount of by-products being formed. The CVD method uses hydrocarbon (liquid and gases) as a sources of carbon[15] and catalyst particles that serve as seeds to nucleate the growth of CNTs. By positioning catalyst seeds in arrayed fashion, organised patterns of nanotube structures can be produced on surfaces, comprising both SWNTs and MWNTs[15].

Biodefence issues have become prominent because of the potential bio-, chemical-lethal weapons attacks from terrorists. Since, the 9/11 attack in United State, research on development of biosensing and chemical sensing technologies have been stimulated, especially for internal security. However, currently available sensing technologies are not sensitive enough to meet the requirement of rising demand for small, fast, highly sensitive, highly selective biosensors. Therefore, new technologies have to be explored to reach this goal and in this context CNT is very promising material.

Received 4 May 2007, revised 28 January 2008

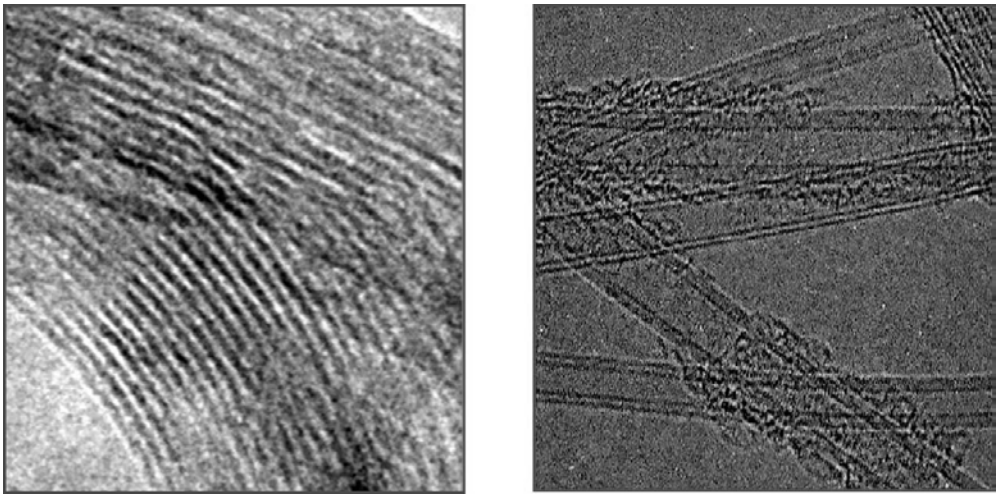


Figure 2. A transmission electron micrograph of, A: SWNT and B: Double wall carbon nanotubes. (Reprinted with permission from Shenzhen, Nanotech Port Co. Ltd., China).

Recent years have witnessed significant interest in nano materials, such as nanocrystals, nanowires and nanotubes for application in life sciences and biotechnology. CNTs are well-ordered and all carbon hollow graphitic nanomaterials have exceptional structural, electrical, mechanical, and thermal properties. These properties can be very useful for many promising applications of nanotechnology including the detection of chemical or biological warfare agents in the future.

2. DIFFERENT TYPE OF CNTS:

2.1 Single-walled Carbon Nanotubes

The SWNTs can be imagined as a perfect graphene sheet (graphene being the same ploy-aromatic mono-atomic layer made of an hexagonal display of Sp2 hybridized carbon atoms that genuine graphite is built up with) rolled up into a cylinder, with the hexagonal rings put in contact to join seamlessly[16,17]. Considering this simple procedure, there are countless different ways to roll up a graphene sheet in order to form a nanotube. The way a nanotube is built from a graphene sheet does not only have an influence on the diameter and the chirality of a nanotube (Fig. 2) but also plays an important role for the electronic properties of the nanotube[18,19].

2.2 Multi-walled Carbon Nanotubes

The MWCNTs, as implied by their name, consist of multiple graphene sheets rolled up in concentric CNTs, filling each other's inner cavities to end up with nanotube-filled nanotubes[20-22]. The intertube distance in a MWCNT is approximately that of the intergraphene distance in turbostratic, polyaromatic solids, i. e. 0.34 nm (as opposed to 0.34 nm in graphite) (Fig. 3 and 4). As a result of this, many layers that MWCNTs are built from, are much more rigid than that of SWCNTs[23,24].

3. PREPARATION OF CNTS

Various techniques have been developed to produce carbon nanotubes and well known techniques are arc discharge,

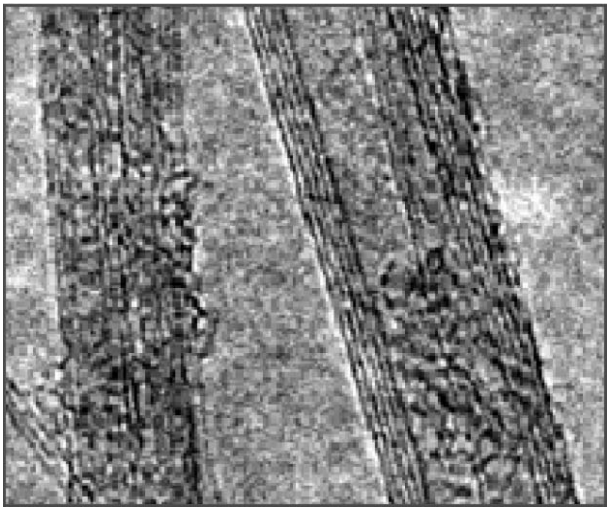


Figure 3. A transmission electron micrograph of MWCNTs. (Reprinted with permission from Shenzhen, Nanotech Port Co. Ltd., China).

Figure 4. A transmission electron micrograph of aligned MWCNTs. (Reprinted with permission from Shenzhen, Nanotech Port Co. Ltd., China).

laser ablation and chemical vapour depositin (CVD) [2,20,21,25]. Most of these processes take place in vacuum or with inert gases mixed with precursor flow. CVD growth of CNTs can take place in vacuum or at atmospheric pressure. Large quantities of nanotubes can be synthesised by these methods; however, advances in catalysis, and continuous growth processes are making CNTs more commercially viable[22] .

3.1 Arc Discharge Method

Iijima observed CNTs under microscope in 1991 in the carbon soot of graphite electrodes during an arc discharge, by using a current of 100 amps intended to produce fullerenes[26]. During this process, the carbon contained in the negative electrode sublimates because of the high temperatures caused by the discharge. Because nanotubes were initially discovered using this technique, it has been the most widely used method of nanotube synthesis. The yield by this method is up to 30 per cent by weight and it produces both single and multi walled nanotubes, however these are quite short (50 μ)[22, 26].

3.2 Laser Ablation Method

Laser ablation method was developed by Smalley[20] *et al*. In the laser ablation process, a pulsed laser vapourises a graphite target in a high temperature reactor while an inert gas is bled into the chamber. The nanotubes develop on the cooler surfaces of the reactor, as the vapourised carbon condenses. A water-cooled surface may be included in the system to collect the nanotubes. This method yields around 70 per cent and produces primarily CWCNTs, with a controllable diameter determined by the reaction temperature. However, it is more expensive than either arc discharge or chemical vapour deposition[22] methods.

3.3 Chemical Vapour Deposition Method

In CVD method, a substrate is prepared with a layer of metal catalyst particles or in metals combination[22]. The metal nanoparticles can be produced by reduction of oxides or solid solutions of oxides. The diameters of the nanotubes to be grown are related to the size of the metal particles[20]. This can be controlled by patterned (or masked) deposition of the metal, annealing, or by plasma etching of a metal layer. The substrate is heated to approximately 700 °C. To initiate the growth of nanotubes, two gases are bled into the reactor: a process gas (ammonia, nitrogen, hydrogen, etc) and a carbon-containing gas (such as acetylene, ethylene, etc.) [27-30]. Nanotubes grow at the sites of the metal catalyst; the carbon-containing gas is broken down at the surface of the catalyst particle, and the carbon is transported to the edges of the particle, where it forms the nanotubes. This mechanism is still under investigation. The catalyst particles can stay at the tips of the growing nanotube during the growth process, or remain at the nanotube base, depending on the adhesion between the catalyst particle and the substrate[26,27].

The CVD method shows the most promise for industrial scale deposition in terms of its price/unit ratio. There are additional advantages to the CVD synthesis of nanotubes. Unlike the above methods, CVD is capable of growing nanotubes directly on a desired substrate, whereas the nanotubes must be collected in the other growth techniques. The growth sites are controllable by careful deposition of the catalyst. Additionally, no other growth methods have been developed to produce vertically aligned nanotubes.

4. FUNCTIONALISATION OF CNTS

Due to their extraordinary properties, CNTs are attractive candidates in various nanotechnological applications, such as fillers in polymer matrices, hydrogen-storage devices, biosensors, electrochemical super capacitors, field-emitting devices, pharmaceutical scaffolds, etc.[31-35]. However, lack of solubility of as-produced nanotubes and the difficult manipulation in almost all solvents have imposed great limitations to the use of CNTs. Indeed, as-produced CNT are insoluble in all organic solvents and aqueous solutions. These can be dispersed in some solvents by sonication, but precipitation almost immediately occurs when this process is interrupted. To overcome these limitations, a chemical functionalisation can be applied which offers the possibility modifying the surface of the nanotubes that is in contact with the solvent or surrounding matrix to integrate the nanotubes using interactive attraction forces. The field of functionalisation of CNTs has been rapidly expanding over the last few years, which is reflected by the large number of papers published on this subject. Following are the possible ways to functionalise the CNTs:

4.1 Endohedral Functionalisation

This technique involves the filling of the inner cavity of the CNTs with atoms or small molecules. One of the first species that could be found inside a SWCNT was C_{60} molecules[36]. High resolution transmission electron microscopy (HRTEM) images sowed that encapsulated C_{60} self-assembles into chains of the spherical molecule and that single C_{60} molecules can spontaneously jump nanometer distances along the axis of the containing nanotube when excited with a electron beam[37].

A better nomenclature was proposed to be X@SWCNT in accordance with the nomenclature for the filled fullerenes (e. g. $Gd@C_{82}$)[38]. Upon thermal annealing, the encapsulated fullerenes fuse in the interior of the SWCNT, which results in a new, concentric endohedral nanotube with a diameter of 0.7 nm. In general, endohedral functionalisation can either be achieved *in situ* during nanotube growth or as a separated functionalisation step via solution-phase chemistry, by taking advantage of capillary effects or the sublimation of the guest molecules[38]. Besides the filling with fullerenes, the endohedral functionalisation with metal halides became an important topic as the metal-filled SWCNTs provide interesting electronic properties. The first material which was successfully inserted into a SWCNT was $RuCl_{13}$, which was subsequently reduced to metallic ruthenium by heat treatment (45 °C) in a hydrogen atmosphere[39]. Many other metal halides have been encapsulated so far and a very interesting and peculiar property is the formation of single crystals of these substances inside the SWCNTs, due to the constraining 1D space provided by the

nanotubes inner cavity. Hence, the inner cavity of CNTs provides a special space where materials can have properties different from what these would have under normal conditions and offers the opportunity to investigate molecules separated from each other.

Sidewall Functionalisation

Sidewall functionalisation of CNTs involves the direct covalent addition of reactive molecules to the nanotube sidewalls, and therefore leads to partial destruction of the conjugated Sp^2 lattice by the formation of Sp^3 carbon atoms in the sidewalls. This can totally destroy the electronic band-structure of metallic and semi-conducting nanotubes and result in insulating CNTs, if the reaction conditions are too harsh. One of the first reactions taking advantage of the sidewall approach was the fluorination of CNTs at temperatures between room temperature and 600 °C. Considering the reaction mechanism for the fluorination, Kelly[40] et al. proposed two possible addition patterns, consisting of a 1,2-addition or 1,4-addition. Up to now, there is still controversy about the addition pattern, but it can be assumed that due to the low energy barrier between these, both patterns exist during the reaction. The importance of the fluorination reaction lies in the fact that the fluorine atoms can subsequently be substituted by alkyl groups using Grignard[41] or organolithium reagents[42]. These alkylated nanotubes can very easily be dispersed in various organic solvents as their modified surfaces can interact with the solvent molecules. Additionally, bundling of the CNTs is reduced as the addend molecules prevent p-stacking. Oxidation of the functionalised nanotubes in hot air resulted in the removal of the alkyl addends giving back the pristine nanotubes. Another important reaction is the [2+ 1] cycloaddition of carbenes. *In situ*- generated dichlorocarbene readily reacts with SWCNTs[43]. With this approach a functionalisation of 16 per cent of the carbon atoms could be achieved, with drastic effects on the optical properties of the nanotubes[44]. The reactive species were carrying a wide variety of functional groups, such as alkyl chains, dendrimers, different crown ethers and oligoethylene glycols, which led to increased solubility of the modified nanotubes and to the complexation of metal ions (*Cu* and *Cd*). Recently, reductive alkylation of CNTs under Birch conditions became a very challenging field of research[45]. In this approach, SWCNTs have been dissolved in liquid ammonia at low temperatures (~ –35 °C). The addition of alkali metals such as lithium, sodium or potassium instantly results in the dissolution of the metal and the production of M^+SWCNT-salts. The latter readily reacted with alkyl halides to give alkylated SWCNT derivatives in good yields.

Non-covalent Functionalisation

The non-covalent functionalisation of CNTs is based on van der Waals and π-π stacking interactions between the functional molecules and the large π-system of the CNTs. If these interactions are strong enough, the nanotubes can easily be de-bundled and rendered hydrophilic using mild ultra-sonication[46,47]. One advantage which makes this approach particularly attractive is that the electronic structure of the nanotubes is not affected by the functionalisation, thus conserving their special electronic and optical properties. The non-covalent approach started in 1998, when scientists tried to dissolve CNTs in an aqueous medium using various surfactants (LDS, SDS, SDBS, and Triton-X ®[48]. O'Connell[49] *et al* showed that even long-chain polymers like polyvinyl pyrrolidone or polystyrene sulphonate could effectively be wrapped around de-bundled nanotubes.

Defect-site Functionalisation

The sidewalls of CNTs are perfect Sp^2 systems without any distortions in the carbon lattice, but in reality there are of course dislocated carbon atoms generated during the production process. These areas of disorder are prevalently located at the ends of the nanotubes where the growth process was started (e.g., disordered fullerene-caps or even dangling bonds) or where the catalyst particle was attached to the nanotube. Defects can also be located directly in the CNTs sidewalls if there were significant irregularities of the parameters during the growth process. In most cases, the result was a so-called pentagon-heptagon[50] defect, which leads to strain and bending of the CNT and has significant effects on the electronic and mechanical properties of the nanotube. The strain evoked by the defects represents a location of higher reactivity towards aggressive chemicals like oxidising agents and thus opening of the nanotubes at these positions is possible. Oxidative treatment also produces oxidised carbon species like alcohols, aldehydes, ketones or carboxylic acid groups covalently connected to the sidwalls which offer the opportunity for subsequently chemical modifications using well-known organic chemistry[44,51].

Liu, *et al*. reported the first use of a 3:1 concentrated H_2SO_4/HNO_3 mixture to cut the highly-tangled long ropes of SWCNTs into short, open-ended pipes to produce many carboxylic acid groups at the opened ends[52]. The oxidation of CNTs with HNO_3, O_3 , $KMnO_4$, OSO_4, and RUO_4 was also reported[53-56]. The oxidation processes begin mainly with the oxidation of the initial defects which arise during the growth of the SWCNTs and are accompanied by processes that can be roughly divided into two steps: firstly, the defect-generating step and secondly, the defect-consuming step. Specifically, during the defect-generating step, the oxidants attack the graphene structure by electrophilic reactions and generate active sites such as -*OH* and -*C=O* functions. This step depends on the oxidant's ability to generate -*C-OH* groups and to transform them into -*C=O* functions and finally into -*C=O*(*OH*) groups. During the defect-consuming step, the graphene structure of the nanotube is destroyed by the oxidation of the generated active sites in the first step by the formation of gaseous CO_2. The defect-consuming step is mainly based on the ability of the oxidant to etch or destroy the graphite-like structure around the already generated -*C=O*(*OH*) functions an neighbouring atoms. After workup, the newly-introduced functionalities can be subsequently modified. As most of the carbon atoms are oxidized to carboxylic groups, the most commonly use reactions

Table 1. Functionalisation and binding of various biomolecules to CNTs

Functional group to be created on CNTs	Cross linker to be used	Binding of various biomolecules to CNTs	Reference
–COOH	EDC (1-ethyl-3-3-dimethyl aminopropyl] carbodiimide)	DNA, proteins, enzymes, and antibodies	57, 58
–COOH	SMCC (Succinimidyl 4-[n-maleimidamethyl]-cyclohexane-1-carboxylate	DNA, proteins, enzymes, and antibodies	59
–CONH2	Biotin	DNA, proteins, enzymes, and antibodies	60
–CONH	EDC (1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide)	DNA, proteins, enzymes, and antibodies	61
–COO–HN 3-PEG–NH2	Ionic interaction	DNA, proteins, enzymes, and antibodies	62, 63

are esterification and amidation reactions. Chen[43] et al and Hamon[51] et al showed that SWCNT-*C=O*(*OH*) derivatives can be functionalised with amines to yield SWCNT *C=O*(*NH-R*) via the activated acid chlorides SWCNT-*C=O*(*CI*). Despite the partial destruction of the CNT backbone, functionalisation of oxidatively-introduced defect sites has attracted great interest in the last decade as it allows very versatile chemical functionalisation that lead to almost any material property possible for the nanotube derivatives produced by this approach. The functionalisation of CNTs for binding of various biomolecules is summarises in Table 1[57-63].

5. DETECTION OF CHEMICAL AND BIOLOGICAL WARFARE AGENT USING CNTS

For the detection of chemical and biological warfare agents, functionalised CNTs may be cast on suitable sensitised electrode by thick film techniques and can be exposed to enzymes solution for immobilistion procedure[64]. This working electrode with a reference electrode (*Ag/AgCI*) and counter electrode (*Pt*) is to be immersed into a phosphate buffer

Table 2. Various types of CNTs used for the development of sensors for chemical warfare agents

CNT	Agents
SWCNT[65]	Foot and mouth disease
Nano wire[66]	Anthrax
Nano wire[68]	Smallpox
Nano wire[68]	Ricin
Nano wire[68]	Botulinum
Nano wire[68]	Anfluenza A
Ag nanoparticles[67]	Bacilus globigii
Ag nanoparticles[67]	Erwinia herbicolo
Ag nanoparticles[67]	Bacillus thuringiensis

Table 3. Various types of CNTs used for the development of sensors for biologial warfare agents

CNT	Agents
SWCNT[69]	DIMP (Diisopropyl methylphosphonate) Simulant of Sarin
SWCNT[69]	DMMP (Dimethyl methylphosphonate) Simulant of Soman
MWCNT[70]	VX (O-ethyl-S-2-diisopropylaminoethyl methylphosphonothionate)
MWCNT[70]	DMAET (2-(Dimethylamino)ethanethiol)
MWCNT[70]	R-VX (O-isobutyl-S-2-diethylaminoethyl methylphosphonothionate
MWCNT[70]	DEAET (2-(Diethylamino)ethanethiol) (Hydrolysis product of R-VX)
SWCNT[71]	DMMP (Dimethyl methylphosphonate) Simulant of Soman
Ag nanoparticles[71]	Dimethyl methylphosphonate Simulant of Sarine
Ag nanoparticles[67]	Pinacolyl methylphosphonate Simulant of Soman
Ag nanoparticles[67]	Diethyl phosphoramidedate Simulant of Tabun
Ag nanoparticles[67]	2-choloroethyl ethylsulfide Simulant of sulphur musrtard
Ag nanoparticles[72]	Cyanide

under the condition of constant stirring. Low concentration of organophorous compound solution to be added to detect the oxidation current. It is also expected that quantitative measurement of organophosphorus compounds can be realised by measuring the intensity of oxidation current. The developed device can be used for detection of these chemicals[63]. Tables 2 and 3 summarises the detection of various biological and chemical warfare agents using CNTs and other nano particles.

5.1 Detection of Toxic Organophosphorus Compounds

Organophosphorus compounds are generally used in insecticides, pesticides, and also as chemical warfare agents. It is well known that these compounds affect the central nervous system by inhibiting acetylcholine esterase (AchE), which functions as regulator for the neurotransmitter acetylcholine. An efficient technique to monitor these organophosphorus compounds is electrochemical detection[64]. Enzyme-immobilised carbon nanotube based biosensors are promising with high stability, sensitivity and selectivity. CNTs as the electrode material in biosensors has the possibility of promoting electron transfer reactions at a lower over-potential as well as enzyme immobilisation. In the sensing process, the enzyme which is AchE., is immobilized on nanotube surface and catalyses hydrolysis of thiocholine ester, acetylthiocholine, to form thiocholine. The electrochemical oxidation of thiocholine can be detected by electrochemical techniques[73]. When organophosphorus compounds exist, the catalytic ability of AchE is reduced, hence, the oxidation of thiocholine is inhibited, which can be detected by monitoring amperometric measurements.

5.2 Detection of AIkylating Agents Containing Sulphur and Nitrogen

Alkylating agents work by three different mechanisms, e.g., attaching to DNA bases, forming cross-bridge and inducing mispairing, all of which disrupt of DNA function and cause cell death. There are six groups of alkylating agents: nitrogen mustards; ethylenimes; alkylsulfonates; triazenes; piperazines; and nitrosureas. Detection of alkylating agents can be achieved by DNA sensing as the biological recognition element which could have numerous potential applications including decentralized clinical testing, environmental monitoring, and food safety.

Among the DNA biosensors, the electrochemical DNA biosensors, regarded as rapid, inexpensive candidate for diagnosis of genetic diseases, relying on the conversion of the base-pair recognition event into a useful electrical signal[74-79].

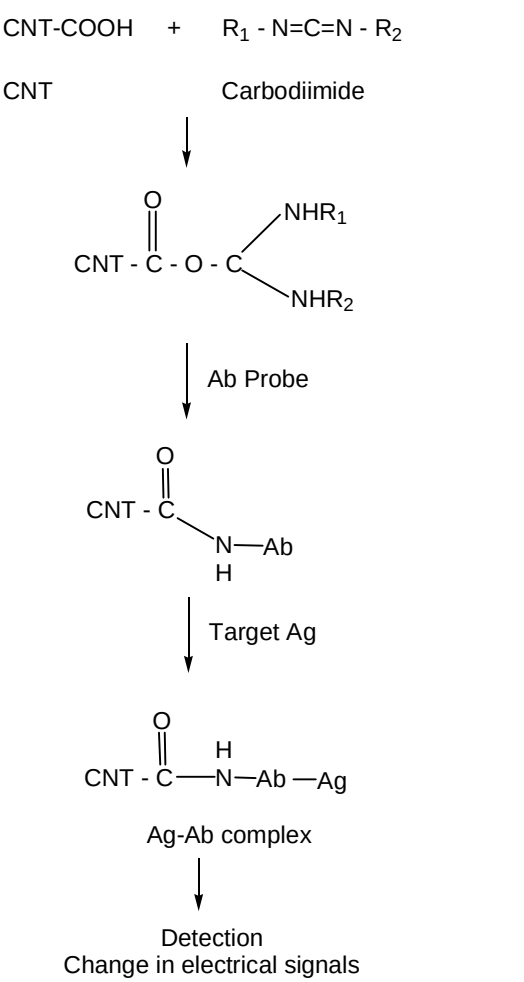
To improve the sensitivity, aligned CNTs should be used as nanoelectrode array for DNA recognition[79]. A DNA probes of approximately 15 to 20 base pairs may be designed, and covalently bonded on the tip of functionalised CNTs. Then these electrodes will be dipped into a suspension of single strand DNA[74]. Hybridisation[79] is performed by incubating the probe with complementary target DNA oligonucleotides with and without introducing alkylating agents containing sulphur and nitrogen, respectively. After the incubation, the electrodes will be washed to get rid of unhybridised DNA strands and electrochemical testing to be performed.

5.3 Detection of Various Toxic Proteins, Bacteria, and Viruses

Biological warfare agents such as toxins and various pathogenic organisms[80-83], such as *Salmonella typhi, Vibrio cholerae*, etc, are extremely dangerous for human health[62]. Very low concentration of these toxins/bacteria in the air, food or water can cause illness or even death. The best way to reduce or prevent the incidences of infection is by providing effective monitoring systems. Therefore, high sensitive detection system or biosensors are required to detect various toxic proteins at fast speed. The use of Antibodies[83] as probes for the detection of bacteria and other biological agents CNTs will be explored extensively (Scheme I). The capture of specific toxin/bacteria by particular antibodies results in a mass change that can be detected by different sensor platforms. The CNT is a potential candidate to be applied in the development of next generation biosensors for toxic protein detection[83-84].

Antibodies are high-molecular weight soluble proteins produced by organisms to fight against foreign substances, antigen, with which they form immunochemical complexes. The antibody molecule has the shape of a "Y". Two antigen binding sites are located on the upper parts of antibody. Antibody and antigen interaction proceed by relatively weak non-covalent forces such as hydrogen bonds, hydrophobic or van der Waals forces and ionic interactions.

The CNTs can be used as a measuring platform for



Scheme I. Schamatic presentation of target antibody (Ab) binding on–COOH-generated CNTs

various toxic proteins[85] which will be immobilised on the functionalised nanotubes both covalently and noncovalently[85-88]. The immobilisation of antibodies on the sensor platform is crucial for improving the performance of these antibody-antigen based biosensors[85,89,90] due to their conversion of a non-electrical, physical or chemical, quantity into an electrical signal for detection. Significant efforts are being input to improve the immobilisation procedure of antibody to CNT platforms. The monoclonal or polyclonal antibodies are coated on nanotube platforms followed by washing and drying processes. Then bacterial suspensions are added and incubated under proper conditions. As some of the characterisation techniques, scanning electron microscope (SEM) and electrochemiluminescence (ECL) can be used to test the bonding of toxic proteins to the antibodies on nanotube platforms. The detection can be done by integrating these sensor tips to a signal conditioning and processing circuit and measure the conductance, and electrical signal obtained due to the presence of toxic proteins[91-92].

CONCLUSIONS

The CNTs have wide applications in various fields. The sorption properties of CNTs and their chemical

functionalisation can be utilised in the development of various types of biosensors for the detection and identification of chemical and biological warfare agents in future.

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