Review

Recent Advances in Carbon Nanotubes Based Biosensors

T. Basu [1]**, Pratima R. Solanki** [2] **and B. D. Malhotra** [2,*]

1 Amity Institute of Applied Sciences, Amity University (UP), Noida-201303(UP), India.
E-mail: basu002@yahoo.com; tbasu@ase.amity.edu.
2 Biomolecular Electronics & Conducting Polymer Research Group, National Physical   Laboratory,
Dr K.S.Krishnan Marg, New Delhi 110012, India.
E-mail: pratimasolanki@yahoo.com; bansi.malhotra@gmail.com.

* Author to whom correspondence should be addressed. bansi.malhotra@gmail.com.

Abstract: Carbon nanotubes (CNTs) have recently aroused world-wide interest. This has been attributed to the combination of structure, size and morphology of CNTs resulting in their reasonable surface and mechanical properties, semi-conducting and metallic properties. We discuss recent developments relating to the methods and techniques that use carbon nanotubes as transducers and mediators for fabrication of biosensors. Besides this, attempts have been made to describe in brief the preparation, characterization and applications of nano-materials to both health care diagnostics and environmental biosensing.
Keywords: Carbon nanotube, Biosensor, Glucose, Cholesterol, DNA.

1. Introduction

Nanomaterials such as colloidal metal particles, metal oxides, nano-structured conducting polymers and carbon nanotubes have recently attracted much interest owing to their applications in nano-scaled devices, sensors and detectors [1-5]. Among these, carbon nanotubes have aroused much attention due to high aspect ration of the order of 1000, high conductivity, biocompatibility and chemical inertness [1]. Besides this, these novel electronic materials have attractive chemical, mechanical and electronic properties. Since the discovery of carbon nanotubes (CNTs) by Iizima in 1991[6], there has been increased interest in exploiting these miniaturized entities for applications as field emitters, batteries,

nanotube actuators , probe tips, reinforced composites, nanoelectronics display devices, sensors and biosensors etc .

Applications of carbon nanotubes to biosensors have attracted the maximum attention. Carbon nanotubes (CNT) are well-ordered and are allotropes of carbon. The two main variants, single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) both posses a high tensile strength (Young's modulus > 1 terapascal, making CNTs as stiff as diamond and flexible along the axis), are ultra-light weight, and have excellent chemical and thermal stability. They are known to possess semi- and metallic-conductive properties [6]. This startling array of features has led to many proposed applications in the biomedical field, including biosensors, drug and vaccine delivery and the preparation of unique biomaterials such as reinforced composites and/or conductive polymer nanocomposites. Both SCNTs and MWCNTs are typically a few nanometers in diameter and several micrometers (10 μm) to centimeters long. SWCNT has a cylindrical nanostructure with high aspect ratio, formed by a rolling up a single graphite sheet called grapheme into a tube form. It has a tendency to aggregate to form bundles consisting of 10 to 100 of nanotubes in parallel and in contact with each other. MWCNT comprises of several layers of graphite cylinders concentrically nested like rings with an interlayer spacing of 3.4 A^0 [Fig. 1], can be considered as a mesoscale graphite system, whereas the SWCNT is truly a single large molecule.
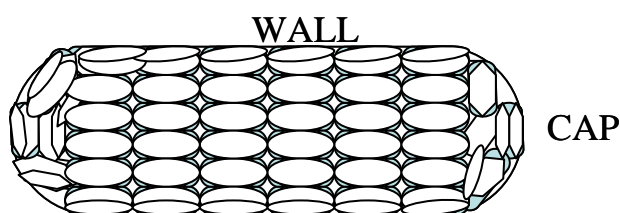

Figure 1. Schematic of single Carbon nano tube (SWCNT).

Biosensors are electronic devices that yield quantitative or semi-quantitative analytical information using a biological element. For the last about ten years, biosensors are finding numerous applications in the field of clinical diagnosis, drug discovery, detection of environmental pollutants, biotechnology, military and civil defense due to their smart size, quick and dependable response compared to the conventional systems. A biosensor consists of three parts (i) biological detection element that recognizes the substance of interest, (ii) transducer that converts a biorecognition event into measurable signal and (iii) a signal processing system that converts the output into a processable signal (Fig. 2).
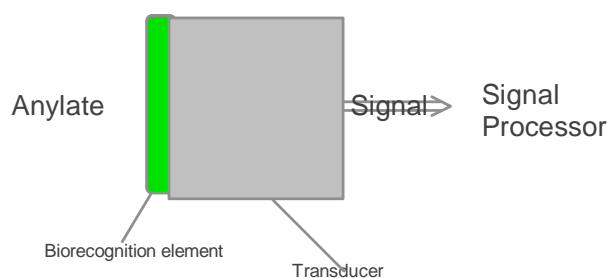

Figure 2. Schematic of a biosensor.

Biological recognition elements include enzymes, antibodies, organelles, photosensitive membranes from plants and bacteria, protoplasts, whole-cells, tissue slices, antibodies, deoxyribonucleic acid (DNA) or cell membrane receptors etc. The biological molecules are ordinarily immobilized in close proximity to a transducer surface thereby facilitating direct or mediated signal transfer to the transducer. The efficiency of a biosensor depends on the effective electron transfer, stability of the biomolecule etc, reusability, linearity and sensitivity etc. It has been found that transduction efficiency is a key factor that yields information on analytical characteristics of sensor such as signal stability, reproducibility, detection limit and in specific cases operational stability and selectivity etc.

The recent advancements in nanomaterials have provided a platform to develop efficient transduction matrices for biosensors. The integration of nanoparticles, having unique electronic, optical and catalytic activities with biomaterials that exhibit unique recognition, catalytic and inhibition properties, may result in novel new generation biosensors with of synergic properties and functions. A living organism comprises of cells of about 10 μm. Metals and semiconductor nanoparticles or nano rods (1-100 nm) have similar dimensions such as proteins (enzymes, antigens, antibodies) or DNA. The cell parts are even smaller than that of a protein molecule of typical size of 5 nm which is comparable with the smallest man-made nanoparticles.

In this paper, we review recent developments relating to the preparation, characterization and application of carbon nanotubes based biosensors reported in literature in the last about five years.

2. Preparation of carbon nanotubes

Some of the techniques of synthesis and characterizations of carbon nanotubes are described below [6,7].

(i) Laser evaporation

The carbon nanotubes can be prepared by a dual pulsed laser vaporization technique of a carbon target in a furnace at 1200 °C. The two successive laser beams are used to vaporize a target more uniformly. A cobalt-nickel catalyst helps the growth of the nanotubes, presumably because it prevents the ends from being "capped" during synthesis, and about 70-90% of the carbon target can be converted to single-wall carbon nanotubes. The method has several advantages, such as the high quality of the diameter and controlled growth of the SWCNTs. The change of the furnace temperature, catalytic metals and flow rate directly affect the size (diameter) of SWCNTs.

(ii) Carbon arc method

In this case, gas is ignited by passing high currents through carbon electrodes. Typical operating conditions for a carbon arc used for the formation of carbon nanotubes include the use of carbon rod electrodes of 5-20 nm diameter separated by a distance of 1 nm with a voltage of 20-25 V across the electrodes and a continuous DC electric current of 50-120 A flowing between the electrodes. For preparation of MWCTs, no catalyst is used and for the SWCNT transition metals (Fe, Co, Ni) and rare earths (Y, Gd) have been used. This method can produce SWCNT of diameter 1-5 nm with a length of 1 μM. When a graphite rod containing a metal catalyst (e.g., Fe and Co) is used as the anode and the cathode is pure graphite, SWCNTs are generated instead of MWCNTs. Large-scale synthesis of MWCNTs by arc-discharge can be achieved in a helium atmosphere. The drawback of arc-discharge

method is purification of CNTs. Removal of non-nanotube carbon and metal catalyst material is much more expensive than the production itself.

   (ii) Chemical vapor deposition

   In the CVD method, a catalyst is heated up to high temperature in a furnace with a flow of hydrocarbon gas through the tube reactor. For MWCNT growth, ethylene or acetylene is used at a temperature of 550-750 ^{0}C whereas for SWCNT (70-80 % yield), methane is used in the presence of metal particles (Fe, Co, Ni) on MgO at a temperature of 1000^0C. The width and peak of the diameter distribution of CNTs depend on the composition of the catalyst, the growth temperature and various other growth conditions.

(iv) Ball milling

   It is a simple method of production of carbon nanotubes. The graphite powder (99.8 % purity) is ball milled at room temperature for about 150 h in a stainless steel container containing 400 balls in the presence of argon gas (300 kPa). After that the powder is annealed at 1400 ^{0} C for about 6 h under the purging of nitrogen gas [6-7].

(v) Miscllaneous methods

   CNTs can be formed by diffusion flame synthesis, solar energy, heat treatment of a polymer and low temperature solid pyrolysis [6-7].

3. Characterization of carbon nanotubes

   Transmission electron microscope (TEM) studies have shown that carbon nanotubes prepared by the arc method consist of multi-layered concentric cylinders of single graphite sheets. The diameter of the inner tube is a few nanometers. The outer most tube is as large as 10-30 nm. During the curling of graphene sheet into a cylinder, helicity is introduced. Electron diffraction studies reveal helicity, suggesting the growth of carbon nano tube occurs in the spiral growth of the crystals. The gap between the cylinders in MWCNT is 3.45 A^0 which is close to the separation between the planes of graphite [1]. The ring like patterns of CNTs are shown by the high resolution electron microscope of images due to individual tubes consisting of cylindrical graphite sheets that are independently oriented with helical symmetry for the arrangement of the hexagons. Similar ring morphology has been observed in atomic force microscopy (AFM) and SEM studies. In the electron microscope image, SWCNT is observed as a combination of rings [8]. Fullerenes have been found inside a laser-synthesized SWCNT by high resolution TEM [9]. X-ray diffraction measurements are used to characterize CNTs. X-ray diffraction views many ropes at once and show that the diameters of the carbon single-wall nanotubes have a narrow distribution with a strong peak. STM has been used to probe the electronic structure of CNTs deposited on various substrates. Scanning Tunneling Microscope (STM) studies have brought out that carbon nanotubes have sp^3 defect structure, closure of the tubes and pentagon induces changes in the electronic structure. Raman spectroscopy has provided important insights about the structure of a CNT. In a scanning electron microscope, the carbon nanotubes produced by any of the above methods look like a mat of carbon ropes. The ropes are between 10 and 20 nm across and up to 100 μM long. When examined in a transmission electron microscope, each rope is found to consist of a bundle of single-wall carbon nanotubes aligned along a single direction.

4. Importance of carbon nanotubes in biosensors

The unique structure of a CNT, different from graphite and conventional carbon has attracted many researchers from chemistry, physics and materials science. All the carbons in graphene sheets are sp^2 hybridized and have delocalized π electron structure that is responsible for extraordinary electronic properties.

Luo et al. have investigated electrochemical behavior of CNT modified glassy carbon electrode for the oxidation of biomolecules like dopamine, epinephrine and ascorbic acid resulting in considerable improvement in the electro oxidation of these biomolecules [10]. Recent studies have reported that surface-confined CNTs can dramatically accelerate the electron transfer reactions of important anylates, including catecholamine neurotransmitters, hydrogen peroxide, cyctochrome C, NADH and hydrazine compounds [11-16].

A thermoelectric carbon nanotube nose has been constructed from tangled bundles of single walled carbon nanotubes [17]. The response is specific to gases such as He, N_2, H_2 could be easily detected.

The storage modulus and water stability of chitosan con be improved by incorporating chitosan-grafted carbon nanotubes (CNTs-g-CS) for interesting biochemical and electrochemical applications [18].

The application of CNTs to biosensors has led to increased stability of the immobilized enzymes resulting in enhanced biosensor response. Besides this, the CNT modified biosensor exhibits enhanced stability and approximately eight-fold sensitivity [19]. The aflatoxin–detoxifizyme (ADTZ) –MWNTs electrode is used to detect sterigmatocystin (ST), a carcinogenic mycotoxin with toxicity second to aflatoxins, usually contaminated in food-stuff [20].

It has been reported that SWCNT modified electrodes can be used to promote electron transfer reactions of biomolecules like dopamine, 3-4 hydroxy phenyl acetic acid(dopac), uric acid, ascorbic acid, cytochrome C, homocysteine (HcySH) and norepinephrine [11,13,21-25]. The peak current linearly varies with analyte concentration. It can be seen that CNTs not only amplify the signal but are very selective in detection.

Amperometric biosensors based on MWCNT modified electrodes have been constructed for the determination of phenolic compounds such as phenol, o-cresol, p-cresol, m-cresol, catechol, dopamine, and ephinephrine. The measurement of phenolic compounds is based on the signal produced by electrochemical reduction of o-quinones, the product of the enzymatic reaction [26].

The direct electron transfer of hemoglobin (Hb) has been observed using an Hb-MWNT composite electrode, which exhibits excellent electrocatalytic activity to detect H_2O_2 to construct a third-generation mediator-free H_2O_2 biosensor [27-29].

The CNT-tyrosinase based biosensor can be used to estimate concentration of one of the most hazardous endocrine disruptors bisphenol A (BPA) which is a pollutant of the drinking and surface water as well as many products of the food chain [30].

The well-defined graphite chemistry of CNT helps to selectively functionalize CNT at the ends. Functionalization of carbon nanotubes can further improve the performance of an electrochemical biosensor. So far, carboxylic acid, carbodiimide ester, fluorine, amines, alkyl groups have been functionalized on a CNT. Functionalization of single wall carbon nanotubes (SWNTs) with an enzyme

via the formation of a covalent bond as shown by Fourier-transform infrared (FTIR) spectroscopy offers architecture of a three-dimensional enzyme array for application to biosensors [31]. Functionalized carbon nanotubes (CNTs) can be employed in an electrochemical cell to serve as a biosensor to specifically detect either lactate or pH in an electrolyte solution of artificial sweat [32]. The functionalized and shortened CNT can be vertically aligned on the electrode through covalent binding. These are called carbon nanotube array electrodes and can be uniquely suited to analytical applications by coupling sensing biomolecules to carboxyl-terminated ends [33-34] of the nanotubes.

CNT arrays are also referred to as microelectrodes. These microelectrodes have been utilized for molecular diagnosis [35-36] and these can be directly integrated with microelectronics and microfluidics systems to provide miniaturization and multiplex detection systems with high sensitivity. The observation can be explained by the size similarity of shortened functionalized carbon nanotubes and the biomolecules since the nano-scale diameter may promote intimate contact of SWCNTs within the electron tunneling distance of redox site of biomolecule.

Chen et al. have fabricated composite electrodes consisting of functionalized MWCNTs, Au NP nanoparticles and hydroxypropyl-b-cyclodextrin (HPbCD), deposited on glassy carbon electrode, gold and ITO surfaces, respectively for simultaneous determination of tyrosine (TYR), guanine (GU), adenine (AD) and thymine (THY) present in pH 7.4 aqueous solutions. These electrodes have the advantages of ease of fabrication, high reproducibility and long-term stability [37]. However, the biomolecules based sensors suffer from the stability point of view. It has been reported that the vertically aligned MWCNT modified glassy carbon electrode can be used to act as a glucose sensor without the enzyme immobilization in alkaline medium [38-40]. A substantial decrease (+ 400 mV) in the glucose oxidation over voltage has been reported. The characteristics of this electrode are high sensitivity, fast response time and stability. It gives a high sensitivity in presence of chloride ion up to 0.20 M and has the limited selectivity towards other bioactive molecules [39].

5. Fabrication of carbon nanotubes based bioelectrodes

5.1 Amperometric carbon nanotubes based bioelectrodes

Carbon nanotubes have been found to play important role for the fabrication of biosensing electrodes. Most commonly used to fabricate CNT based electrodes are CNT coated electrodes [41-42] and CNT/binder composite electrodes [43-44].

The insolubility of CNTs in common organic and inorganic medium has limited its applications in biosensors. However, CNTs dispersed in bromoform, Teflon, mineral oil or soluble in sulfuric acid and Nafion polymer can be utilized for the development of desire amperometric biosensors [11, 44-45].

Carbon nanotubes paste based amperometric electrode (CNTPE) can be prepared by using the CNTs-mineral oil (60/60 % w/w dispersion) paste into a cavity of Teflon or glass tube (3.0 nm diameter). The desired biomolecules can be immobilized by immersing these micro tubes in a buffer solution containing required biomolecules in specific amount by applying a constant potential for a given time. These microtubes possess higher loading capacity due to large surface area of CNT and enhanced electron transfer rate. CNTPE detects hydrogen peroxide at 0.95 V as compared to 1.33 V of

CP [46]. Interestingly, a binderless biocomposite microelectrode has been reported [47]. CNT, graphite powder and GOx mix is filled into a polyimide tube (300 μm) by dipping the tube into a mixture of CNT, graphite and GOx. The resulting composite is inserted into a 21 gauge needle. The surface is smoothened and coated with Nafion film. This binderless composite needle electrode provides higher stability, mechanical strength, conductivity, reproducibility, shorter response time as compared to CNTPE since the binder mineral oil slows down the electron transfer process.

CNTs cast electrode can be fabricated by dispersing CNTs into conc. H_2SO_4 (1 mg/ml). A solution of biomolecule alone or accompanied with a covalent binder can be physically adsorbed on the cast electrode. The screen printer can be used for fabricating transducer containing Ag/AgCl and the CNTs as working electrode. CNTs based screen printing ink is printed on a polyester substrate through a patterned stencil to yield a definite number of strips [48]. The enzyme or protein molecule can be physically adsorbed on this electrode.

The challenge of solubilizing CNTs has been solved either by their covalent modification or non-covalent functionalization [49-50]. However, wrapping of CNTs by a polymeric chain is found to improve the solubility without impairing its physical properties. Composite materials based on wrapping of CNTs with poly (p-phenylenevinylene or m-phenylenevinylene)-co-[2,5-dioctyloxy-(p-phenylene)-vinylene] have been reported. Nafion film (perflurosulfonated polymer) has been used to solubilize both SWCNTs and MWCNTs with dramatic enhancement in the electron transfer rate is observed in the detection of hydrogen peroxide and NADH [51] due to unique ion exchange property and biocompatibility.

The MWCNTs can be dispersed into PMMA binder wrapped by a cationic polymer poly (diallydimethylammonium chloride) (PDDA) to fabricate a MWCNT/PMMA composite without affecting the properties of CNTs. The conducting medium is CNTs and the whole composite serves as enzyme reservoir [52].

5.2 Functionalized carbon nanotubes based bioelectrodes

CNTs can be functionalized by applying a suitable potential or treating with oxidizing agents like H_2SO_4, HNO_3 or air to produce functional groups like COOH so that its potentiality towards bonding and electron transfer for biodevices can be improved. Goodling et al. have functionalized a CNTs modified gold electrode [53] as per reaction scheme (Fig. 3). The electrochemically treated gold electrode is dipped in an ethanol solution of cysteamine to produce a self-assembled monolayer (SAM). The functionalized and shortened carbon nanotubes on treatment with dimethylformamide (DMF) with dicyclohexyl carbodiimide (DCC) converts carboxyl groups at the end of the shortened SWCNT into active carbodiimde esters. The SAM modified gold electrode is placed in a carbon nanotube solution while the amines at the terminus of the SAM formed amide bonds with one of the end of the carbon nanotubes. The aligned CNT on the electrodes are placed in the form of a bundle of 5-20 tubes. These aligned CNTs can act as molecular wires between the electrode and the redox site of the biomolecule resulting in the enhanced electroactivity.
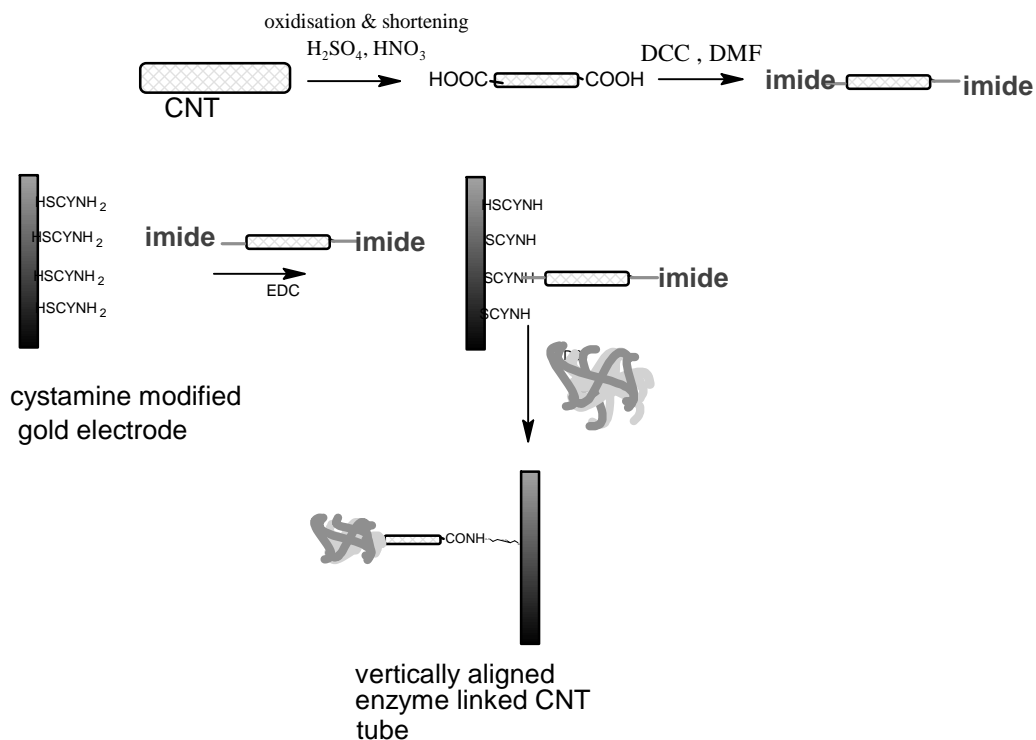
Figure 3. Scheme for the preparation of aligned CNTs functionalized with biomolecules.

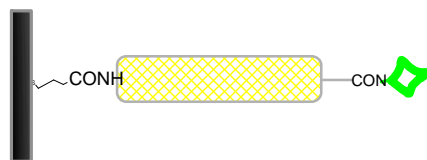These aligned CNTs are also referred as nano-electrodes (Fig.4).

Figure 4. CNT nanoelectrode.

Lin et al have reported the development of CNTs-nanoelectrode ensembles (NEEs) based amperometric biosensor [54]. Such NEEs comprise of millions of nanoelectrodes (each of them has less than 100 nm diameter). These NEEs are placed in epoxy polymer which is on chromium coated silicon substrate. The enzyme is immobilized on these surfaces by the reaction between carboxylic acid group of CNT and amine residue of enzyme through a linking molecule.

The immobilization of biomolecules into CNT modified matrix can be done by two ways: (i) small biomolecules are entrapped into the inner channel of open carbon nanotubes by physical adsorption and (ii) attachment of the biomolecule on the outer surface of carbon nanotube either by hydrophobic or electrostatic interactions or through covalent bonding [55-56].

The CNT modified electrodes find potential applications in clinical diagnosis, food processing, detection of toxicants, military and defense etc.

6. Applications of carbon nanotubes to biosensors for clinical diagnostics
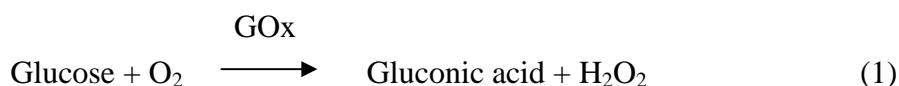
6.1 Enzymes based carbon nanotube electrodes

Enzyme based electrodes have received great attention because they are very sensitive, highly selective and fast responding. They are effective analytical tools for industrial, environmental and clinical applications. At present, enzyme sensors for determination of glucose, lactate, pesticides, urease based on electrochemical oxidation or reduction of hydrogen peroxide and NADH have been widely studied [57-63].

These are formed by the oxido-reductase enzyme. These sensors are used to analyse important chemicals like glucose, lactate, cholesterol, amino acids, urate, pyruvate, glutamate, alcohol, hydroxybutyrate to generate electrochemical detectable products NADH and hydrogen peroxide.

6.1.1 Carbon nanotubes based glucose biosensor

Glucose biosensor is one of the most studied biosensors. Glucose biosensor is based on glucose oxidase that catalysis the oxidation of glucose to gluconic acid:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \tag{1}$$

At the electrode:

$$O_2 + 2e + 2H^+ \longrightarrow H_2O_2 \tag{2}$$

The glucose sensor can be fabricated by monitoring (i) oxygen concentration (ii) pH of the medium and (iii) hydrogen peroxide concentration. Incorporation of carbon nano- particles can enhance stability, sensitivity and decrease response time.

CNTs modified GOx electrode can reduce the detection potential of hydrogen peroxide resulting in enhanced stability of the electrode [64-65]. CNTs/Nafion/GOx coated electrodes have been found to offer a highly selective low potential (-0.05 vs Ag/AgCl) glucose biosensor electrode [12]. The deposition of Pt nano particles on Nafion containing CNT/GOx film particles have resulted in detection limit upto 0.25 nM as compared to 1.5 µM and 150 µM with Pt/GOx and CNTs/GOx alone and response time is 3s [66]. Pt nano particles are used to keep the electrical contact through SWCNTs with the glassy carbon electrode enabling the composite structure to fabricate glucose oxide sensor that could be reused. When CNTs are used in combination with other metallic NP or quantum dot, a synergistic effect is observed [67-75]. Recently, Chen et al have developed Nafion biocomposite glucose oxidase electrochemiluminescence (ECL) biosensor which is found to be very useful for determination of glucose concentrations in real serum samples with satisfactory results [76].

The CNTs–mineral oil paste electrode exhibits a high linear range upto 30 mM glucose and low detection level up to 0.6 mM [44]. No interference is observed in the presence of ascorbic acid and uric acid. The non-conducting medium mineral oil reduces the overall electroactivity of the electrode. This problem can, however, be overcome by a needle biosensor for continuous glucose monitoring

based on packing of binderless GOx-CNT composite within 21 gauge needle by Wang et al. [47]. The sensitivity increases rapidly upto 25 % CNT loading. This biocomposite CNTs/GOx electrode con be used to provide detection of hydrogen peroxide at very low potential (-0.1 V vs Ag/AgCl). As the enzyme is protected within a CNTs-needle, the electrode could sustain thermal stress upto 90^0 C for about 24 hrs. This biosensor has been found to have high sensitivity, selectivity, higher linear range (upto 40 mM), prolonged life time and oxygen independence.

Bestman et al. have reported a field-effect-transistor based glucose biosensor [77]. GOx is attached to a SWCNT side wall through a linking molecule. Such enzyme immobilization is found to have decreased electrical conductivity due to change in the total capacitance of SWCNT. The resulting electrode can act as pH sensor. The conductivity of the sensor is increased by adding glucose when a gate voltage is applied between standard electrode and the semiconducting SWCNT.

Intrinsic conducting polymer based biosensors are known as the third generation biosensors as the conducting medium offers efficient electron transfer pathway. Incorporation of CNTs into the conducting polymer produces an advanced level of third generation biosensors [78-79]. The low-potential detection of the liberated hydrogen peroxide for glucose biosensor based on iron loaded nanotube tips, coated with conducting polymer (polypyrrole) has been reported by Wallace et al. [80]. The intimate contact of the biorecognition element with the polymer is obtained as the GOx is immobilized during electropolymerization of pyrrole enabling efficient electron transduction. It has been reported that incorporation of ferrocene mediator in PPy based GOx biosensor increases the sensititvity up to 1.5 A $mM^{-1}cm^{-2}$ and decreases the response time to 2s [81]. Interestingly, the glucose electrode, constructed by co-immobilization of HRP and GOx in an electropolymerized pyrrole (PPy) film on a single-wall carbon nanotubes (SWNTs) coated electrode has shown excellent performance in the actual blood serum but it has limitation of poor shelf life [82].

Carbon nanotube based biosensors eliminate the need of mediator resulting in the decreased response time and increased shelf-life. The difficulty in GOx enzyme is that the redox center is entrapped deep into the glycoprotein shell and the enzyme cannot be oxidized or reduced at any potential. A direct electron transfer between GOx and annealed SWCNT has been reported by Guiseppi et al. [83]. It is observed that both GOx and FAD are adsorbed spontaneously on unannealed CNT, cast on a glassy carbon surface and exhibits quasi-reversible one electron transfer. Similarly, GOx when adsorbed on annealed CNTs, shows a quasi-reversible one electron transfer. The decrease in the over potential of hydrogen peroxide as well as direct electron transfer of glucose oxidase (GOx) has been observed at a CNT modified electrode since CN tubes are positioned within the tunneling distance of cofactors resulting in reduced denaturation.

The carboxylated open-ends of nanotubes have been used for the immobilization of the enzymes. Aligned reconstituted GOx on the edge of SWCNTs can be linked with an electrode through covalent bonding. These aligned SWCNTs can act as a molecular wire between electrode and immobilized GOx at the end of a nanotube. It has been found that electrons can travel a distance more than 150 nm through the lengths of SWCNTs and the rate of interfacial electron transfer depends on the length of SWCNTs. The rate constants are 42 s^{-1} and 19 s^{-1} for 50 nm and 100 nm long SWCNT, respectively. The efficient electron transfer has been possible due to the covalent coupling of GOx on the electrode [84]. Xu et al have reported a high rate of single electron transfer of 1500 s $^{-1}$, exceeding the rate of

oxygen reduction by glucose oxidase for the CNTs array electrode [85]. The enhanced electron transfer rate of GOx (7.73/s) in the CNTs/chitosan system, which is more than one-fold higher than that of flavin adenine dinucleotide (FAD) adsorbed on the carbon nanotubes ($3.1s^{-1}$) is explained by the conformational change of GOx in the microenvironment enabling accessibility of active sites of GOx to the electrode [86].

Luong et al. have observed enhanced electron transfer rate between GOx and MWCNT-modified glassy carbon electrode [42]. The 3-amino-propyltriethoxysilane (APTES) is used for both modifying CNT and as the immobilizing medium for GOx to fabricate a reagentless glucose biosensor. The bundles of ultra micro electrodes actually facilitate access active site of FAD for direct electron transfer. The biosensor shows glucose response at -0.45 V (Ag/AgCl) (which is much lower than the non-covalent bonded electrode) in the presence of the common interfering species, uric acid, ascorbic acid, and acetaminophen, respectively. Amperometric biosensor based on multi-walled (MWCNT) grown on a platinum (Pt) substrate has been reported by Chaniotakils et al. [87]. The opening and functionalization by oxidation of the carbon nanotube array allows efficient immobilization of glucose oxidase enzyme. The carboxylated open-ends of nanotubes are used for the immobilization of the enzymes while the platinum substrate provides the direct transduction platform for signal monitoring. Two types of CN tube arrays have been utilized e.g. (1) acid oxidized and (2) air oxidized. It has been observed that acid treated showed better performance than the air oxidized.

Chen et al. have reported a glucose biosensor based on a nine multiplayer films made by MWCNT, GNP and GOx on platinum electrode [88]. The resulting biosensor exhibits wide linear range (0.1-10 mM), high sensitivity (2.527 μA $M^{-1}cm^{-2}$), detection limit 6.7 μM and quick response time (within 7 s). Khor et al. have fabricated transparent and flexible glucose biosensor consisting of MWNTs and glucose oxidase layer-by-layer (LBL) self-assembled film on a PET substrate [89]. This performance, combined with the large area preparation process, demonstrates that this CNT-based multilayer biosensor can be exploited for commercial applications.

A direct electron transfer between enzyme and electrode has been reported by Okuma et al. in SWCNTs-GOx-11-(ferrocenyl)-undecyltrimethylammonium bromide (FTMA) amperometric biocomposite electrode. It is found that 11-(ferrocenyl)- undecyltrimethylammonium bromide (FTMA) has value of different critical micelle concentration (CMC) in its reduced and oxidized forms (7×10^{-5} and 1×10^{-3} mol L^{-1}). The formation and disruption of vesicles in FTMA has been monitored at constant potential. The electron-transfer coefficient and electron-transfer rate constants are found to be 0.70±0.3 and 2.69±0.03/s, respectively. The electrode retains 78.62% of its initial response after 50 cycles [90].

The GOx and horse radish peroxidase (HRP) have been co-immobilized in functionalized MWCNT- poly (amidoamine) dendrimer (PAMAM). The bi-enzymatic CNT-PAMAM nano-composites are highly dispersible in water and show high sensitivity to glucose with current response of 2200 nA mM^{-1} and fast response (1s). The negative electrode potential of 0.34 V is suitable for glucose detection in real samples without interference caused by other biomolecules [91].

Gorton et al. have developed a new type of glucose biosensor based on glucose dehydrogenase (GDH) and diaphorase (DI) co-immobilized with NAD+ into a carbon nanotube paste (CNTP) electrode, modified with an osmium functionalized polymer (Poly(1-vinylimidazole)12-[osmium(4,4-

dimethyl-2,2'-dipyridyl)$_2$Cl$_2$]$^{2+/+}$). The biosensor can be used for the determination of glucose in different samples of sweet wine and the results are in good agreement with the commercial spectrophotometric enzymatic kit [92].
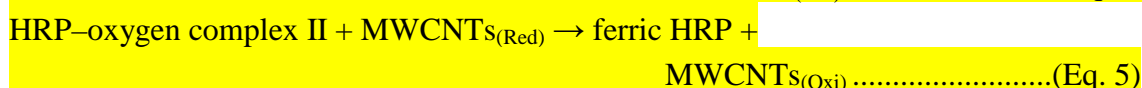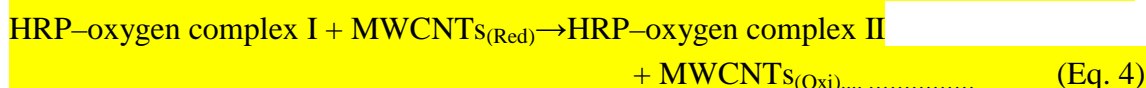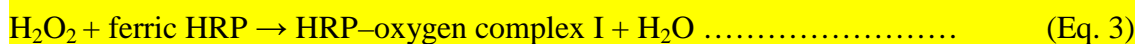

6.1.2 Carbon nanotube based cholesterol biosensor

Total cholesterol in blood is determined by a cholesterol sensor, constructed by co-immobilization of cholesterol esterase, cholesterol oxidase, peroxidase, potassium ferrocyanide and MWCNTs on a carbon paste electrode. The testing results have shown a fairly good correlation with clinical laboratory method while only 2 μL blood sample is required for a test [93].

A new type of amperometric cholesterol biosensor based on sol-gel chitosan/silica and multi-walled carbon nanotubes (MWCNTs) organic–inorganic hybrid composite material has been developed. The hybrid composite film is used to immobilize cholesterol oxidase on the surface of Prussian blue-modified glass carbon electrode. This sensor has been used to estimate free cholesterol concentration in real human blood samples [94].

6.1.3 Carbon nanotubes based horseradish peroxidase biosensors

Hydrogen peroxide is the main by-product of many enzyme catalyzed reactions based on glucose oxidase, choline oxidase, alcohol oxidase, cholesterol oxidase and lactate oxidase etc. It is also an essential in food, pharmaceutical and environmental analysis. Ampereometric biochemical sensors based on horseradish peroxide enzyme, for the determination of hydrogen peroxide are the most promising due to their high sensitivity and simplicity. CNT modified sensors offer many more advantages due their unique electronic, physical and mechanical properties. Besides this, nano-sized CNTs has other advantages like unique size distribution and hollow geometry. CNTs can provide excellent electronic communication with redox centers of the immobilized enzyme which in fact provides reversibility, low response time, signal amplification [95-96]. The mechanism of electrocatalysis reduction to H$_2$O$_2$ with MWCNTs–HRP electrode can be explained as follows:

$$H_2O_2 + \text{ferric HRP} \rightarrow \text{HRP–oxygen complex I} + H_2O \dots\dots\dots\dots\dots\dots \quad \text{(Eq. 3)}$$

$$\text{HRP–oxygen complex I} + \text{MWCNTs}_{(Red)} \rightarrow \text{HRP–oxygen complex II} + \text{MWCNTs}_{(Oxi)}\dots\dots\dots\dots \quad \text{(Eq. 4)}$$

$$\text{HRP–oxygen complex II} + \text{MWCNTs}_{(Red)} \rightarrow \text{ferric HRP} + \text{MWCNTs}_{(Oxi)} \dots\dots\dots\dots\dots\dots \text{(Eq. 5)}$$

$$\text{MWCNTs}_{(Oxi)} + 4e \rightarrow \text{MWCNTs}_{(Red)}\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(Eq. 6)}$$

The oxidized MWCNTs are electrochemically reduced at the electrode yielding an enhanced reduction current. CNTs modified electrode can detect trace levels of H$_2$O$_2$ and used to construct a compound enzymatic biosensor.

Yammoto et al. [97] have reported funtionalized MWCNT modified glassy carbon electrode wherein HRP along with glutaraldehyde is immobilized by physical adsorption [CNT+HRP]. This

electrode shows excellent response of reduction current for determination of H_2O_2 at -300 mV (*vs.* Ag/AgCl) which is much below the interference from ascorbic acid, uric acid and other electroactive substances. This biosensor has been used for on-line monitoring of hydrogen peroxide

Rustling et al. [98] have reported horseradish peroxide immobilized CNT modified amperometric array electrode wherein HRP is covalently linked to carboxy modified SWCNT by following conventional carbodiimide chemistry. Quasi-reversible $Fe^{(III)}/Fe^{(II)}$ voltammetry is observed around 0.25 V (vs. SCE) for the HRP based SWCNT array electrode with detection limit of 100 nM and stability of a few weeks. These carbon nanotube based array electrodes behave electrically similar to a metal, wherein conducting electrons emanate from the external circuit to the redox sites of the enzymes. These ultra-sensitive microelectrodes have the possibility of fabrication of multi-element nanobiosensor arrays.

HRP immobilized electrochemical biosensor has been reported by Xu et al. [99]. In this assembly HRP and methylene blue are coimmobilized on MWCNT coated glassy carbon electrode and the methylene blue acts as an electron carrier between enzyme and the surface. The magnetic multi-walled carbon nanotubes (M-MWNTs) can be prepared by introducing Fe_2O_3 nanoparticles into the carbon nanotubes. The three-dimensional M- MWNTs/HRP multilayer films have showed satisfactory stability, biocompatibility and electrochemical properties [100].

6.1.4 Carbon nanotubes based nicotinamide adenine dinucleuotide hydride (NADH) biosensors

Carbon Nanotubes based biosensors have been fabricated by coimmobilization of dehydrogenase enzyme and nicotinamide adenine dinucleuotide (NAD^+) as the cofactor. The biochemical reaction that governs the sensor activity relates to the oxidation of NADH to NAD^+. The basic problems associated with these types of sensors are (i) high overvoltage of NADH oxidation (ii) biofouling effect and (iii) accumulation of reaction products. Use of CNTs can result in the decrease of the oxidation potential of NADH [43, 101-103]. The CNT modified electrodes have shown substantial decrease in overvoltage due to the incorporation of SWCNT and MWCNT. CNT modified non-enzymatic glassy carbon electrode exhibit strong and stable electrocatalytic response towards NADH [15]. A substantial (490 mV) decrease in the overvoltage of the NADH oxidation reaction (compared to ordinary carbon electrodes) is observed using single-wall and multi-wall carbon-nanotubes coatings. The peak currents show an increase of 1.6 fold for MWCNT and SWCNT 2.3 fold larger than the corresponding peak at the bare surface. The CNTs result in enhanced electron transfer rate for NADH oxidation. The CNTs-coated electrodes provide a highly sensitive, low-potential and stable amperometric sensor. Wang and Musamach [45] have reported amperometric ethanol biosensor based on CNTs/Teflon matrix. This sensor is prepared by the coimmobilization of alcohol dehydrogenase (ADH) and NAD+. The oxidation potential of NADH is reduced to 0.6 V. The CNT modified electrode can substantially reduce the detection potential of alcohol. The reagentless biocomposite responds favorably to the 1 mM ethanol additions at potential of +0.20 V. Response time is 60 s and no response is observed for the carbon nanotube electrode under identical conditions. Another biosensor is fabricated based on alcohol dehydrogenase (ADH) into a colloidal gold (Aucoll) - MWCNTs composite electrode using Teflon as binding material [104].

6.2 Carbon nanotubes based DNA biosensors

DNA biosensors are getting increased importance for detection of genetic and contagious disease, drug discovery or warning against bio-warfare agents due to rapidity, simplicity and accuracy. This biodetection commonly relies on hybridization or antigen-antibody (Ag-Ab) interactions.

The carbon nanotubes (CNTs) can be used to amplify the enzyme-based electrical sensing ability of proteins and DNA. The CNTs modified DNA electrode eliminates the need for indicator addition, association and detection steps. A direct reading of electrochemical DNA biosensors has the advantage of rapid and multi-analytes detection.

The electrochemical response of natural DNA is poor as the redox site of guanine and adenine in DNA are hidden in the double helix structure whereas the denatured DNA can give better electrochemistry. It has been assumed that in the denatured DNA, double helical structure of DNA is broken and the hidden redox site is opened. SWCNTs modified electrode shows remarkable improvement in the signal enhancement of the oxidation peaks of adenine and guanine in comparison with those other reference electrodes reported [105-107]. The high aspect ratio of CNTs, conjugated π electron systems, topological defects provide well defined electrochemistry of natural DNA at CNTs modified electrode. Two distinct peaks at 0.7 V and 0.97 V pertaining to the oxidation peak of guanine and adenine are observed in cyclic voltammogrammes of DNA at SWCNTs modified electrode [108]. The accumulation of natural DNA at the SWCNT-modified electrode causes a DNA/SWCNTs interaction, resulting in the unwrapping of the DNA double helix structure and exposure of the primary redox sites of adenine and guanine residues in natural DNA. Similar phenomenon is observed for the guanine DNA response at the MWCNTs paste electrode. In some cases, guanine amplification signal can be increased even by 11 folds by MWCNTs modified CG electrode [109]. The amplified purine signal is used to find ultratrace measurements of various nucleic acids and the detection of the purine content in DNA and RNA samples including estimates of the guanine/adenine ratio. The similar principle is used for the rapid detection of indicator free DNA and RNA concentration using screen printed carbon electrode modified with MWCNTs. With an accumulation time of 5 min, it can be used to detect *calf thymus* ssDNA ranging from 17.0 to 345 μg mL^{-1} with detection limit of 2.0 μg mL^{-1} and tRNA ranging from 8.2 μg m L^{-1} to 4.1 mg mL^{-1} [110].

Rivas and Pedano have found remarkable increase in the guanine oxidation signal from oligo and polynucleotides using functionalized MWCNT paste electrode as compared to the normal graphite electrode (CPE). The electrode is able to detect 2μg L^{-1} for 21 bases oligonucleotides and 170 μg L^{-1} for *calf thymus* ds DNA. The interaction between MWCNTPE and nucleic acid is considered to be of hydrophobic type. The stability of the electrodes in different environment like air and in various buffers solution from pH 5.0 to 7.8 have led its environmental stability [46].

Trace of dopamine assay is an important neurotransmitter in the mammalian system since it can cause Perkinson's disease and other similar diseases. DNA immobilized CNTs paste has been used to monitor dopamine ion concentration. The signal of the above assembly has been found to be very sensitive compared to other system. Under optimum analytical condition the above sensor can detect a very low value upto a level of 4.0 ng L^{-1} at the 3.0 mg L^{-1} dopamine concentration [111]. This system exhibits a wide linear range upto 150 mg L^{-1}.

6.2.1 Carbon nanotubes based DNA hybridization biosensors

   Human genome project with an objective of detecting gene sequence has brought out a new area in the field of medical diagnosis. DNA hybridization (fig 5a) arrays are a common form of screening technology allowing the analysis of hundred to thousands genes in parallel.
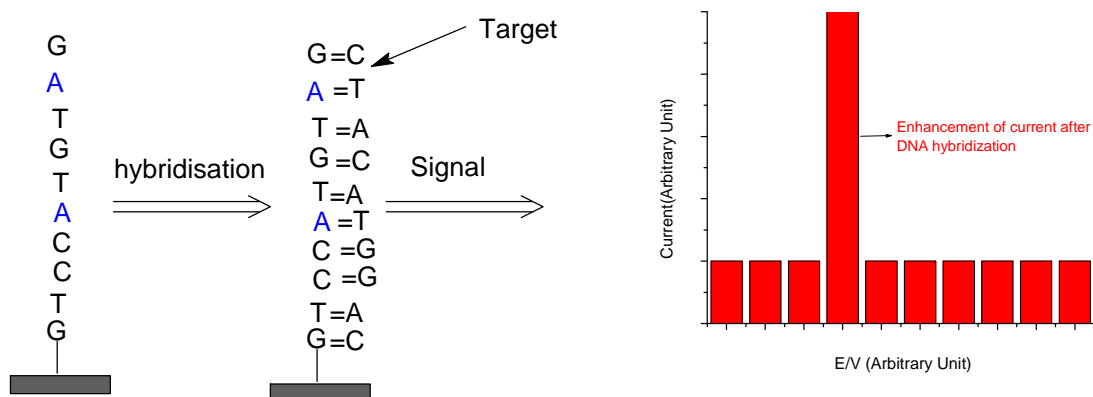

Figure 5. (a) DNA hybridization in a biosensor (b) Electrochemical detection of DNA hybridization.

   An oligonucleotide of 15 to 21 bases is usually immobilized in a transducer. The sensitivity, selectivity, reproducibility and rapidity of DNA biosensor depends on the method of immobilization adopted, i.e it should not restrict the free configuration of probe and the target which in fact can restrict the movement during hybridization.   Fig. 5 (a,b) show the DNA hybridization and corresponding electrochemical detection. Many protocols have been proposed for electrochemical monitoring of DNA hybridization.

   Oligonucleotides labeled with enzymes such as horseradish peroxidase or alkaline phosphotase or electroactive tags such as ferrocene or anthraquinone have been employed in the hybridization detection protocols [112]. External labels such as anticancer drugs, metal complexes and organic dyes are used wherein electro activity of guanine, adenine or dopamine bases are exploited. The robust electronic structure, biocompatibility and chemical inertness of CNT can simplify DNA hybridization assays so that label-free DNA hybridization sensor can be fabricated which can offer an instantaneous detection of duplex formation and eliminate the need for the indicator addition/association/detection steps. Surface modified MWCNT glassy carbon electrode is found to improve remarkable signal amplification of guanine and label free electrical detection of DNA hybridization. In the presence of a target DNA, substantially high signal is obtained even at a very low concentration (250 µg L^{-1}) as compared to non- complementary DNA (25 mg L^{-1}). No signal has been observed for a control experiment without target DNA. Linearity upto 250 µg L^{-1} for hybridization is reported (20 min hybridization time) [109].

   The electrochemical biosensors based on immobilization of ss-DNA on the electrode surface, can be used for hybridization and duplex DNA [105]. Use of CNTs in this type of device enhances electrical signal and sensitivity. An application of conducting polymer along with CNTs offer a real challenge in the field of biosensors. Oligonucleotide-substituted conducting polymeric (polypyrrole film) film

changes its electrical properties after DNA hybridization. Nucleic acids can be incorporated into the growing conducting polypyrrole polymer chain [113]. The anionic oligonucleotide has been used as a dopant. The dopant can provide the necessary changes during polypyrrole oxidation maintaining its ability to recognize complementary target DNA, which is the basis of DNA hybridization. Cai et al. [114] have reported a label-free DNA probe doped polypyrole film over carboxy modified MWCNTs-glassy carbon electrode. After hybridization reaction with complementary DNA sequence, a decrease of impedance is attributed to the decrease of the electrode resistance which is reflected by the concomitant change in the electrical conductivity of the composite electrode. Usually DNA duplex formation does not produce significant change in electrical signal. Carboxy-MWCNTs modified conducting polymer allows intimate association between a biological recognition element and electrical conducting medium. The sensitivity is found to be 5 folds more in CNT modified device than without CNTs.

Cai et al. have developed an electrochemical DNA biosensor based on (MWCNT –COOH) modified glassy carbon electrode (GCE). Oligonucleotide is linked with CNTs through a covalent bonding between 5' amino group and COOH group. The biosensor is found to be useful to monitor hybridization reaction by the differential cyclic voltammetry (DPV) technique using daunomycin as an electroactive indicator. Large surface area of CNTs and covalent linking enhanced electroactivity, stability and loading capacity as compared to ordinary GCE electrode [115].

A novel and sensitive electrochemical DNA biosensor based on MWNTs/Nano ZrO_2/chitosan-modified glassy carbon electrode (GCE) has been fabricated and the hybridization reaction on the electrode monitored by differential pulse voltammetry (DPV) analysis using electroactive daunomycin as an indicator. The biosensor signal increases linearly with the increase of the logarithm of the target DNA concentration in the range of 1.49×10^{-10} to 9.32×10^{-8} mol L^{-1} with the detection limit of 7.5×10^{-11} mol L^{-1} [116].

Genomic information demands quick, reliable and robust detection technique. It has been shown that nanoscale sensing elements can be introduced to provide ultrahigh sensitivity. When functionalized, vertically aligned, carbon nanotubes can be embedded in some matrix like SiO_2, the resulting MWCNTs are considered as nano electrode arrays wherein open ends of MWCNTs are exposed at the dielectric surface act as nanoelectrodes. Combining the CNTs nanoelectrode array with $Ru(bpy)_3^{2+}$ mediated guanine oxidation method [117] hybridization of less than a few attomoles of oligonucleotide targets can be easily detected. The performance of the electrode is inversely proportional to the radius of the electrode. The open end of a MWCNT shows a fast electron transfer rate (ETR) similar to the graphite edge-plane electrode while the sidewall is inert like the graphite basal-plane. This nano electrode can be used for the detection of trace redox chemicals (such as metal ions, toxic contaminants, and neurotransmitters), immunoassay based pathogen detection, and EC detectors in microfluidic devices.

Fang et al. [118] have designed a bio protocol for DNA hybridization detection using mercapatoacetic acid (RSH)-coated magnetite nanoparticles, capped with 5-(NH_2) oligonucleotide as DNA probe. The DNA probe combined with functionalized MWNTs/PVP CME, proposed in this protocol is used successfully for the identification of the complementary sequence, non-complementary

sequence, and three bases mismatched sequence which indicates that it is useful in electrochemical immunoassays.

Highly sensitive bioelectronic devices have been designed to detect protein and DNA based on the coupling of several CNT-derived amplification process [119]. In this bioaffinity device the CNTs amplify both the biorecognition and the transduction, namely as a carrier for numerous enzyme tags and for accumulating α napthol products of enzymatic reaction. (Fig.6). The CNT array in combination with alkaline phosphatase (ALP) enzyme as enzyme a tracer to generate electrical signals are found to be extremely useful for ultrasensitive electrochemical bioaffinity assays of proteins and DNA. Such bioaffinity assays depend on the hybridization or antigen and antibody interaction. Coverage of 9600 molecules per CNT is estimated. Such bioaffinity protocols con detect DNA as well as proteins upto the 1.3 and 160 zmol in 25-50 μL samples indicating a great promise for PCR free DNA analysis. Such nano level detection can be explained by the large enhancement of signal amplification by the linking of CNT amplifier (ALP loaded CNT) to the magnetic beads through sandwich hybridization or antigen and antibody interaction.
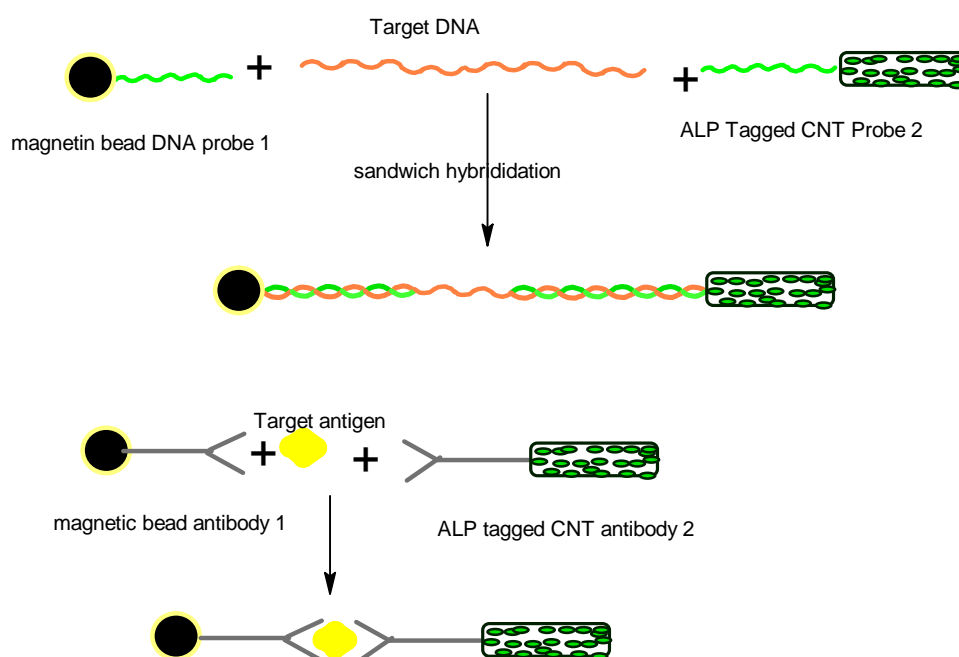
Figure 6. CNTs-derived amplification of the recognition and transduction events.

The MWCNT modified label-free DNA hybridization detector is evaluated on the basis of measurements of nucleic-acid segments related to the breast-cancer BRCA1 gene [109].

6.3 Carbon nanotubes based immunosensors

An immunosensor is used for selective estimation of proteins based on the specific antibody-antigen interaction. This analytical device works on changes in mass, heat, electrochemical, or optical properties. Park et al. [120] have developed carcinoembryonic antigen (CEA) immunosensor by immobilizing monoclonal antibodies on the side walls of SWCNTs on an FET using CDI-Tween 20 as

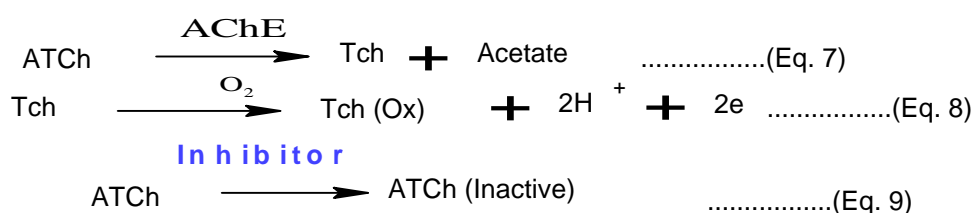the linker. Chen et al. have fabricated CNT based FET sensors for the biomarker detection with focus on the early diagnosis of cancer [121] and also for human autoimmune disorder [122]. The detection limit is as low as 1 nM L^{-1}. CNTs are found to be directly grown on the silicon substrate and the hemaglutinin antigen is immobilized on the reverse side of the substrate. The current-voltage (I-V) curves between the source and drain electrode of the FET is measured by changing the potential across the back gate of the control system [123].

A label-free electrochemical microelectrode has been reported by Okuno et al. [124] where in a Pt working microelectrode modified with SWCNTs has been utilized for the detection of monoclonal antibodies against total prostate specific antigen (T-PSA). Based on differential pulse voltammetry, the current increases with an increase in the T-PSA concentration. Functionalized CNTs have been incorporated into polyethylene vinyl acetate to form a composite [125] for the attachment of biotinylated antibodies, which could be extruded into sheets. Immunosenors have been developed by using SWCNT arrays on glass or silicon wafers with native oxide or quartz crystal microbalance resonators having gold electrodes [126]. It has been demonstrated that a nanosensor can be fabricated using highly oriented SWCNT forests in a configuration with HRP and myoglobin (Mb) linked to the ends of the nanotubes. The value of sensitivity to the changes in the concentration of H_2O_2 have been found as 0.049 and 0.033 $\mu A\ \mu M^{-1}$ with the detection limit of 50 and 70 nM for SWCNT/HRP and SWCNT/Mb, respectively [127-128].

6.4 Carbon nanotubes based biosensors for toxicant detection

Detection of organophosphorous (OP) compounds in micro level in food, water is an absolute necessity due to high toxicity. They are widely used in pesticides, insecticides and chemical warefare agents. These compounds affect the nervous system by inhibiting acetylcholinesterase (AChE) function of regulating the neurotransmitter acetylcholine. The world-wise increase in terrorist activities demands an urgent need for rapid, ultrasensitive (in the nanoscale) and cheap detection technique for these types of compounds. The AchE inhibition is monitored through the electrochemical oxidation of thiocholine or p-aminophenol and hydrogen peroxide. Inhibition of AchE by OP is irreversible, OP biosensor should be disposable, ultra-sensitive and low cost. The carbon nano tube based biosensor can fulfill all the needs except the cost [129]. Joshi et al have reported a disposable acetylchlolinase immobilized MWCNT modified thick film strip electrode [130]. The degree of inhibition of the enzyme acetylcholinesterase (AChE) by OP compounds is determined by measuring the electrooxidation current of the thiocholine (TCh) generated by the AChE catalyzed hydrolysis of acetylthiocholine (ATCh). The reaction scheme is shown as below:

$$ ATCh \xrightarrow{\text{AChE}} Tch\ +\ Acetate \qquad \dots\dots\dots(Eq.\ 7) $$

$$ Tch \xrightarrow{O_2} Tch\ (Ox)\ +\ 2H^+\ +\ 2e \qquad \dots\dots\dots(Eq.\ 8) $$

$$ ATCh \xrightarrow{\text{Inhibitor}} ATCh\ (Inactive) \qquad \dots\dots\dots(Eq.\ 9) $$

A disposable CNT functionalized biosensor has been developed and has high sensitivity, large linear range and low detection limit for the analysis of organophophorous compounds [130]. The biosensors using acetytchlolinesterase (AchE)/Choline oxidase (CHO) enzymes provided phosphorous compounds. The enzymes were coimmobilized on the CNTs by following carbodiimide chemistry through EDC as a coupling agent. The biosensor exhibits broad dynamic linear range up to 200 M, high sensitivity 0.48 % and low detection limit for methyl parathion (organophosphorous compounds).

The MWCNT modified electrode reduces oxidation potential upto 200 mV without any electron transfer medium. This biosensor can detect 0.5 nM of the model organophosphate nerve agent paraxon. An acetylcholinesterase (AChE) biosensor has been developed by Du et al. based on MWCNT-chitosan matrix for the investigation of pesticide based on the change in electrochemical behavior of enzymatic activity induced by pesticide. Four pesticides of carbaryl, malathion, dimethoate and monocrotophos have been used to demonstrate their inhibition efficiencies to AChE [131]. The inhibited AChE can be reactivated in a short time by using pralidoxime iodide.

Biosensor for the detection of organophosphorous compounds based on CNTs modified transducer has been reported by the immobilization of organophosphorous hydrolase biocatalyst. The CNTs layer improves anodic detection of the enzymatically generated p-nitro phenol product with a high a level of accuracy and sensitivity. This biosensor is able to detect as low as 0.15 μM paraxon and 0.8 μM methyl parathion with sensitivities of 25 and 6 mA $M^{-1}cm^{-2}$, respectively [132]. The high sensitivity is achieved due to promoted electron transfer activity of CNT which in fact reduces the detection potential and hence increases the stability of the immobilized enzyme. This is also associated with elimination of surface fouling effect which occurs during the oxidation of phenolic product.

Zhang et al. have reported a sensitive, fast and stable amperometric sensor for quantitative determination of organophosphorous insecticide by immobilization of acetylcholinesterase (AChE) on MWNTs–chitosan (MC) composite. Atomic force microscopic studies have showed that this matrix possesses homogeneously netlike structure, which prevents the enzyme from leaching [133].

Jun Wang et al. [134] have reported amperometric choline biosensor based on the electrostatic assembly of the choline oxidase (ChO) enzyme and a bienzyme of ChO and horseradish peroxidase (HRP) onto multiwall carbon nano tubes (MWCNT) modified glassy carbon electrodes. These choline biosensors are fabricated by immobilization of enzymes on the negatively charged MWCNT surface by following layer by layer technique using alternate layer of cationic poly(diallyldimethylammonium chloride, PDDA) layer and an enzyme layer. The ChO/HRP/CNT biosensor is more sensitive than ChO/CNT biocomposite. The linear range is 5×10^{-5} to 5.0×10^{-3} M and the detection limit is about 1.0×10^{-5} M.

An amperometric biosensor based on choline oxidase (ChOx) is fabricated by immobilization of ChOx into a sol–gel silicate film on the MWCNT modified platinum electrode. The catalytic property of MWCNT is further exploited as a selective determination scheme for choline in the in the presence of other electroactive compounds. This biosensor has been used to detect choline released from Lecithin by phospholipase D (PLD) in serum samples [135].

The choline biosensors, fabricated by layer-by- layer assembled functionalized MWNTs and PANI multilayer film, have exhibited a wide linear range 1×10^{-6} to 2×10^{-3} M and the response time is 3s [136].

6.5 Other carbon nanotubes based biosensor

Catalase is a heme protein in the group of oxidoreductases with ferriprotoporphyrin-IX at the redox center, and it catalyzes the disproportionation reacation of hydrogen peroxide. Like other enzymes, it is difficult for catalase to transfer electrons as they have big and complex structures, where the redox centers deeply immerse in the bodies. CNTs modified electrodes enhance the electrical contact between the electrode and the catalase.

The catalase/CNT modified gold electrode has been reported by Zhou and coworkers [137]. The catalase modified electrode displayed catalytic wave upon addition of hydrogen peroxide. Salimi et al have shown direct electron transfer in catalase immobilized MWCNT biosensor for a pair of well-defined and nearly reversible cyclic voltammetry peaks for Fe(III)/Fe(II) redox couple of catalase. The high value of Michaelis–Menten constant (1.70 mM) indicates potential applicability of the electrode as a reagentless biosensor based on the direct electrochemistry of the catalase enzyme [138].

An amperometric lactate dehydrogenase (LDH) biosensor based on MWCNT-chitosan at glassy carbon electrode has been developed for the determination of lactate with a sensitivity of $0.0083A\ M^{-1}cm^{-2}$ and a response time of about 3s [139]. This biosensor can retain 65% of its original response after 7 days. This work should include the effect of interfering agents. Li et al. have constructed a positively charged chitosan/PVI-Os(polyvinylimidazole-Os)/CNT/LOD (lactate oxidase) nano-composite on gold electrode for detection of lactate. The positively charged chitosan and PVI-Os are used as the matrix and the mediator to immobilize the negatively charged LOD and to enhance the electron transfer, respectively. FESEM (field emission scan electron microscopy) and electrochemical characterization have proved that CNT has behaved as a cross-linker to network PVI and chitosan due to its nanoscaled and negative charged nature. This biocomposite has shown improved conductivity. This enzyme biosensor has potential applications in diagnostics, life science and food analysis [140]. Lactate biosensors show potential commercial applications [141-145].

Gorton et al. have immobilized pseudomonas putida DSM 50026 cells as the biological component on the MWCNTs modified carbon paste electrodes (CPE) by using a redox osmium polymer, viz. poly(1-vinylimidazole)12-[Os-(4,40-dimethyl-2,20-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ and the measurement is based on the respiratory activity of the cells estimated from electrochemical measurements. The electrode has been characterized by using glucose as substrate. Furthermore, the microbial biosensor can be prepared using phenol adapted bacteria for detection of phenol in an artificial waste water sample. The main disadvantages are the increase in the background current and the diffusion problem of electrons that occur due to overlapping of the diffusion layers formed at closely spaced CNT in the film [146]. Table I shows the characteristics of various CNT based biosensors reported in literature.

7. Future scope

In spite of spectacular advancement in understanding the carbon nanotubes structure-property relationship, there are still many challenges for making well-defined carbon nanotubes with narrow size distribution including studies on structure-topology-property relations. The fact that CNTs and biomaterials such as enzymes, antibodies or nucleic acids and proteins are of similar dimensions

making the nano tube biomolecule assembly attractive elements for biosensor devices. It is clear that in the field of biosensors wherein CNTs can be used as a transducer matrix or matrix modifier, there are a lot of opportunities. The CNT-derived bioaffinity protocol can open new horizon for medical diagnostics and protein analysis such as (i) carbon nanotubes may help us to develop a biosensor without the immobilization of biomolecules so that the restrictions associated with the biomolecules including denaturation of the biomolecules, suface fouling effect by the accumulation of enzyme catalyzed products effects, poor response time can be overcome. (ii) vertically aligned functionalized CNTs can be used for size miniaturization and multianylates detection in the direction of fabrication of biochips which is the ultimate goal of biosensor. (iii) CNT modified sensor have potential application in the fabrication of electronic nose, ear etc and (iv) DNA and organophorous based bio sensors have wide scope in the field of gene sequence detection and warning against terrorist activities which has got a big appeal in the welfare of mankind. It is expected that CNT modified bio-devices have promising applications in clinical diagnostics, environmental monitoring, security surveillance and for ensuring food safety.

Acknowledgements

Table 1. Characteristics of various carbon nanotubes based biosensors as reported in the literature.

S. No.	Biomolecules	Matrix	Linear range	Detection limit	Sensitivity	Response time (sec)	Reference
1	GOx	Nafion-CNTs-CdTe-GOD	0-5mM		$1.018 \mu \tilde{A}\ mM^{-1}$		[73]
2	GOx	Pt-CNT-GOx	1.2×10^{-4} to 7.5×10^{-3} M	1.0×10^{-5} M		< 10	[74]
3	GOx	GOD-CNT-PtNP-CS-MTOS-GCE	1.2×10^{-6} - 6.0×10^{-3} M	3×10^{-7}	$2.08\ \mu A\ mM^{-1} cm^{-2}$	5	[67]
4	GOx	Nafon-GOx-GNP-PtNP-CNT-GOx	0.5 to 17.5 mM				[68]
5	GOx	PtNW-CNT-CHIT-GOx	5×10^{-6} to 1.5×10^{-2} M	3.0 Mm	$30mA\ mM^{-1}cm^{-2}$	<10	[72]
6	GOx	Pd-GOx-Nafion CNT	Up 12 mM	0.15mM			[75]
7	GOx	MWNT/GOx	5.0×10^{-6} ~ 8.0×10^{-4} mol	2.0×10^{-6} mol L^{-1}	-	-	[76]

			L^{-1}				
8	GOx	CNT/Mineral oil/GOx	0-30	0.6 mM L^{-1}	-	-	[87]
9	GOx	PPy–cMWNTs–GOx	up to 4 mM	-	95 nAmM cm^{-2}	~8	[78]
10	GOx	PPy/CNT/GOx	up to ca. 50mM	0.2mM	2.33 nA mM^{-1}cm^{-2}	~15	[79]
11	GOx	MWCNT/GOx/GC	Up to 5	25 µM L^{-1}		6-15	[48]
12	GOx	Aligned MWCNT/GOx/GC	0.25-2.5		93.9×10^{-6} mM cm^{-2}		[94]
13	GOx	MWCNT/GNP/Chitosen (LBL film)	0.1-10	6.7 µM L^{-1}	2.527 µA/M/cm^2	7	[95]
14	GOx	MWNT–GOx multilayer membrane	0.02–2.2 mM	10µM	3.9 µA mM^{-1} cm^{-2}	20	[52]
15	GOx, HRP	GOx-HRP immobilized CNT-PAMAM	4.0 µM to 1.2 mM		2200 nA mM^{-1}	1	[91]
16	Cholesterol esterase, cholesterol oxidase, HRP	CNT modified screen printed carbon electrode	100–400 mgdL^{-1}	-	0.0059 and 0.0032 µA mg^{-1}dL^{-1}	-	[93]
17	Cholesterol oxidase	GC/CS-SiO2-COx-MWCNTs electrode	8.0 x10^{-6} to 4.5 ·x10^{-4} M	4.0 x10^{-6} M	0.54µA mM^{-1}	25	[94]
18	HRP	MWCNT/HRP/GC	0.3×10^{-3} - 0.2	0.1 µM L^{-1}	-	-	[97]
19	HRP	MWCNT/HRP-MB/GC	4 × 10^{-3} - 2	1 µM L^{-1}			[73]
20	ADH	poly(vinyl alcohol)–multiwalled carbon nanotube (PVA–MWCNT) composite	up to 1.5 mM		196 nA mM^{-1}	8	[101]
21	LDH	LDH/MWCT-MB	0.10–10 mmol	7.5×10^{-6}	(3.46µAcm^{-2}	-	

			L^{-1}	mol L^{-1}	mmol L^{-1}		[102]
22	ssDNA	ssDNA/MWNTs/ZrO2/CHIT/GC	1.49×10^{-10} to 9.32×10^{-8} mol L–1		7.5×10^{-11} mol L^{-1}	-	[116]
23	DNA	MWNT–COOH/ppy	6.9×10^{-14} to 8.6×10^{-13} mol/L	0.023 pmol L^{-1}	-	-	[118]
24	Antibody	SWNT-modified microelectrodes		0.25 ng mL^{-1}			[124]
25	Acetytchlolinesterase (ATChE)	CNT/GC	2 x10^{-5} to 2 x10^{-4} M	3 x 10^{-7} M	-	-	[129]
26	Acetytchlolinesterase (AchE)/Choline oxidase(CHO)	MWCNT/AchE/CHO	Up to 200 M L^{-1}		48 %		[130]
27	Organophosphorous hydrolase(OPH)	OPH/CNT		0.8μM (methyl parathion)	6.0		[132]
28	Choline oxidasae (CHOD)	CHO/CNT	5×10^{-2} - 5.0	1 X 10^{-5} M L^{-1}			[134]
29	Choline oxidase (ChOx)	ChOx/MWCNT/Pt	5×10^{-6} to 1×10^{-4}M	7×10^{-7} M		15	[135]
30	Choline oxidasae CHOD	(MWNTs/PANI)5/(PANI)3/GC electrode	1×10^{-6} to 2×10^{-3} M	0.3 μ M		3	[136]
31	Catalase	MWCNTs-modiWed GC electrode	0.05-0.08 mM	50 μM	2.4×10^{-10} mol cm^{2}	<2	[138]

32	LDH	MWCNT-CHIT-LDH	5 μM-0.2mM	0.76 μM	0.0083AM^{-1} cm^{-2}	3	[139]
33	LOD	CNTs/chitosan/PVI-Os/LOD	0-1.0 mM	5 μM	19.7μAmM^{-1} cm^{-2}	7	[140]
34	LOD	MWCNTs/PtNP/GCE	2.0×10^{-3} M	0.2–2.0 mM	6.36 μA mM^{-1}	5	[141]
35	ADH	ADH–PDDA–SWNTs	0.5 to 5.0 mM	~90 μM		5	[142]
36	LOD	LOD/sol-gel/GCE	0.2 to 2.0 mM	0.3×10^{-3} mM	6.031 μAmM^{-1}	5	[143]
37	ADH	ADH/MWCT-MB	0.05–10 mM L^{-1}	5×10^{-6} mol L^{-1}	4.75 μAcm^{-2} mmol L^{-1}	-	[144]
38	Lactase	lactase/CNTs–CS/GC electrode	0 to 0.2mM	7.8 μM	27.31 μA mM^{-1}		[145]

Symbols: CNTs, Carbon nanotubes; MWCNTs, Multiwalled carbon nanotube; PtNP, Platinum nanoparticles ADH, Alcohol dehydrogenase; LOD, lactate oxidase; HRP, Horseradish peroxidase; GC, Glassy carbon; GOx, Glucose oxidase; MB, Methylene blue; GCE, Glassy carbon electrode; PANI, Polyaniline.

References and Notes

1. Wang, J. Carbon-Nanotube Based Electrochemical Biosensors: A Review, *Electroanalysis,* **2005**, *17*, 7-14.

2. Wanekaya, A.K.; Chen, W.; Myung, N.V.; Mulchandani, A. Nanowire-based electrochemical biosensor. *Electroanalysis* **2006,** *18*, 533-550.

3. Luo,X.;Morrin, A.;Killard A.J.; Smyth, M.R. Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis*. 2006, 18, 319-326.

4. Wang, J. Nanoparticles-based electrochemical bioassays of proteins. *Electroanalysis*. **2006**, *19*, 769-776.

5. Pandey, P.; Datta, M.; Malhotra, B.D. Prospects of nanomaterials in biosensor. *Anal.Lett*. **2008**, *41*,157-207.

6. Nanoscience and Nanotechmnologies I July 2004, The Royal Society and The Royal Academy of Engineering p-8-23.

7. Carbon nanotube and Related Structured: New materials for the Twenty First Century, Harris, P.J.F.Cambridge, University Press, 1999.

8. Martel, R.; Shea, H. R.; Avouris, P. Ring Formation in Single-Wall Carbon. *J. Phys. Chem. B*, **1999**,*103*, 7551-7556.

9. Smith, B. W.; Monthioux, M.; Luzzi, D. E. Encapsulated C60 in carbon nano tubes. *Nature*, **1998**, *396*, 323-324.

10. Luo, H.; Shi, Z.; Li, N.; Gu, Z.; Zhuang, Q. Investigation of the Eelectrochemical and Electrocatalytic Behavior of Single-Wall Carbon Nanotube Film on a Glassy Carbon. *Electrode Anal. Chem.* **2001**, *73*, 915-920.

11. Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Electrocatalytic Oxidation of Norepinephrine at a Glassy Carbon Electrode Modified with Single Wall Carbon Nanotubes.   *Electroanalysis*, **2002**,14, 225-230.

12. Wang, J.; Musamech, M.; Lin, Y. Solubilization of Carbon Nanotubes by Nafion toward the Preparation of Amperometric Biosensors. *J. Am. Chem. Soc*. **2003**, *125*, 2408-2409.

13. Wang, Ji.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Direct Electrochemistry of Cytochrome *c* at a Glassy Carbon Electrode Modified with Single-Wall Carbon Nanotubes. *Anal. Chem.* **2002,** *74*, 1993-1997.

14. Wang, Z.H.; Liu, J.; Liang, Q.L.; Wang, Y.M.; Luo, G. Carbon nanotube-modified electrodes for the simultaneous determination of dopamine and ascorbic acid. *Analyst* **2002,***127* , 653-658.

15. Musameh, M.; Wang, J.; Merkoci, A.; Lin, Y. Low-potential stable NADH detection at carbon-nanotube-modified glassy carbon electrodes. *Electrochem. Commun.* **2002**, 4, 743-746.

16. Zhao, Y.D.; Zheng, W.D.; Chen, H.; Luo, Q. M. Anodic oxidation of hydrazine at   carbon nanotube powder microelectrode and its detection. *Talanta* **2002***, 58*, 529-534.

17. Adu, C.K.W.; Sumanasekera, G.U.; Pradhan, B.K.; Romero, H.E.; Eklund,P.C. Carbon nanotubes: A thermoelectric nano-nose. *Chem. Phys. Lett*. **2001**, *337*, 31-35.

18. Shieh,Y.T.; Yang, Y.F. Significant improvements in mechanical property and water stability of chitosan by carbon nanotubes, *Euro. Poly. Jour.* **2006**,*42*, 3162–3170.

19. Luo, X.; Killard, A. J.; Morrin, A.; Smyth, M.R. Enhancement of a conducting polymer-based biosensor using carbon nanotube-doped polyaniline. *Anal. Chim. Acta* **2006**, *575*, 39–44.

20. Yao, D.S.; Cao, H.; Wen, S.; Liu, D.; Bai, Y.; Zheng, W.A novel biosensor for sterigmatocystin constructed by multi-walled carbon nanotubes (MWNT) modified with aflatoxin–detoxifizyme (ADTZ). *Bioelectrochem.* **2006**, *68*,126 – 133.

21. Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Electrocatalytic oxidation of 3,4-dihydro-xyphenylacetic acid at a glassy carbon electrode modified with single-wall carbon nanotubes. *Electrochimica Acta* **2001**,*47*,651-657.

22. Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Investigation of the electrocatalytic behavior of single-wall carbon nanotube films on an Au electrode. *Microchem.J* .**2002**,*73*, 325-333.

23. Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Direct Electrochemistry of Cytochrome *c* at a Glassy Carbon Electrode Modified with Single-Wall Carbon Nanotubes. *Anal. Chem.* **2002**,*74*,1993-1997.

24. Rubianes, M.D.; Rivas, G.A. Dispersion of multi-wall carbon nanotubes in polyethyle- nimine: A new alternative for preparing electrochemical sensors. *Electrochem. Commun.* **2007**, *9*, 480–484.

25. Gong, K.; Dong, Y.; Xiong, S.; Chen, Y.; Mao, L. Novel electrochemical method for sensitive determination of homocysteine with carbon nanotube-based electrodes. *Biosensor. Bioelectron.***2004**, *20*, 253–259.

26. Tsai, Y.C., Chiu, C.C. Amperometric biosensors based on multiwalled carbon nanotube-Nafion-tyrosinase nanobiocomposites for the determination of phenolic compounds. *Sensors. Actua. B* **2007**, *125*, 10–16.

27. Zhang, R.; Wang, X.; Shiu, K.K. Accelerated direct electrochemistry of hemoglobin based on hemoglobin–carbon nanotube (Hb–CNT) assembly. *J.Colloid Interface Sci.* **2007**, (in press)

28. Chen, S.; Yuan, R.; Chai, Y.; Zhang, L.; Wang, Na; Li, X. Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles. *Biosens. Bioelectr..* **2007**, *22,* 1268–1274.

29. Yin Y.; Lü Y.; Wu P. ; Cai C. Direct Electrochemistry of Redox Proteins and Enzymes Promoted by Carbon Nanotubes, *Sensors* **2005***, 5,* 220-234.

30. Mita, D.G.; Attanasio, A.; Arduini, F.; Diano, N.; Grano, V.; Bencivenga, U.; Rossi, S., Amine, A.; Moscone D. Enzymatic determination of BPA by means of tyrosinase immobilized on different carbon carriers. *Biosens. Bioelectron.* **2007**, *23*,60–65.

31. Wang, Y.; Iqbal, Z.; Malhotra, S.V. Functionalization of carbon nanotubes with amines and enzymes. *Chem. Phys. Lett.* **2005**, *402*, 96–101.

32. Weber, J.; Kumar, A.; Kumar, A.; Bhansali, S. Novel lactate and pH biosensor for skin and sweat analysis based on single walled carbon nanotubes. *Sens. Actuat.B* **2006**, *117*, 308–313.

33. Withey, G.D.; Lazareck, A.D.; Tzolov, M.B.; Yina, A.; Aich, P.; Yeh, J.I.; Xua, J.M. Ultra-high redox enzyme signal transduction using highly ordered carbon nanotube array electrodes. *Biosens. Bioelectron.* **2006**, *21*,1560–1565.

34. Yu, X.; Chattopadhyay, D.; Galeska, I.; Papadimitrakopoulos, F.; Rusling, J.F. Peroxidase activity of enzymes bound to the ends of single-wall carbon nanotube forest electrodes. *Electrochem. Communi.* **2003**, *5* , 408–411.

35. Popovich, N.D.; Thorp, H.H. New strategies for electrochemical nucleic acid detection. *Interface* **2002**,*11*,30-34.

36. Umek, R.M.; Lin, S.W.; Vielmetter, J.; Terbrueggen, R.H.; Irvine, B.; Yu, C.J.; Kayyem, J.F; Yowanto, H.; Blackburn, G.F.; Farkas, D.H.; Chen, Y.P. Electronic detection of Nucleic acid : A Versatile Platform for Molecular Diagnostics**.** *J. Mol. Diagn.* **2001**, *3*, 74-84.

37. Yogeswaran, U.; Thiagarajan, S.; Chen, S.M. Pinecone shape hydroxypropyl-β- cyclodextrin on a film of multi-walled carbon nanotubes coated with gold particles for the simultaneous determination of tyrosine, guanine, adenine and thymine. *Carbon* **2007**,*45,*2783-2796.

38. Rong, L.Q.; Yang, C.; Qian, Q.Y.; Xia, X.H, Study of the nonenzymatic glucose sensor based on highly dispersed Pt nanoparticles supported on carbon nanotubes. T*alanta* **2007**,*72*, 819-824.

39.   Ye, J.S.; Wen, Y.; De, Zhang W.; Gan, L.M.; Xu, G.Q. Sheu, F.S. Nonenzymatic glucose detection using multi-walled carbon nanotube electrodes. *Electrochem. Comm.* **2004**, *6*,66-70.

40.   Lyons M. E. G.; Keeley G. P., The Redox Behaviour of Randomly Dispersed Single Walled Carbon Nanotubes both in the Absence and in the Presence of Adsorbed Glucose Oxidase, *Sensors* **2006**, *6*, 1791-1826

41.   Wang, J.; Musameh, M.; Lin, Y. Solubilization of Carbon Nanotubes by Nafion toward the Preparation of Amperometric Biosensors. *J. Am. Chem. Soc.* **2003**,*125*, 2408-2409.

42.   Luong, J.H.; Hrapovic, S.; Wang, D.; Bensebaa, F.; Simard, B. Solubilization of Multiwall Carbon Nanotubes by 3-Aminopropyltriethoxysilane Towards the Fabrication of Electrochemical Biosensors with Promoted Electron Transfer. *Electroanalysis* **2004**,*16*, 132-139.

43.   Wang, J., Musameh, M. Carbon Nanotube/Teflon Composite Electrochemical Sensors and Biosensors. *Anal. Chem.* **2003**,*75*, 2075-2079.

44.   Rubianes, M.D.; Rivas, G.A. Carbon nanotubes paste electrode. *Electrochem. Commun.* **2003**, *5*, 689-694.

45.   Wang, J.; Musameh, M. Carbon Nanotube/Teflon Composite Electrochemical. *Anal. Chem.* **2003**, *75*, 2075 -2079.

46.   Pedano, M.L.; Rivas, G.A. Adsorption and Electrooxidation of nucleic acids at carbon nanotubes paste electrodes. *Electrochem. Comm.* **2004,** *6*, 10-16.

47.   Wang, J.; Musameh, M. Enzyme-dispersed carbon-nanotube electrodes: a needle   microsensor for monitoring glucose. *Analyst* **2003**,*128*,1382 -1385.

48.   Wang, J.; Musameh, M. Carbon nanotube screen-printed electrochemical sensors. *Analyst* **2004,***129*,1-2.

49.   Star, A.; Stoddart, J.S.; Streuerman, D.; Diehl, M.; Boukai, A.; Wong, E.W.; Yang, X.; Chung, S.W.; Choi, H.; Heath, J.R. Preparation and Properties of Polymer-Wrapped Single-Walled Carbon Nanotubes. *Angew. Chem. Int. Ed.* **2001**,*40*,1721-1725.

50.   Riggs, J.E.; Guo, Z.; Carroll, D.L.; Sun, Y.P. Strong Luminescence of Solubilized Carbon Nanotubes. *J. Am. Chem. Soc.* **2000***,122*,5879-5880.

51.   Wang, J.; Musameh, M.; Lin, Y. Solubilization of Carbon Nanotubes by Nafion toward the Preparation of Amperometric Biosensors. *J. Am. Chem. Soc.* **2003**,*125*, 2408-2409.

52.   Manesh, K.M.; Kim, H.T.; Santosh, P.; Gopalan, A.I., Lee, K.P.   A Novel glucose biosensor based on immobilization of glucose oxidase into multiwalled nanotube-polyelectrolyte-loaded electrospun nanofibrous membrane. *Biosens Bioselectron. 2007* (in press).

53.   Gooding, J.J; Wibowo, R.; Liu, J.; Yang, W.; Losic, D.; Orbons, S.; Mearns, F.J.; Shapter, J.G.; Hibbert, D.B. Protein Electrochemistry Using Aligned Carbon Nanotube Arrays. *J. Am. Chem. Soc.***2003**,*125*, 9006-9007.

54.   Lin, Y.; Lu, F.; Tu, Y.; Ren, Z. Glucose Biosensors Based on Carbon Nanotube Nanoelectrode Ensembles. *Nano Lett.*, **2004**, *4,* 191-195.

55.   Chen, R.J.; Zhang, Y.; Wang, D.; Dai, H. Noncovalent Sidewall Functionalization of Single-Walled Carbon Nanotubes for Protein Immobilization.*J. Am. Chem. Soc.* **2001,***123*, 3838-3839.

56.   Huang, W.; Taylor, S.; Fu, K.; Lin, Y.; Zhang, D.; Hanks, T.W.; Rao, A.; Sun, Y. Attaching Proteins to Carbon Nanotubes via Diimide-Activated Amidation.*Nano. Lett.* **2002**,*2*, 311-315.

57. Sharma, S.K.; Singhal, R.; Malhotra, B.D.; Sehgal, N.; Kumar, A. Lactose biosensor based on Langmuir–Blodgett films of poly(3-hexyl thiophene). *Biosens. Bioelectron.* **2004**,*20*,651-657.

58. Singhal, R.; Gambhir, A.; Pandey, M.K.; Annapoorni, S.; Malhotra, B.D. Immobilization of urease on poly(N-vinyl carbazole)/stearic acid Langmuir–Blodgett Fims for application to urea biosensors. *Biosens. Bioelectron.* **2002**, *17*,697-703.

59. Gerard, M.; Chaubey, A.; Malhotra, B.D. Application of conducting polymers to biosensors.*Biosens.Bioelectron.***2002**,*17*,345-359.

60. Shi, G.Y.; Liu, M.; Zhu, M.; Zhou, T.S.; Chen, J.S.; Jin, L.T.; Jin, J.Y. The study of Nafion/xanthine oxidase/Au colloid chemically modified biosensor and its application in the determination of hypoxanthine in myocardial cells *in vivo.Analyst* **2002**,*127*,396-400.

61. Hong, M.Y.; Chong, J.Y.; Yoon, H.C.; Kim, H.S. Development of a screen-printed amperometric biosensor for the determination of l-lactate dehydrogenase level. *Biosens. Bioelectron.* **2002**,*17*, 13-18.

62. Hubalek, J.; Hradecky J.; Adam V.; Krystofova O.; Huska D.; Masarik M.; Trnkova L.; Horna A.; Klosova K.; Adamek M.; Zehnalek J.; Kizek R. Spectrometric and Voltammetric Analysis of Urease – Nickel Nanoelectrode as an Electrochemical Sensor**,** *Sensors* **2007, *7*,** 1238-1255

63. Chailapakul, O.; Ngamukot, P.; Yoosamran, A.; Siangproh,W.; Wangfuengkanagul N. Recent Electrochemical and Optical Sensors in Flow-Based Analysis, *Sensors* **2006***, 6*, 1383-1410

64. Hrapovic, S.; Liu, Y.; Male, K.; Luong, J.H. Electrochemical Biosensing Platforms Using Platinum Nanoparticles and Carbon Nanotubes. *Anal. Chem.* **2004,***76*, 1083-

65. Wang, S.G.; Zhang, Q.; Wang, R.; Yoona, S.F. A novel multi-walled carbon nanotube-based biosensor for glucose detection. *Biochem.Biophys. Rese.Commun.* **2003**, *311*, 572–576.

66. Hrapovic, S.; Liu, Y.; Male, K.; Luong, J.H. Electrochemical Biosensing Platforms Using Platinum Nanoparticles and Carbon Nanotubes. *Anal.Chem.* **2004**,*76*,1083-1088.

67. Kang, X., Mai, Z., Zou, X.*,* Cai, P., Mo, J. Glucose biosensors based on platinum nanoparticles-deposited carbon nanotubes in sol–gel chitosan/silica hybrid. *Talanta* **2007** (in press)

68. Liu, Q.; Lu, X.; Li, J.; Yao, X.; Li, J. Direct electrochemistry of glucose oxidase and electrochemical biosensing of glucose on quantum dots/carbon nanotubes electrodes. *Biosens. Bioelectron.* **2007**, *22*,3203–3209.

69. Wu, Z.; Xu, Y.;Zhang, X.; Shen, G.; Yu, R. Microwave plasma treated carbon nanotubes and their electrochemical biosensing application. *Talanta* **2007,** *72*,1336–1341.

70. Manso, J.; Mena, M.L.; Yanez-Sedeno, P.; Pingarron, J. Electrochemical biosensors based on colloidal gold–carbon nanotubes composite electrodes. *J.Electroanal.Chem.* **2007**,*603*,1–7.

71. Chu, X.; Duan, D.E.; Shen, G.; Yu, R. Amperometric glucose biosensor based on electrodeposition of platinum nanoparticles onto covalently immobilized carbon nanotube electrode. *Talanta* **2007**,*71,* 2040–2047.

72. Qu, F.; Yang, M.; Shen, G.; Yu, R. Electrochemical biosensing utilizing synergic action of carbon nanotubes and platinum nanowires prepared by template synthesis. *Biosens. Bioelectron.* **2007**,*22* ,1749–1755.

73.   Yang, M.; Jiang, J.; Yang, Y.; Chen, X.; Shen, G.; Yu, R. Carbon nanotube/cobalt hexacyanoferrate nanoparticle-biopolymer system for the fabrication of biosensors. *Biosens. Bioelectron.* **2006**, *21*,1791–1797.

74.   Carrara, S.; Bavastrello, V.; Ricci, D.; Stura, E.; Nicolini, C. Improved nanocomposite materials for biosensor applications investigated by electrochemical impedance spectroscopy. *Sens.Actuator. B,* **2005,***109,* 221–226.

75.   Lima, S.H.; Wei, J.; Lin, J.; Lib, Q.; You, J.K. A glucose biosensor based on electrodeposition of palladium nanoparticles and glucose oxidase onto Nafion-solubilized carbon nanotube electrode. *Biosens. Bioelectron.* **2005**,*20*, 2341–2346.

76.   Lin, Z., Chen, J., Chen, G. An ECL biosensor for glucose based on Carbon-nanotube/Nafion film modified glass carbon electrode. *Electrochim. Acta* **2007** (in press)

77.   Besteman, K.; Lee, I.; Wiertz, F.; Heerin, H.; Dekker, C. Enzyme-Coated Carbon Nanotubes as Single-Molecule Biosensors. *Nanoletters* **2003**, *3*,727-730.

78.   Tsai, Y.C.; Li, S.C.; Liao, S.W. Electrodeposition of polypyrrole–multiwalled carbon nanotube–glucose oxidase nanobiocomposite film for the detection of glucose. *Biosens. Bioelectron.* **2006**,*22*, 495–500.

79.   Wang, J.; Musameh, M. Carbon-nanotubes doped polypyrrole glucose biosensor, Carbon-nanotubes doped polypyrrole glucose biosensor. *Anal. Chim.Acta* **2005**, *539*, 209–213.

80.   Gao, M.; Dai, L.; Wallace, G.G. Biosensors Based on Aligned Carbon Nanotubes Coated with Inherently Conducting Polymers. *Electroanalysis* **2003**,*15*,1089-1094.

81.   Fiorito, P.A.; Cordoba de Torresi, S.I. Glucose Amperometric Biosensor Based on the Co-immobilization of Glucose Oxidase (GOx) and Ferrocene in Poly(pyrrole) Generated from Ethanol / Water Mixtures. *J. Braz. Chem. Soc.* **2001**, *12,* 729-733.

82.   Zhu, L.; Yang, R.; Zhai, J.; Tian, C. Bienzymatic glucose biosensor based on co-immobilization of peroxidase and glucose oxidase on a carbon nanotubes electrode, *Biosens. Bioelectron.* **2007**,*23*, 528–535.

83.   Guiseppi –Elie, A.; Lei, C.; Baughman, R. Direct electron transfer of glucose oxidase on carbon nanotubes. *Nanotech.*2002,*13*,559 -564.

84.   Patolsky, F.; Weizmann, Y.; Willner, I. Long-Range Electrical Contacting of Redox Enzymes by SWCNT Connectors. *Angew. Chemie. Int Edn.* **2004**, *43,* 2113-2117.

85.   Withey, G.D.; Lazareck, A.D.; Tzolov, M.B.; Yin, A.; Aich, P.; Yeh, J.I.; Xu, J.M. Ultra-high redox enzyme signal transduction using highly ordered carbon nanotube array electrodes. *Biosens. Bioelectron.* **2006**,*15* ,1560-1565.

86.   Liu, Y., Wang, M., Zhao, F., Xu, Z., Dong, S. The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix. *Biosens. Bioelectron.* **2005**, *21*, 984–988.

87.   Sotiropoulou, S.; Chaniotakis, N. A. Carbon nanotube array -based biosensor. *Anal. Bioanal. Chem.* **2003**, *375,* 103-105.

88.   Wu, B.Y.; Hao, S.H.; Yin, F.; Zhao, Z.X.; Wang, Y.Y.; Wang, S.H.; Chen, Q. Amperometric glucose biosensor based on multilayer films via layer-by-layer self-assembly of multi-wall carbon

nanotubes, gold nanoparticles and glucose oxidase on the Pt electrode. *Biosens. Bielectron.* **2007** (in press)

89. Yan, X.B.; Chen, X.J.; Tay, B.K.; Khor, K.A. Transparent and flexible glucose biosensor via layer-by-layer assembly of multi-wall carbon nanotubes and glucose oxidase. *Electrochem. Communic.* **2007**, *9* , 1269–1275.

90. Sato, N.; Okuma, H. Development of single-wall carbon nanotubes modified screen-printed electrode using a ferrocene-modified cationic surfactant for amperometric glucose biosensor applications. *Sens. Actuat. B* **2007** (in press)

91. Zeng, Y.L.; Huang, Y.F.; Jiang, J.H.; Zhanga, X.B.; Tang, C.R.; Shen, G.L.; Yu, R.Q. Functionalization of multi-walled carbon nanotubes with poly(amidoamine) dendrimer for mediator-free glucose biosensor. *Electrochem. Communic.* **2007**, *9,* 185–190.

92. Antiochia, R.; Gorton, L. Development of a carbon nanotube paste electrode osmium polymer-mediated biosensor for determination of glucose in alcoholic beverages. *Biosens. Bioelectron.* **2007**, *22,* 2611–2617.

93. Lia, G.; Liao, J.M.; Hu, G.Q.; Ma, N.Z.; Wu, P.J. Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood. *Biosens. Bioelectron.* **2005,** *20*, 2140–2144.

94. Tan, X., Li, M., Cai, P., Luo, L., Zou, X. An amperometric cholesterol biosensor based on multiwalled carbon nanotubes and organically modified sol-gel/chitosan hybrid composite film. *Anal. Biochem.* **2005**,*337*,111–120.

95. Liu, Y., Lei, J., Huangxian, J. Amperometric sensor for hydrogen peroxide based on electric wire composed of horseradish peroxidase and toluidine blue-multiwalled carbon nanotubes nanocomposite. *Talanta* **2007**(in press)

96. Qian, L., Yang, X. Composite film of carbon nanotubes and chitosan for preparation of amperometric hydrogen peroxide biosensor. *Talanta* **2006**,*68* ,721–727.

97. Yamamoto, K.; Shi, G.; Zhou, T.; Xu, F.; Xu, J.; Kato, T.; Jind, J.Y.; Jin, L. Study of carbon nanotubes–HRP modified electrode and its application for novel on-line biosensors. *Analyst*, **2003**,*128*, 249-254.

98. Yu, X.; Chattopadhyay, D.; Galeska, I.; Papadimitrakopoulos, F.; Rusling, J.F.   Peroxidase activity of enzymes bound to the ends of single-wall carbon nanotube forest electrodes. *Electochem. Commun.* **2003**,*5*, 408-411.

99. Xu, J.Z.; Zhu, J.J.; Wu, Q.; Hu, Z.; Chen, H.Y. An Amperometric Biosensor Based on the Coimmobilization of Horseradish Peroxidase and Methylene Blue on a Carbon Nanotubes Modified Electrode. *Electroanalysis* **2003**,*15*, 219-224.

100. Qu, S.; Huang, F.; Chen, G.G.; Yu, S.; Kong, J. Magnetic assembled electrochemical platform using Fe_2O_3 filled carbon nanotubes and enzyme. *Electrochem. Communic.* **2007**, *9*,2812–2816.

101 Tsai, Y.C.; Huang, J.D.; Chiu, C.C. Amperometric ethanol biosensor based on poly(vinyl alcohol)–multiwalled carbon nanotube–alcohol dehydrogenase biocomposite, *Biosens. Bioelectron.* **2007**,22, 3051–3056.

102 Santos, A.S.; Pereira, A.C.; Duran, N.; Kubota, LT. Amperometric biosensor for ethanol based on co-immobilization of alcohol dehydrogenase and Meldola's Blue on multi-wall carbon nanotube. *Electrochimica Acta.* **2006**,*52* , 215–220.

103   Radoi, A.; Compagnoneb, D.; Valcarcel, M.A.; Placidi, P.; Materazzi, S.; Mosconea, D.; Palleschi, G. Detection of NADH via electrocatalytic oxidation at single-walled carbon nanotubes modified with Variamine blue. *Electrochim. Acta*, **2007** (in press).

104   Manso, J.; Mena, M.L.; Yanez-Sedeno, P.; Pingarron, J.M. Alcohol dehydrogenase amperometric biosensor based on a colloidal gold-carbon nanotubes composite electrode. *Electrochimica Acta*, **2007** (in press).

105   Goodling, J.J. Electrochemical DNA Hybridization Biosensors. *Electroanalysis,* **2002**, *14*, 1149 - 1156.

106   Wang, Z.; Liu, D.; Dong, S. Study on Adsorption and Oxidation of Calf Thymus DNA at Glassy Carbon Electrode. *Electroanalysis* **2000**,*12*, 1419-1421.

107   Wang, H.; Ju, H.; Chen, H. Voltammetric Behavior and Detection of DNA at electrochemically Pretreated Glassy Carbon Electrode. *Electroanalysis* **2001**,*13*, 1105-1109.

108.   Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. Electrochemistry of DNA at Single-Wall Carbon Nanotubes. *Electroanalysis* **2004**,*16,*140-144.

109.   Wang, J.; Kawde, A.; Mustafa M. Carbon-nanotube-modified glassy carbon electrodes for amplified label-free electrochemical detection of DNA hybridization. *Analyst* **2003**,*128*, 912-916.

110.   Ye, Y.; Ju, H. Rapid detection of ssDNA and RNA using multi-walled carbon nanotubes modified screen-printed carbon electrode. *Biosens. Bioelectron.* **2005**, *21*,735-741.

111   Ly, S.Y. Detection of dopamine in the pharmacy with a carbon nanotube paste electrode using voltammetry. *Bioelectrochem.* **2006**,*68*, 227-231.

112.   Singh, A.K.; Willner, I. Liposomes Labeled with Biotin and Horseradish   Peroxidase: A Probe for the Enhanced Amplification of Antigen-Antibody or Oligonucleotide-DNA Sensing Processes by the Precipitation of an Insoluble Product on Electrodes. *Anal. Chem.***2001**, *73*, 91-102.

113.   Wang, J.; Jung, M. Toward Genolelectronics: Nucleic Acid Doped Conducting   Polymers. *Langmuir* **2000**,*16*,2269-2274.

114.   Cai, H.; Xu, Y.; He, P.; Fang, Y.Z. Indicator Free DNA Hybridization Detection by Impedance Measurement Based on the DNA-Doped Conducting Polymer Film   Formed on the Carbon Nanotube Modified Electrode. *Electroanalysis* **2003**,*15*,1864-1870.

115.   Cai, H.;Cau, X.; Jiang, Y.;. He, P.; Fang, Y. Carbon nanotube-enhanced electrochemical DNA biosensor for DNA hybridization. *Anal. Bioanal. Chem.***2003**, 375, 287-293.

116.   Yang, Y.; Wang, Z.; Yang, M.; Li, J.; Zheng, F.; Shen, G.; Yu, R. Electrical detection of deoxyribonucleic acid hybridization based on carbon-nanotubes/nano zirconium dioxide/chitosan-modified electrodes. *Anal. Chim. Acta* **2007**, 584, 268–274.

117.   Li, J.; Ng, H.T.; Cassell, A.; Fan, W.; Chen, H., Ye, Q.; Koehne, J.; Han, J.;   Meyyappan, M. Carbon Nanotube Nanoelectrode Array for Ultrasensitive DNA   Detection .*Nano Lett*. **2003**,*3*,597-602.

118.   Cheng, G.; Zhao, J.; Tu, Y.; He, P.; Fang, Y. A sensitive DNA electrochemical biosensor based on magnetite with a glassy carbon electrode modified by muti-walled carbon nanotubes in polypyrrole. *Anal. Chim. Acta* **2005**,*533,* 11–16.

119. Wang, J.; Liu, G.; Jan, M.R. Ultrasensitive Electrical Biosensing of Proteins and DNA: Carbon-Nanotube Derived Amplification of the Recognition and Transduction Events. *J.Am.Chem.Soc.* **2004,** *126*, 3010-3011.

120. Park, D.W.; Kim, Y.H.; Kim, B.S.; So, H.M.; Won, K.; Lee, J.O.; Kong, K.J.; Chang, H. Detection of tumor markers using single-walled carbon nanotube field effect transistors. *J. Nanosci. Nanotechnol.* **2006**,*6* 3499-3502.

121. Chen, R.J.; Choi, H.C.; Bangsaruntip, S.; Yenilmez, E.; Tang, X.; Wang, Q.;   Chang, Y.L.; Dai, H. An investigation of the mechanisms of electronic sensing of protein adsorption on carbon nanotube devices. *J.Am.Chem.Soc.* **2004**,*126*, 1563-1568.

122. Chen, R.J.; Bangsaruntip, S.; Drouvalakis, K.A.; Kam, N.W.; Shim, M.; Li, Y.; Kim, W.; Utz, P.J.; Dai, H. Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc.Natl.Acad.Sci. U.S.A.*, **2003,***100*, 4984-4989

123. Takeda, S.; Sbagyo, A.; Sakoda, Y.; Ishii, A.; Sawamura, M.; Sueoka, K.; Kida, H.; Mukasa, K.; Matsumoto, K. Application of carbon nanotubes for detecting anti-hemagglutinins based on antigenantibody interaction. *Biosens. Bioelectron.* **2005.** *21,* 201-205.

124. Okuno, J.; Maehashi, K.; Kerman, K.; Takamura, Y.; Matsumoto, K.; Tamiya, E. Label-free immunosensor for prostate-specific antigen based on single-walled carbon nanotube array-modified microelectrodes. *Biosens. Bioelectron.***2006**, *22*, 2377-2381.

125. Wohlstadter, J.N.; Wilbur, J.L.; Sigal, G.B.; Biebuyck, H.A.; Billadeau, M.A.; Dong, L.; Fischer, A.B.; Gudibande, S.R.; Jameison, S.H.; Kenten, J.H.; Leginus, J.; Leland, J.K.; Massey, R.J.; Wohlstadter, S.J. Carbon nanotube based biosensor. *Adv. Mater.* **2003**,*15,* 1184-1187.

126. O'Connor, M.; Kim, S.N.; Killard, A.J.; Forster, R.J.; Smyth, M.R.; Papadimitrakopoulos, F.; Rusling, J.F. Mediated amperometric immunosensing using single walled carbon nanotube forests. *Analyst,* **2004.***129*,1176-1180.

127. Yu, X.; Kim, S.N.; Papadimitrakopoulos, F.; Rusling, J.F. Peroxidase activity of enzymes bound to the ends of single-wall carbon nanotube forest electrodes. *Electrochem. Commmun.* **2003,** 5: 408-411.

128. Uda, T., Kanmei, R., Akasofu, S., Hifumi, E., and Ohtaki, M. Application of polymer-protected ultrafine platinum particles to the immunological detection of human serum albumin. *Anal. Biochem,* **1994,***218,* 259-264.

129. Liu, G.; Riechers, S.L.; Mellen, M.C.; Lin, Y. Sensitive electrochemical detection of enzymatically generated thiocholine at carbon nanotube modified glassy carbon electrode. *Electrochem. Communicat.* **2005,***7,*1163–1169.

130. Joshi, K.A.; Tang, J.; Haddon, R.; Wang, J.; Chen, W.; Mulchandan. A. A Disposable Biosensor for Organophosphorus Nerve Agents Based on Carbon Nanotubes Modified Thick Film Strip Electrode. *Electroanal.* **2005,***17*, 54-58.

131. Dua, D.; Huang, X.; Cai, J.; Zhang, A. Comparison of pesticide sensitivity by electrochemical test based on acetylcholinesterase biosensor. *Biosens. Bioelectron.* **2007,***23,* 285–289.

132   Deo, R.P.; Wang, J.; Block, I.; Munchandani, A.; Joshi, K.A.; Trojanowicz, M.; Scholz, F.; Chen, W.; Lin, Y. Determination of organophosphate pesticides at a carbon

nanotube/organophosphorus hydrolase electrochemical biosensor. *Anal. Chim. Acta* **2005,** *530*, 185-189.

133. Dua, D.; Huanga, X.; Cai, J.; Zhang, A. Amperometric detection of triazophos pesticide using acetylcholinesterase biosensor based on multiwall carbon nanotube–chitosan matrix. *Sens.Actuat.B* **2007** (in press).

134. Wang, J.; Liu, G.; Lin, Y. Amperometric choline biosensor fabricated through electrostatic assembly of bioenzyme/polyelectrolyte hybrid layers on carbon nano tubes. *Analyst* **2006**, *131*, 477-483.

135. Song, Z.; Huang, J.D.; Wu, B.Y.; Shi, H.B.; Anzai, J.I.; Chen, Q. Amperometric aqueous sol–gel biosensor for low-potential stable choline detection at multi-wall carbon nanotube modified platinum electrode. *Sens.Actuat.B* **2006,***115*,626–633.

136. Qu, F.I.; Yang, M.; Jiang, J.; Shen, G.; Yu, R. Amperometric biosensor for choline based on layer-by-layer assembled functionalized carbon nanotube and polyaniline multilayer. *Anal. Biochem.* **2005,***344,1*08–114.

137. Zhou, L.; Wang, J.; Zhou, F. Electroanalysis, 16, 672 (2004)

138. Salimi, A.; Noorbakhsh, A.; Ghadermarz, M. Direct electrochemistry and electrocatalytic activity of catalase incorporated onto multiwall carbon nanotubes-modified glassy carbon electrode. *Anal. Biochem.* **2005,** *344,*16–24.

139. Tsai, Y.C.; Chen, S.Y.; Liaw, H.W. Immobilization of lactate dehydrogenase within multiwalled carbon nanotube-chitosan nanocomposite for application to lactate biosensors. *Sens. Actuat. B* **2007,***125,* 474–481.

140. Cui, X.; Li, C.M.; Zang, J.; Yu, S. Highly sensitive lactate biosensor by engineering chitosan/PVI-Os/CNT/LOD network nanocomposite. *Biosens. Bioelectron.* **2007,** *22,* 3288–3292

141. Huang, J.; Li, J.; Yang, Y.; Wang, X.; Wu, B.; Anzai, J.; Osa, T.; Chen, Q. Development of an amperometric L-lactate biosensor based on L-lactate oxidase immobilized through silica sol–gel film on multi-walled carbon nanotubes/platinum nanoparticle modified glassy carbon electrode. *Mat. Sci.Eng.C.* **2007** (in press).

142. Liu, S.; Cai, C. Immobilization and characterization of alcohol dehydrogenase on single-walled carbon nanotubes and its application in sensing ethanol. *J. Electroanal. Chem.* **2007,***602*,103–114.

143. Huang, J.; Song, Z.; Li, J.; Yang, Y.; Shi, H.; Wu, B.; Anzai, J.I.; Osa, T.; Chen, Q. A highly-sensitive L-lactate biosensor based on sol-gel film combined with multi-walled carbon nanotubes (MWCNTs) modified electrode. *Mat. Sci.Eng.C.* **2007,***27,* 29–34.

144. Liu, Y.; Qu, X.; Guo, H.; Chen, H.; Liu, B.; Dong, S. Facile preparation of mperometric laccase biosensor with multifunction based on the matrix of carbon nanotubes–chitosan composite. *Biosens.Bioelectron.* **2006,***21,* 2195–2201.

145. Pereira, A.C.; Aguiar, M.R.; Kisner, A.; Macedoa, D.V.; Kubota, L.T. Amperometric biosensor for lactate based on lactate dehydrogenase and Meldola Blue coimmobilized on multi-wall carbon-nanotube. *Sens.Actuat. B* **2007,***124,* 269–276.

146. Timur, S.; Anik, U.; Odaci, D.;Gorton, L. Development of a microbial biosensor based on carbon nanotube (CNT) modified electrodes. *Electrochem. Communic.* **2007,** *9*, 1810–1815.