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REVIEW

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Biosensors based on carbon nanotubes

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Abstract Carbon nanotubes (CNTs) exhibit a unique combination of excellent mechanical, electrical and electrochemical properties, which has stimulated increasing interest in the application of CNTs as components in (bio) sensors. This review highlights various design methodologies for CNT-based biosensors and their employment for the detection of a number of biomolecules. In addition, recent developments in the fields of CNT-based chemiresistors and chemically sensitive field-effect transistors are presented. After a critical discussion of the factors that currently limit the practical use of CNT-based biosensors, the review concludes with an outline of potential future applications for CNTs in biology and medicine.

Keywords Carbon nanotubes · Enzymatic biosensors · Electrochemical biosensors · Amperometric detection · DNA sensing · Chemiresistors

Introduction

Sensors are a class of devices that have found widespread use, ranging from the detection of gas molecules to the real-time tracking of chemical signals in biological cells. In general, a sensor comprises an active sensing element and a signal transducer, and produces an electrical, optical, thermal or magnetic output signal. While the sensing element is responsible for the selective detection of the analyte, the transducer converts a chemical event into an appropriate signal that can be used with or without amplification to determine the analyte concentration in a given test sample.

Biosensors—first reported in the 1960s [1]—differ from classical chemical sensors in the following two ways: (a) the sensing element consists of a biological material such as proteins (e.g., cell receptors, enzymes, antibodies), oligo- or polynucleotides, microorganisms, or even whole biological tissues [2, 3], and (b) the sensor is used to monitor biological processes or for the recognition of biomolecules. For in vitro biosensing, the sample solution (such as blood serum, urine, milk products etc.) is dropped atop the biosensor, and the output signal gives information on the composition of the solution. By contrast, in vivo biosensing addresses dynamic systems, aiming for instance to measure the rate of uptake or efflux of relevant species or to estimate the spatial distribution of the concentration of an analyte in a living organism [2].

Electrochemical biosensors are currently among the most popular of the various types of biosensors. Carbon materials have been used as components in electrochemical biosensors for over a decade. Carbon nanotubes (CNTs) are promising materials for sensing applications due to several intriguing properties. In particular, their large length-to-diameter aspect ratios provide for high surface-to-volume ratios. Moreover, CNTs have an outstanding ability to mediate fast electron-transfer kinetics for a wide range of electroactive species, such as hydrogen peroxide or NADH. In addition, CNT chemical functionalization can be used to attach almost any desired chemical species to them, which allows us—for instance—to enhance the solubility and biocompatibility of the tubes. This has permitted the realization of composite electrodes comprising CNTs well-dispersed in an appropriate polymer matrix [4]. Among other factors, ongoing progress in the development of novel biomaterials such as functional polymers, sol-gel materials and so on has led to an upsurge of research into the use of CNTs as components in biosensors.

This review is organized as follows. First, an overview of various design architectures used in the fabrication of CNT-based biosensors is presented. This is followed by a collection of electrochemical biosensors that have proven valuable for the detection of specific biomolecules. The next section describes single-wall carbon nanotube (SWCNT)-based field-effect transistors (FETs) and chemiresistors that have been used for biosensing. Next, complications associated with the potential real-life and day-to-day use of CNT-based sensors

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are outlined, and finally, future application perspectives are discussed.

Design principles of CNT-based biosensors

Biosensing can be performed using a broad spectrum of techniques. Many classical approaches like mass spectrometry and biolabeled fluorescence usually require a number of steps before the biomolecule is detected [5]. Such methods are highly sensitive, but are difficult to miniaturize. Electronic and electrochemical detection techniques, in comparison, are advantageous in this aspect. CNTs, with their interesting electrochemical and electrical properties, are ideal both as electrodes and as transducer components in biosensors. Individual SWCNTs are extremely sensitive to their surrounding environment. Both chemiresistors and chemically sensitive field-effect transistors (FETs) incorporating pristine or specifically functionalized CNTs have been shown to be capable of detecting biomolecules. In the following, a survey of the various types of molecular architectures that have been implemented for CNT-based biosensors over the last decade is presented.

CNT-based paste electrodes

Carbon paste electrodes (CPE) are fabricated by mixing carbon powder with mineral oil. In a similar manner, CNT paste electrodes (CNTPE) have been obtained (see Fig. 1) by mixing CNT powder with deionized water [6], bromoform [7] or mineral oil [8]. For biosensing purposes, an enzyme is added to the mixture to obtain a CNTPE with incorporated enzymes [8]. Biosensors have also been fabricated by modifying the classical CPE with multiwall carbon nanotubes (MWCNTs) and subsequently immobilizing enzymes [9].

Electrodes modified by CNTs

Conventional electrochemical biosensors are based on either glassy carbon electrodes (GCE) or metal electrodes (Au, Pt or Cu for example) for amperometric or voltammetric analyte detection. Such electrodes have a series of disadvantages, including poor sensitivity and stability, low reproducibility, large response times and a high overpotential for electron transfer reactions [10]. CNTs can overcome most of these disadvantages owing to their ability to undergo fast electron transfer and the resistance of CNT-modified electrodes to surface fouling.

The simplest route to CNT-modified electrodes is to cast a solution of CNTs onto a GCE. Since CNTs are insoluble in most solvents, ultrasonication is required during preparation in order to effectively disperse the tubes. Electrodes have been fabricated by dispersing CNTs in a phosphate buffer or concentrated sulfuric acid, and subsequent spin-casting onto a polished GCE [11, 12]. The dispersion of CNTs in aqueous solution can be facilitated by an appropriate surfactant. For example, with the aid of dihexadecyl hydrogen phosphate (DHP) or dicetyl phosphate (DCP) as surfactant, homogeneous and stable MWCNT-DHP [13] or MWCNT-DCP [14] film electrodes, respectively, have been obtained through solvent evaporation. Instead of aqueous media, acetone [15, 16] and DMF [17] have also been used as solvents when fabricating electrodes using this technique. Another simple preparation method involves gently rubbing a preheated GCE onto a filter paper supporting the nanotubes [10]. The electrocatalytic activity of the electrodes obtained in this manner can be further improved via electrochemical activation [17].

To strengthen the mechanical connection of the nanotubes to the underlying GCE, Nafion is commonly used. Nafion, a perfluorinated sulfonic acid ionomer with good ion exchange and biocompatibility properties, has proven very effective as a protective coating for glucose sensors [18]. By mixing Nafion with CNT dispersions in concentrated sulfuric acid, followed by casting onto a GCE [11, 12], well-connected CNT networks are obtained. Another example is a carbon-fiber electrode (in place of GCE) modified with MWCNTs, prepared by dipping the fiber into a sonicated phosphate buffer solution containing CNTs and Nafion [19].

An effective method for the fabrication of CNT-modified electrodes is the direct growth of CNTs onto GCE or metallic substrates. This approach not only improves the electrical contact between the active sensing material (CNTs) and the conducting substrate, but also ensures that the sensor is free of impurities originating from the surfactant or the binder [20, 21]. Another advantage of direct growth is the ability to fabricate arrays of CNT nanoelectrodes (see Fig. 2) [22]. Since the edges of the nanotubes are exposed, electrodes with vertically aligned CNT arrays exhibit the highest electrocatalytic activity coupled with fast electron transfer [23, 24].

In order to detect DNA hybridization, amino-terminated oligonucleotides have been covalently linked to oxidized CNTs on a gold substrate [22] or on a GCE [25, 26]. A schematic illustration of the procedure is shown in Fig. 3. Furthermore, adenine- and thymine-containing oligonucleotides have been used to anchor oligonucleotide-modified MWCNTs to a carbon paste electrode [27].

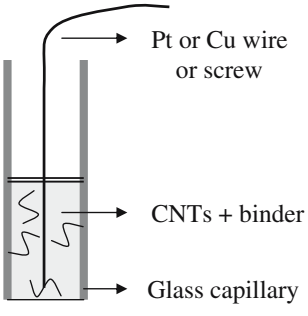

Fig. 1 Schematic drawing of a CNT paste electrode (CNTPE) [6–8]

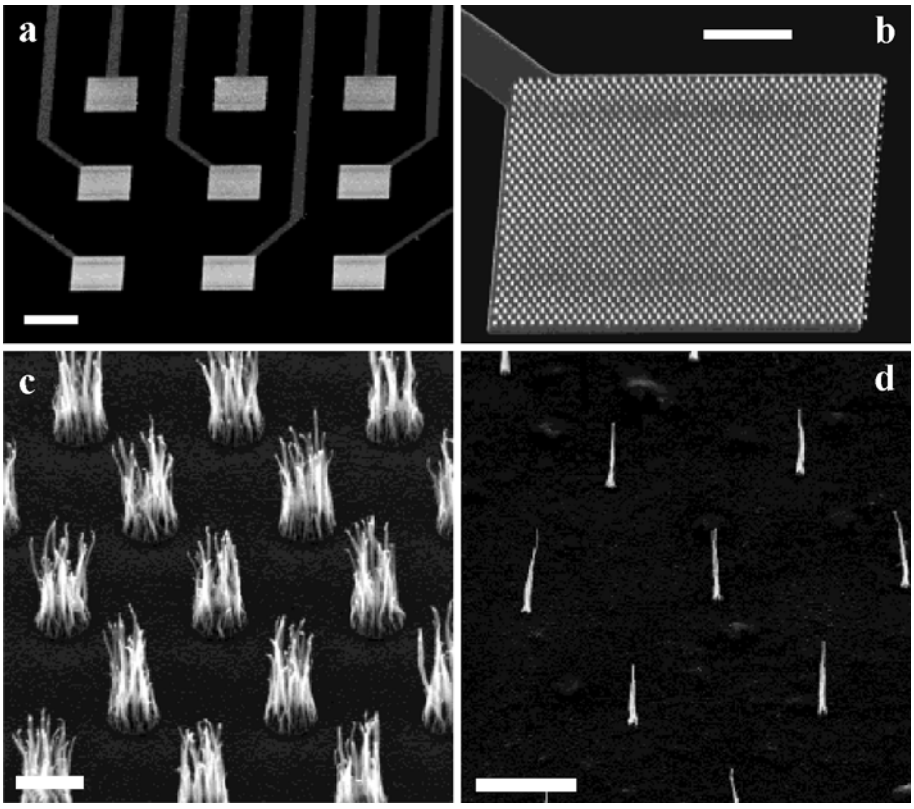

Fig. 2a–d SEM images of MWCNT nanoelectrode arrays: **a** 3×3 electrode array; **b** array of MWCNT bundles on one of the nine pads; **c** and **d** arrays of MWCNTs at UV lithography and e-beam patterned Ni spots, respectively. The scale bars are 200, 50, 2 and 5 μm, respectively. Reprinted with permission from [22]

CNT-based electrodes modified by metallic nanoparticles

Transition metals such as gold, platinum, palladium, copper, silver and nickel are well-known for their high catalytic activity. They have been widely utilized to enhance the performances of electrodes made of carbonaceous materials, and, in particular, to increase their sensitivity towards a specific analyte. However, electrode coatings made of thin metallic films are disadvantageous due to the complicated interplay between the thickness of the film and the sensor response. In addition, such electrodes are prone to severe poisoning or corrosion. A promising alternative is the use of metallic nanoparticles, which help to increase the catalytic activity and provide a larger surface area, thereby imparting a higher sensitivity to the sensing electrode. Furthermore, because nanoparticles can be easily modified with a wide range of biomolecules, they enable the fabrication of biosensors with a plethora of sensing possibilities.

Biosensing electrodes composed of CNTs have been modified with metallic nanoparticles via the adsorption of preformed nanoparticles, or via electrodeposition from metal salt solutions. The latter method is especially attractive since it allows precise control over the degree of modification in a reproducible manner [28, 29]. Figure 4 shows individual SWCNTs decorated with electrodeposited Pt nanoparticles. By controlling the duration and

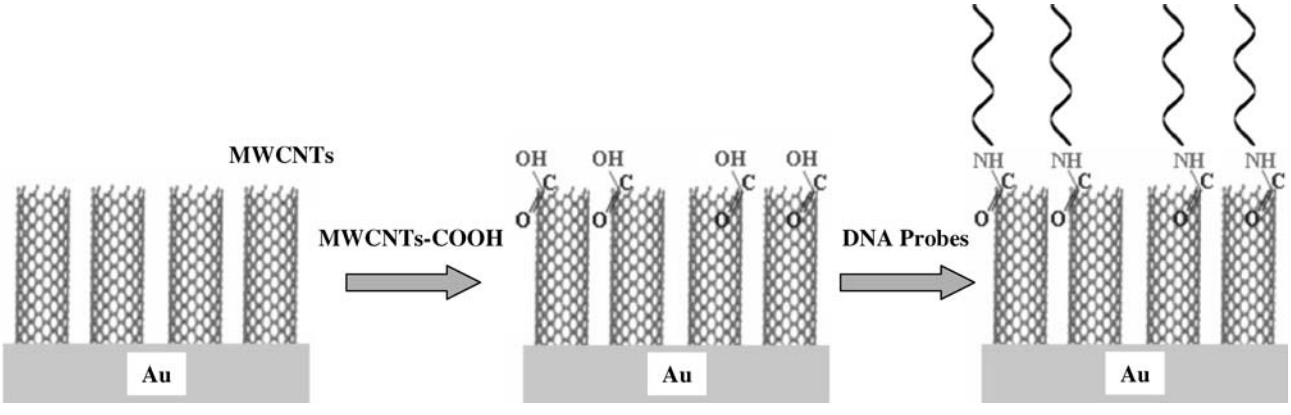

Fig. 3 Schematic representation of the attachment of single-stranded DNA (ssDNA) to the tips of oxidized MWCNTs deposited on a gold substrate. Reprinted with permission from [26]

magnitude of the applied potential in conjunction with the concentration of the salt used, different sizes and densities of such particles can be obtained [29]. Biosensing electrodes containing CNTs homogeneously coated with platinum have been demonstrated using this procedure [21]. Due to the rapid oxidation of H_2O_2—a common by-product of enzymatic reactions—on platinum [2], Pt nanoparticle-modified CNT-based electrodes have been intensively investigated. Excellent amperometric detection of H_2O_2 over a wide range of concentrations has been reported for these electrodes [30].

GCEs modified with nanotubes embedded in a Nafion matrix have also been decorated with copper nanoparticles [31]. Copper has been reported to be superior to other transition metals for the amperometric detection of various carbohydrates and amino acids [32]. Furthermore, gold colloids have been used to decorate MWCNT-modified gold electrodes [33]. Here a suspension of gold colloids in a DHP-stabilized aqueous dispersion of MWCNTs is coated onto a polished gold surface. The nanoparticles were demonstrated to be in electrical contact with the underlying electrode.

CNT-based electrodes with immobilized enzymes

The selectivity and sensitivity of CNT-modified electrodes can be improved through the immobilization of enzymes. In such electrodes, the CNTs mainly serve as transducers, communicating the signal effectively from the active enzyme centers to the substrate. To this end, various techniques relying on noncovalent or covalent bonding of the enzyme have been developed. The noncovalent approach, which has a negligible effect on the activity of the enzyme, can be subdivided into adsorption, entrapment and encapsulation techniques. In the first technique, the enzyme is attached with the help of a binder or an ion-exchange resin onto CNT-modified electrodes. Alternatively, the enzyme can be immobilized using surface groups of self-assembled monolayers or Langmuir–Blodgett (LB) films. In the entrapment method, a (electro)polymerization reaction is carried out during which the enzyme is incorporated into the resulting polymer matrix. The encapsulation method makes use of hydrogels or sol-gels to immobilize the enzyme. In the following subsections, a brief overview of the various immobilization techniques is given.

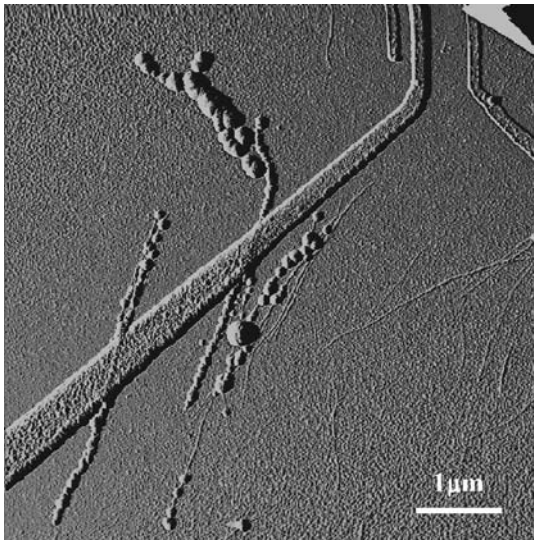

Fig. 4 Controlled electrochemical deposition of platinum nanoparticles onto individual SWCNTs in contact with an electrode. A potential of −0.7 V vs. Pt was applied for 30 s in a solution of H_2PtCl_6 in ethanol. Pt clusters can be identified along the contacted tubes, while the tubes that are not in contact remain unmodified

Enzyme adsorption

In this procedure, the CNT-modified electrode is first prepared by evaporating or casting a CNT dispersion onto a GCE, and then a Nafion-containing solution of the desired enzyme is dropped on top of this electrode and allowed to evaporate. In this manner, a tyrosinase-based amperometric sensor has been realized for the detection of phenolic compounds [34]. As a further example, a sensor for organophosphorus compounds has been obtained by immobilizing the enzyme organophosphorus hydrolase (OPH) onto a CNT-modified GCE [35]. The immobilization of enzymes via adsorption faces several problems, such as the low quantity of adsorbed enzyme, leaching of the enzyme and so on. Some of these limitations can be overcome by adsorbing enzymes onto CNT-modified GCEs decorated with metallic nanoparticles, as has been demonstrated for a Pt-NP/CNT/GC electrode modified with glucose oxidase (GOx) [20, 30]. By subsequently depositing a Nafion film onto the electrode, it is possible to reduce leaching of the enzyme and to improve the stability of the biosensor. Another method for enzyme adsorption involves a layer-by-layer technique wherein alternate layers of oppositely charged polyelectrolyte and enzyme are deposited onto an electrode. This has been exploited in order to fabricate a cholesterol biosensor from a cationic polyelectrolyte (PDDA, poly(diallyldimethylammonium chloride)) and cholesterol oxidase on a MWCNT-modified gold electrode [36].

Covalent attachment

The adsorption method normally yields a random distribution of the enzymes on the electrode. However, direct anchoring of the enzymes to the carbon framework becomes feasible if the covalent immobilization approach is used. In addition, it often enables direct electron transfer to the active center of the enzyme. Covalent attachment has for instance been used to immobilize GOx onto the ends of an array of SWCNTs grown on a gold substrate [37]. In order to achieve direct electron transfer to the redox center of the enzyme molecules, flavin adenine dinucleotide (FAD) is first covalently attached to the SWCNT ends, and then GOx is reconstituted at the im-

mobilized FAD [37, 38]. Figure 5 depicts the fabrication scheme of such an electrode.

Electropolymerization

Electropolymerization is an attractive and well-controlled method for immobilizing enzymes onto electrodes. In this methodology, the enzyme is mixed with a monomer which is electropolymerized at a GCE or a metal electrode, whereupon the enzyme becomes embedded into the polymer matrix. The incorporation of the enzyme into the matrix is often promoted through electrostatic interactions. Advantages of the electropolymerization approach include the good control over the film thickness and the ability to selectively attach biomaterials onto nanoscale electrode surfaces. Numerous enzymes have been incorporated into electropolymerized films [39, 40]. In many cases conductive polypyrrole (PPy) has been used as a polymer matrix. This choice relates to the fact that pyrrole can be electropolymerized at low oxidation potentials in aqueous solutions at neutral pH, which is compatible with a wide range of biological molecules. Polypyrrole has proven effective at electrically wiring the enzymes and CNTs to the underlying electrode. During the fabrication of such biosensors, CNTs bearing carboxylic groups are often used due to their ability to function as an anionic dopant in the matrix. On this basis, glucose biosensors have been fabricated from pyrrole, GOx and oxidized MWCNTs [40]. For this purpose, pyrrole and GOx were added to a solution containing oxidized MWCNTs, followed by electropolymerization on a GCE to obtain a PPy/GOx/MWCNT electrode. The CNTs entrapped in the PPy network were found to retain their electrocatalytic activity towards hydrogen peroxide. PPy-based GOx immobilization has also been performed on aligned carbon nanotube arrays grown on a quartz substrate [24]. By controlling the density of the array, PPy could be deposited either only at the tube ends or both at the ends and on the sidewalls. Representative Scanning Electron Microscopy (SEM) images of such PPy-coated CNT arrays are shown in Fig. 6. Moreover, single-stranded DNA has been incorporated into a PPy matrix through electropolymerization at an MWCNT-GCE, paving the way for a DNA hybridization sensor [41]. Apart from pyrrole, *o*-aminophenol has been used as a monomer when immobilizing GOx by electropolymerization [42]. In this case, the polymerization was performed on a MWCNT electrode precoated with iron phthalocyanine.

Encapsulation

Another method of immobilizing enzymes is to encapsulate them in hydrogels or sol-gel materials. Two different approaches [43] have been pursued for this purpose, as schematically outlined in Fig. 7. In the first procedure, CNTs are first brought onto the substrate followed by the deposition of the hydrogel or the sol-gel matrix containing the immobilized enzyme. Using this methodology, a basal plane pyrolytic graphite electrode modified with MWCNTs has been covered with a sol-gel-derived matrix containing GOx [44]. A broad range of enzymes has been successfully immobilized onto CNT-incorporated redox hydrogels to yield sensitive biosensors [43]. Alternatively, the CNTs can be incubated with the enzymes and then incorporated into the hydrogel or the sol-gel matrix, which is then subsequently brought onto a support material. This scheme has been used in the preparation of a MWCNT-sol-gel matrix for the encapsulation of L-amino acid oxidase (LAAOx) [45]. The main advantage of the encapsulation process is that the entrapped species often preserves its intrinsic bioactivity. Additionally, such sensors exhibit enhanced sensor response, due to an increase in the surface area as well as an improvement in the electrical communication between the redox centers of the hydrogel or the sol-gel-derived matrix and the electrode. Apart from hydrogels and sol-gels, Nafion has also been found to be useful when

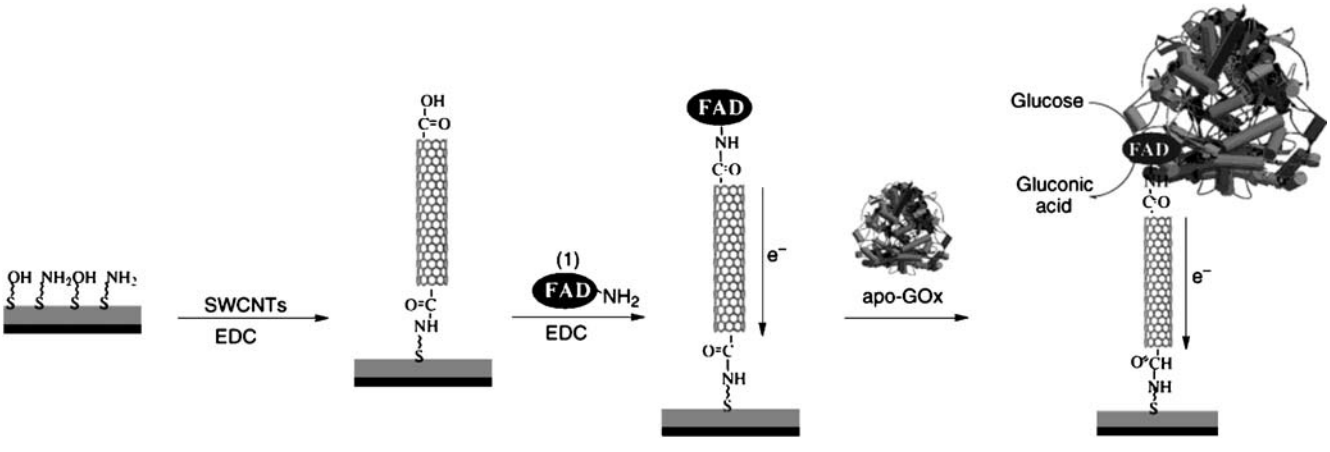

Fig. 5 Assembly of a SWCNT-based GOx electrode. A 2-thioethanol/cystamine (3:1 ratio) mixed monolayer was assembled on an Au electrode, followed by coupling of SWCNTs to the surface in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The amino derivative of the FAD cofactor (1), was then covalently attached to the carboxy groups at the SWCNT tips. In the last step, apo-GOx was reconstituted on the FAD units. Reprinted with permission from [38]

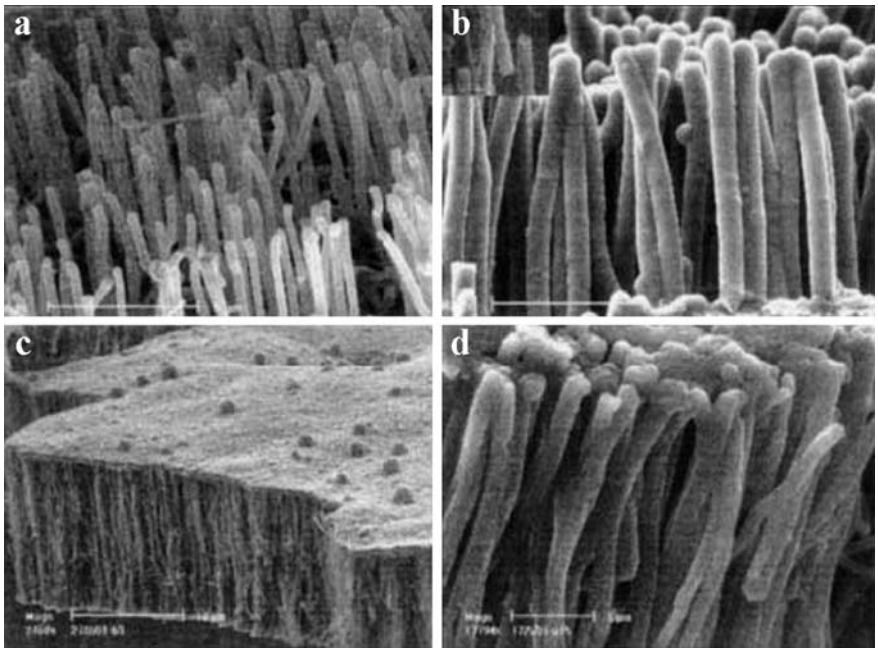

Fig. 6a–d SEM images of: **a** pure CNT array before polypyrrole (PPy) deposition; **b** aligned PPy-CNT coaxial nanowires; **c** PPy deposited only on the top of the CNT surface due to the high density of the tube array; **d** polymer formed on both the tube walls and the surface of the CNT array. Reprinted with permission from [24]

fabricating composite electrodes. A glucose sensor has been obtained by encapsulating GOx and MWCNTs in a Nafion matrix in appropriate amounts [46].

Nanoscale CNT electrodes

Until now, we have been discussing CNT-based microelectrodes for electrochemical biosensing purposes. However, due to their miniscule sizes, it should also be possible to use individual nanotubes as nanoelectrodes in sensor applications. This task has indeed been realized with individual semiconducting SWCNTs contacted in a field-effect transistor (FET) configuration, which enabled the detection of gas molecules [47–49] and humidity [50]. However, commercial realization of such devices has been largely impeded by the inability of the synthesis procedures that are currently available to guarantee the exclusive preparation of semiconducting tubes [51]. One way to overcome this difficulty is to use devices composed of a network of SWCNTs. Such ensembles usually contain an excess of semiconducting tubes, rendering the network weakly semiconducting, which is often sufficient for sensing purposes. An improvement in sensitivity and selectivity towards a specific analyte could be achieved by chemical functionalization of the SWCNTs. Glucose sensors have been demonstrated by immobilizing GOx onto SWCNTs (see Fig. 8a) with the aid of a linker molecule, which binds at one end to the SWCNT through van der Waals interactions, while the carboxylic group at its other end attaches to the enzyme through amide bond formation [52, 53]. Networks of SWCNTs have also been noncovalently modified with receptor molecules on their sidewalls (see Fig. 8b). Specifically, polyethyleneimine (PEI)/polyethyleneglycol (PEG)- [5] and polyethyleneoxide (PEO)-based polymers [54] have been used to coat the nanotubes prior to immobilization of various proteins and enzymes through amide linkages.

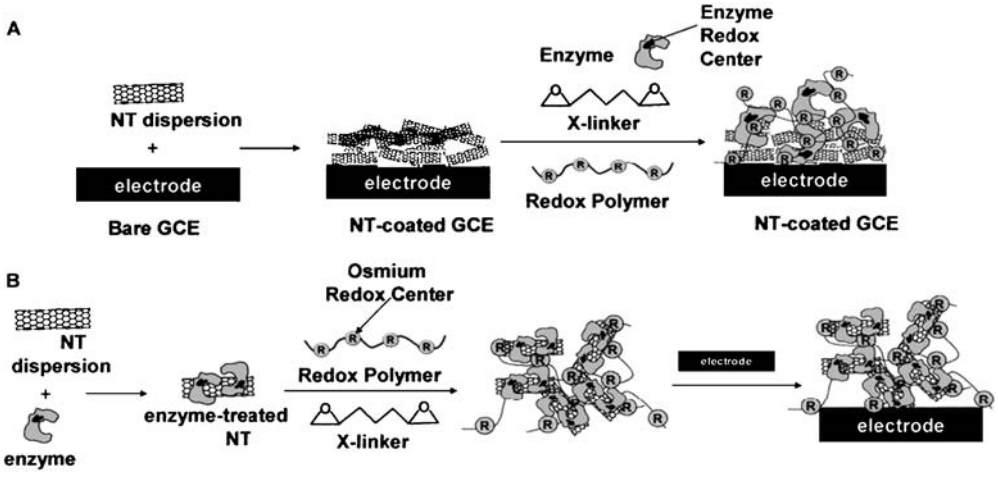

Fig. 7a–b Schematic showing two different methods by which an enzyme is immobilized in a CNT-based hydrogel or sol-gel matrix. In the first method (**a**) the sensors are fabricated by casting a CNT film onto a GCE before the redox hydrogel or the sol-gel composite containing the enzymes is deposited on top of the CNT-coated electrode. The second (**b**) type of sensor is prepared by incubating the CNTs with an enzyme solution, followed by incorporation into the hydrogel or sol-gel matrix, which is then brought onto a substrate. Reprinted with permission from [43]

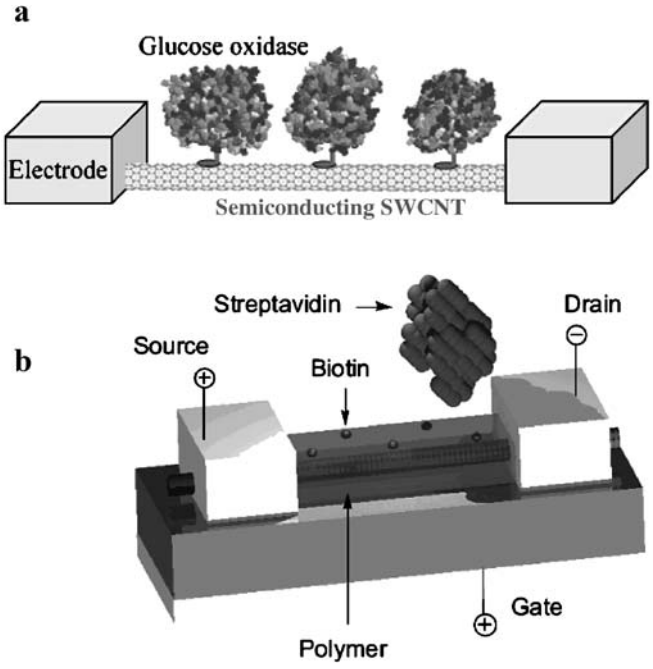

Fig. 8a–b **a** Schematic depiction of a semiconducting SWCNT with GOx immobilized on its surface. The enzyme is attached with the help of a linker molecule (1-pyrenebutanoic acid succimidyl ester). Reprinted in part with permission from [52]. **b** Schematic of a SWCNT field-effect transistor (FET). The SWCNT is coated with a polymeric layer containing biotin, which serves to selectively bind streptavidin and thereby induce a change in the FET characteristics. Reprinted with permission from [5]

Electrochemical detection of biomolecules

Electrochemical methods for detecting biomolecules in solution are highly attractive due to their simplicity and the relative ease of calibration. Electrochemical sensors can be based on potentiometry, amperometry, voltammetry, coulometry, AC conductivity or capacitance measurements [55]. Most of the CNT-based electrochemical biosensors perform the detection of biomolecules amperometrically. In order to have a common framework for analyzing the various biosensors, we discuss sensor parameters and assessment criteria first here. CNT-based biosensors incorporating enzymes are then presented, starting with the glucose biosensors listed in Table 1. Enzymatic biosensors for other biomolecules are summarized in Table 2. Enzyme-free electrochemical biosensors have been successfully applied to the detection of various molecules ranging from nitric oxide to cytochrome *c*. The characteristics of these sensors are collected in Table 3.

Sensor parameters and assessment criteria

There are a number of aspects which need to be considered when analyzing various biosensors. The first important sensor parameter is the range within which the sensor is sensitive. For example, a glucose sensor used to detect diabetes needs to be sensitive in the range of a few μmol/l to 15 mmol/L. This is due to the fact that a level of less than 6 mmol/L of glucose in blood is considered to be normal, while a level of 7 mmol/L or higher suggests diabetes. For environmental studies, sensors for the detection of organophosphorus compounds must have very low detection limits: in the ppb range. A cholesterol sensor must have sensitivity in the range of 2.5–10 mmol/L (100–400 mg/dL). While a total blood cholesterol level of less than ~5 mmol/L is considered to be risk-free, high cholesterol levels of more than ~6 mmol/L are dangerous and this condition is referred to as hypercholesterolemia. Another relevant sensor parameter is sensitivity. For chemicals to be detected in the micromolar range it is required to have a sensitivity that is at least in the μA/mmol/L range. Lower sensitivities than this would require sophisticated electronics to detect low currents, which would increase the cost of the sensor. Another factor that plays a key role is the sensor stability. Since many enzyme-based biosensors contain biodegradable

Table 1 Summary of the various CNT-based glucose sensors based on nanotubes reported in the literature, along with their important parameters

No.	Fabrication methodology	Sensors	Sensitivity $[(mmol/L)^{-1}]$	Linear range [mmol/L]	Response time [s]	Stability
S1	Nanoparticle decoration	CuNP-SWCNT-Nafion/GCE [31]	256 μA	0–0.5	10	
S2	Nanoparticle decoration, Enzyme adsorption	Nafion/GOx/PtNP/CNT/Graphite [20]	14 μA	0.1–13.5	<5	73.5% after 22 days
S3	Nanoparticle decoration, Enzyme adsorption	GOx/PtNP-SWCNT-Nafion/GCE [30]	2.11 μA	0.0005–5	3	
S4	Electropolymerization	POAP-GOx/FePc-MWCNT [42]	735 nA	0.0005–4	<8	120 days
S5	Electropolymerization	PPy-GOx-MWCNT array/Gold [24]	350 nA	2.5–20		70% after 3 days
S6	Encapsulation	GOx-Nafion-MWCNT/GCE [46]	330 nA	0–0.002	<3	
S7	Encapsulation, Sol-gel process	GOx-SGC/MWCNT/bppg [44]	196 nA	0.2–20	<5	3 weeks
S8	Electropolymerization	PPy-GOx-MWCNT/GCE [40]	2.33 nA	0.2–50	15	

The blanks in the columns for response time and stability indicate that these values were not available in the corresponding reported works.
bppg basal plane pyrolytic graphite, *FePc* iron phthalocyanine, *GCE* glassy carbon electrode, *GOx* Glucose oxidase, *POAP* poly-*o*-aminophenol, *NP* Nanoparticle, *PPy* polypyrrole, *SGC* sol-gel composite

Table 2 Overview of enzymatic CNT-based biosensors for various biomolecules

Analyte	Occurrence	Enzyme	Sensor
Glucose	Blood, body fluids	Glucose oxidase (GOx)	See Table 1
Dopamine	Brain tissue, blood	Polyphenol oxidase (PPOx)	MWCNTPE [7]
			PPOx/MWCNTPE [8]
			MWCNT-Nafion/CFE [19]
Other phenolic compounds	Water, food products	Tyrosinase (Ty), PPOx	PPOx/MWCNTPE [8]
			MWCNT-Nafion/GCE [11]
			Nafion/Ty/SWCNT/GCE [34]
Organophosphate pesticides	Water resources, agriculture	Organophosphorus hydrolase (OPH)	OPH/CNT-Nafion/GCE [35]
Cholesterol	Blood	Cholesterol oxidase (ChOx)	ChOx/MWCNT/CPE [9]
			ChOx (LBL)/MWCNT/Gold [36]

CFE carbon fiber electrode, *CNTPE* CNT paste electrode, *CPE* Carbon paste electrode, *LBL* layer-by-layer technique

components, they need to be stored under special conditions to avoid degradation of the active material. In practice, a sensor which loses 50% of its original activity after just two weeks of storage would not have much value.

Glucose

Detection of glucose is one of the most frequently performed routine analyses in medicine. Around 5% of the populations of industrialized nations have diabetes, resulting in a high demand for the detection of glucose in body fluids. Glucose sensors normally incorporate glucose oxidase (GOx), an enzyme which catalyses the oxidation of β-D-glucose to D-glucono-1,5-lactone, using oxygen (O_2) as electron acceptor. The generated hydrogen peroxide (H_2O_2) is then electrochemically detected at an appropriate electrode. GOx shows a very high specificity for β-D-glucose, although the oxidation of 2-deoxy-D-glucose, D-mannose and D-fructose is also catalyzed, albeit with a much lower turnover rate.

The performance characteristics of a range of CNT-based glucose biosensors reported in the literature are compiled in decreasing order of sensitivity in Table 1. It is evident that the sensors incorporating nanoparticles are the ones that possess the highest sensitivity. This can be attributed to the enhanced catalytic activity, good biocompatibility and large surface area obtained by combining the advantages of CNTs and nanoparticles. The Cu nanoparticle-based sensor (S1) has a high sensitivity of 256 μA/mmol/L; however, it can only be used at very low concentrations, below 0.5 mmol/L. The Pt nanoparticle-modified sensors S2 and S3 are preferred in this aspect. S2 can be regarded as an ideal sensor that could be used in practical applications, as it exhibits a very good sensitivity of 14 μA/mmol/L within a large detection range, up to

Table 3 Overview of enzyme-free CNT-based biosensors for various biomolecules

Analyte	Occurrence / Importance	Sensors
NAD+	In vivo processes	MWCNT/GCE [12]
Nitric oxide (NO)	Biological systems, in vivo processes	MWCNT-Nafion/GCE [15]
Cytochrome *c*	In vivo processes	aSWCNT/GCE [17]
		AuNC-MWCNT-DHP/Gold [33]
Morphine	Blood, hair, body fluids	MWCNT/GCE [10]
Cysteine	Consumer products	Pt/CNT-graphite [21]
Indole acetic acid	Plant cells	MWCNT-DHP/GCE [13]
DNA	Bioassays	Dmc; ssDNA/oMWCNT/GCE [25]
		Dmc; PtNP/ssDNA/oMWCNT/GCE [60]
		Dmc; PPy/MWCNT/GCE [61]
		MethBlue; ssDNA/MWCNTNEA [26]
		CNTPE [62]
		SWCNT/GCE [63]
		$Ru(bby)_3^{2+}$; ssDNA/CNTNEA [22]
		MWCNT-CPE [27]
		PPy-ssDNA/oMWCNT-GCE [41]
		MagB-ssDNA/ALP-CNT/GCE [64]

a activated, *o* oxidized, *ALP* alkaline phosphate enzyme, *AuNC* gold nanocolloids, *CNTNEA* CNT nanoelectrode array, *CNTPE* CNT paste electrode, *DHP* dihexadecyl hydrogen phosphate, *Dmc* duanomycin, *MagB* magnetic bead, *MethBlue* methylene blue, *NP* nanoparticles, *PPy* polypyrrole, *ssDNA* single-stranded DNA

13.5 mmol/L, encompassing the clinically important diabetes level. In these electrodes, a permselective Nafion barrier delivers selectivity to the sensors by discarding interferents such as ascorbic acid or D-fructose. The preparation of S3 differs from that of S2 in the way that Nafion is incorporated into the sensing electrode. Figure 9i illustrates a typical amperometric response and a calibration curve obtained for the sensor S3.

Immobilization of GOx into an electropolymerized matrix is the next preferred method for obtaining good sensitivity. S4 has been prepared using poly-*o*-aminophenol (POAP) as the polymer incorporating iron pthalocyanine (FePc), which serves as a redox mediator. Typical amperometric responses with and without FePc are compared in Fig. 9ii. The use of FePc as a redox mediator imparts S4 with twice as much sensitivity as S5, where GOx was immobilized within a PPy matrix on a MWCNT array. In comparison, S8, which is also prepared through the electropolymerization route, exhibits a much lower sensitivity of 2.33 nA/mmol/L. This difference can be understood by the fact that in S4 and S5 the nanotubes are in direct physical contact with the substrate, while in S8 the nanotubes are loosely connected, since they are just suspended in the electropolymerized matrix. Finally, S6 and S7, both prepared by the encapsulation technique, offer relatively low sensitivities of 330 and 196 nA/mmol/L respectively. While the practical application of S6 is limited by its low micromolar detection range, S7 has an acceptable range of up to 20 mmol/L. It should be stressed that the surface area of the electrode is an important factor that determines the sensitivity of an electrochemical biosensing electrode. The total electrode currents were used in the above comparisons since explicit surface area values are not available for most of the reported electrodes.

Another important factor for the practical use of these glucose sensors is the response time. The Pt nanoparticle-based sensors S2 and S3 are able to deliver a signal in less than five seconds. If we take their high sensitivity also into account, they may be regarded as the best alternative for practical purposes. Interestingly, the electrodes prepared by encapsulation (S6 and S7) also offer a fast response in the same range as S2 and S3. In contrast, the sensors consisting of GOx immobilized in an electropolymerized matrix are comparatively slow. The slow response of S1 can be understood by observing that this sensor was prepared without GOx.

In order to get good stability, the electrodes reported here are usually maintained in a phosphate buffer solution under argon atmosphere and/or at low temperatures to avoid contamination and denaturation of the biomaterial. Sensors like S4 and S7 have been reported to fully retain their initial activity under these conditions even after several weeks, while S2 exhibited approximately three-quarters of its initial response after ~3 weeks. On the other hand, a significant loss of signal was observed in S5 after three days of storage in the dry state at 4 °C.

In summary, by looking at the various sensors collected in Table 1 and bearing in mind the assessment criteria described previously, it can be concluded that S1, S3, S4 and S6 have only limited importance due to their sub-5 mmol/L application ranges. S8 on the other hand has a very low sensitivity of a few nA/mmol/L. Thus S2, S5 and S7 are the sensors that appear most promising for real-life applications.

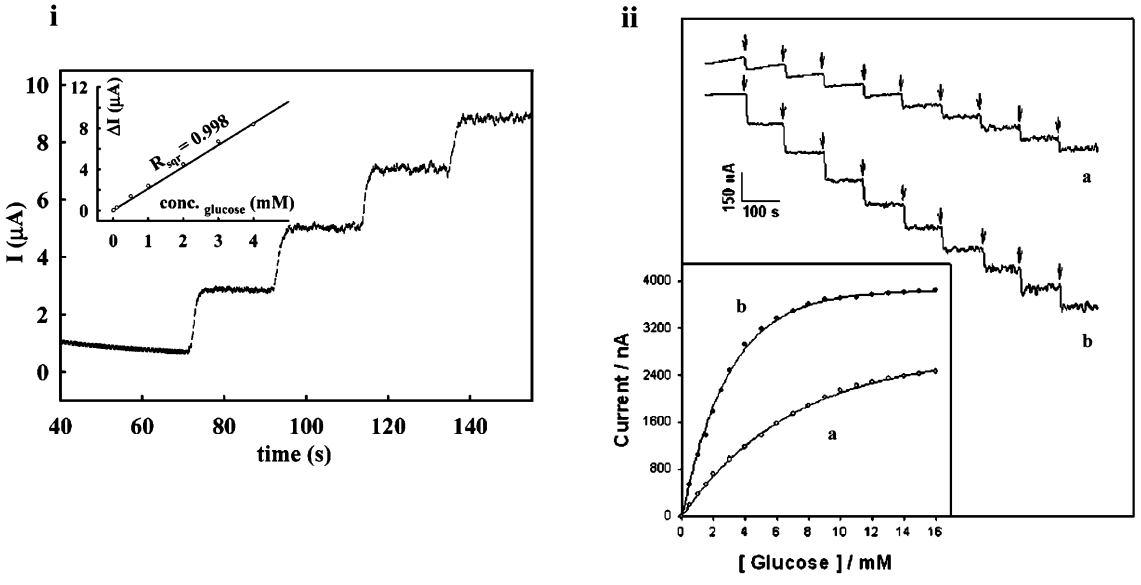

Fig. 9i–ii **i** Amperometric response of S3 [refer to Table 1] to subsequent additions of 1 mmol/L glucose in phosphate buffer solution (PBS) at 0.55 V vs. Ag/AgCl. *Inset* shows the calibration curve for glucose concentrations of between 0.5 mmol/L and 5 mmol/L. Reprinted with permission from [30]. **ii** Amperometric response of S4 [refer to Table 1] without (*a*) and with (*b*) FePc to successive injections of 0.1 mmol/L glucose at 0.6 V vs. Ag/AgCl. *Inset* shows the calibration curve for glucose concentrations of between 0 and 15 mmol/L. Reprinted with permission from [42]

Phenolic compounds

A wide range of phenolic compounds occurs naturally in many food products, plants, vegetables and fruits that have antioxidant properties. High intake of such food products decreases the risk of cardiovascular diseases and cancer. On the other hand, some phenols and cresols are toxic, so their determination in food samples and the environment is of considerable interest. Since phenolic compounds in general are easily oxidized, amperometric detection is the method of choice [56]. The major complication encountered with classical electrodes consists of surface fouling by dimeric or polymeric oxidation products. This problem can be largely alleviated by CNTs, as has been demonstrated by a MWCNT/Nafion-GCE [11]. The sensor response in a phenol solution was found to be highly stable, with 85% of the initial activity retained after 30 minutes, in comparison to complete inhibition of the redox process within six minutes at the bare surface. Similar improvements in stability could be observed with this sensor for other phenolic compounds for a broad range of concentrations.

Apart from the use of bare CNT electrodes, enzyme-modified CNT-electrodes have also been successfully used in the detection of phenolic compounds. One example is the adsorption of the enzyme tyrosinase onto a CNT-modified GCE [34]. In the presence of oxygen, tyrosinase catalyzes the oxidation of phenolic compounds to quinones, which can be electrochemically detected at the electrode. This sensor exhibited a sensitivity of 155 μA/mmol/L for phenol, and allowed for the detection of benzoic acid based on the inhibition of tyrosinase activity. Another example is a CNT paste electrode modified with polyphenol oxidase (PPOx) [8], which enabled the detection of various phenolic compounds, including phenol, catequine and catechol in real pharmaceutical products.

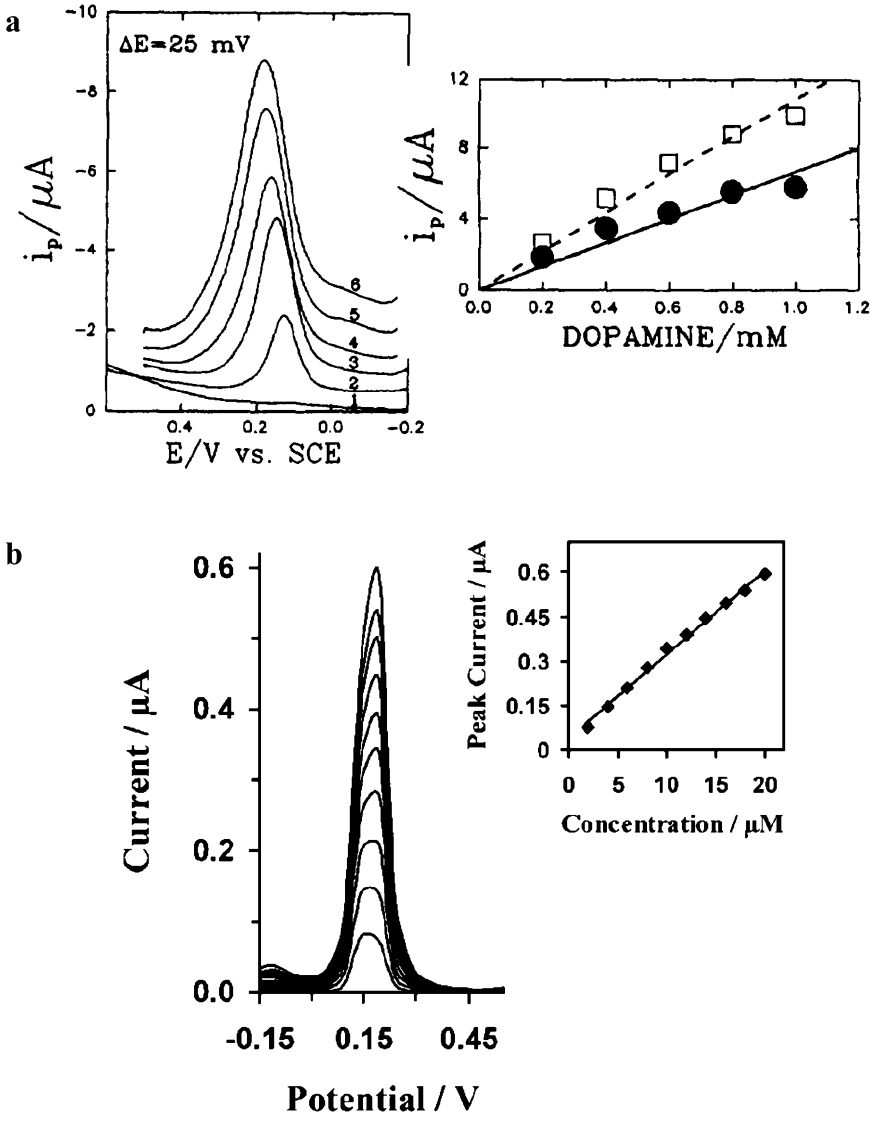

Fig. 10a–b **a** Differential pulse voltammetric (DPV) responses and the corresponding calibration plot of dopamine in PBS at a MWCNT paste electrode. Concentration of dopamine from *1* to *6*: 0, 200, 400, 600, 800, 1000 mmol/L. Reprinted with permission from [7]. **b** DPV responses of ten successive additions of 2 mmol/L dopamine at a MWCNT/Nafion-modified carbon fiber electrode with the resulting calibration plot. Reprinted with permission from [19]

Dopamine

Dopamine is a neurotransmitter and a neurohormone belonging to the catecholamine family of phenolic compounds. Dopamine is used as a drug; it acts on the nervous system to increase heart rate and blood pressure. A deficiency of dopamine in the brain is believed to cause schizophrenia and Parkinson's disease. Thus sensing of dopamine in brain tissue is vital in clinical diagnoses. The first ever reported biosensor using CNT-based electrodes was a dopamine biosensor [7]. It consisted of a MWCNT paste electrode, which was used to detect dopamine in goat's brain tissue homogenate by monitoring its reversible electrochemical oxidation using differential pulse voltammetry (DPV). Figure 10a shows the DPV responses obtained for various concentrations of dopamine and the corresponding calibration plot in an aqueous buffer solution. Using a carbon fiber microelectrode modified with MWCNTs and Nafion, a linear DPV response over a range of 2–20 μmol/L and a detection limit of 70 nmol/L have been achieved, as shown in Fig. 10b [19]. A Nafion membrane was used to block interferents such as ascorbate, which exhibits an oxidation potential close to that of dopamine. Due to its stable response, this sensor may find future application as a disposable dopamine microsensor at physiological concentrations in microvolume samples. Dopamine could also be detected at PPOx-modified CNT paste electrodes with a sensitivity of around 2 μA/mmol/L [8].

Organophosphorus compounds

Organophosphorus (OP) compounds are used as pesticides and chemical warfare agents. Hence the detection of OP neurotoxins is essential for the protection of water resources and food supplies, as well as for monitoring detoxification processes. One attractive method of sensing such compounds involves the use of the enzyme organophosphorus hydrolase (OPH), which converts OP compounds into *p*-nitrophenol, which can be subsequently oxidized at a CNT-modified electrode. On this basis, an amperometric biosensor for OP compounds has been fabricated by adsorbing OPH onto a SWCNT- or MWCNT-modified GCE [35]. Such CNT-modified electrodes yielded a stable anodic current signal for *p*-nitrophenol over a time duration of ~60 minutes. Under optimal conditions, the biosensor could detect as low as 0.15 μmol/L paraoxon and 0.8 μmol/L methyl parathion with respective sensitivities of 25 and 6 nA/mmol/L.

Cholesterol

The analysis of cholesterol levels is of vital importance in clinical diagnoses for both day-to-day control purposes and in cases of chronic cardiovascular diseases. Cholesterol oxidase (ChOx) is an enzyme promoting the oxidation of cholesterol, generating H_2O_2 in the presence of oxygen. A biosensor for cholesterol has been demonstrated where a layer-by-layer adsorption technique is used to immobilize ChOx onto a MWCNT-modified gold electrode. The MWCNTs were oxidized by nitric acid prior to their deposition onto the gold electrode [36]. The sensor response was found to be linear in the range of 0.2–6 mmol/L, with a sensitivity of 559 μA/(cm^2 mol/L). Further to this, a screen-printed carbon paste electrode modified with MWCNTs and ChOx could detect cholesterol in blood in the range of 100–400 mg/dL [9].

NAD^+

Nicotinamide adenine dinucleotide (NAD^+) is an important cofactor of redox reactions inside living cells. The NADH generated can be detected by electrochemical oxidation at appropriate electrodes. The major drawback of using bare electrodes for this purpose is the high oxidation overpotential, combined with problems due to surface fouling by adsorption of reaction intermediates. CNT-modified GC electrodes offer advantages in both these respects. For instance, a stable response with more than 90% initial activity persisting after 60 minutes has been reported [12]. The oxidation potential was found to be much lower (~+0.35 V vs. Ag/AgCl for SWCNTs) compared to a bare GCE (+0.82 V vs. Ag/AgCl).

Nitric oxide

Nitric oxide (NO) represents an important molecular messenger in higher organisms. Biological NO functions include neurotransmission, blood clotting and blood pressure control. Using a MWCNT-modified GCE, NO could be detected amperometrically [15]. A Nafion coating was used to prevent interference from anions such as nitrite. The sensor exhibited a linear response in the range of 0.2–150 μmol/L with a detection limit of 80 nmol/L.

Cytochrome *c*

Cytochrome *c* is a highly conserved protein present in a wide spectrum of plant and animal species. It is a small heme protein that plays an important role in the mitochondrial electron transfer chain, which renders its detection important. The electrochemical response of cytochrome *c* is very poor at bare metal electrodes mainly due to denaturation at the electrode surface. Voltammetric detection of cytochrome *c* has been possible using a GCE modified with activated SWCNTs [17]. The electrode exhibited a linear response in the concentration range of 30 μmol/L to 0.7 mmol/L with a sensitivity of 3 μA/mmol/L. Moreover, using a gold colloid-MWCNT-DHP modified gold electrode, cytochrome *c* could be detected with a faster electron transfer rate than observed for a number of other electrodes [33].

Morphine

Morphine—the principal active agent in opium—is a powerful analgesic drug. The purpose of detecting morphine in biological samples is to monitor drug concentrations in pharmacokinetic studies and forensic cases as an indicator of heroin usage. Morphine has been amperometrically detected using MWCNT-modified GCEs, without requiring an immobilized enzyme [10]. These sensors showed a maximum sensitivity of 10 nA/μmol/L with an estimated detection limit of 0.2 μmol/L.

Cysteine

Cysteine is a sulfur-containing amino acid commonly used in the food and pharmaceutical industry. A platinum-coated CNT/GCE has been utilized to detect cysteine amperometrically in aqueous buffer solution [21]. The sensor exhibited a linear response in the range of 0.5 μmol/L to 0.1 mmol/L and a minimal signal loss of 20% after one month. In addition, the sensor displayed a good selectivity against tyrosine and tryptophan.

Indole acetic acid (IAA)

A plant hormone, indole-3-acetic acid (IAA) is known to regulate numerous developmental processes in the life cycles of many plants [57]. Monitoring the concentration of IAA in plants is of interest when determining their optimal growth conditions. Amperometric IAA sensors have been prepared by coating DHP-stabilized MWCNT dispersions onto a GCE [13]. The oxidation peak current of IAA was found to be strongly enhanced at this electrode in comparison with that at a bare GCE.

Others

Other substances that have been detected with the aid of CNT-based electrodes include hypoxanthine in fish (using a MWCNT-DCP electrode [14]) and ethanol in wine (with alcoholdehydrogenase and NAD+ immobilized on a CNT paste electrode [8]).

DNA

The human genome project has successfully deciphered the human genome, yielding a massive amount of information [58]. This genetic information needs to be analyzed in order to understand the functions of between 20000 and 25000 genes, which would help to revolutionize the treatment of inheritable and infectious diseases. Identifying genomic DNA sequences and detecting mutations is therefore vital in such studies. Electrochemical methods are well-suited to the rapid and simple detection of DNA concomitantly with high sensitivity and quick response. Such sensors have been fabricated by immobilizing single-stranded DNA (ssDNA) onto an electrode, allowing one to monitor a current signal when the complementary sequence hybridizes with the ssDNA. Normally a cationic metal complex or an intercalating organic compound is used as a redox indicator [59], which has a higher affinity for double-stranded DNA (dsDNA), and hence a larger electrochemical response is obtained after hybridization. A schematic of this electrochemical sensing principle is provided in Fig. 11.

In the presence of the electroactive intercalator duanomycin (Dmc), a 24-base pair DNA could be detected using differential pulse voltammetry (DPV) at a MWCNT/GCE electrode modified with complementary ssDNA [25]. The sensor exhibited good selectivity, as concluded from the low currents obtained from oligonucleotide sequences, having a mismatch of only a few bases. The sensitivity was on the order of 4 μA/nmol/L over a linear range of 0.2–50 nmol/L. A superior response was obtained with such electrodes when decorated with Pt nanoparticles [60]. A calibration plot for this PtNP-modified sensor is shown in Fig. 12. Again employing Dmc as an indicator, sub-picomolar concentrations of oligonucleotides have been detected using magnetite nanoparticles at a PPy/MWCNT/GCE [61]. In addition, self-assembled MWCNTs have

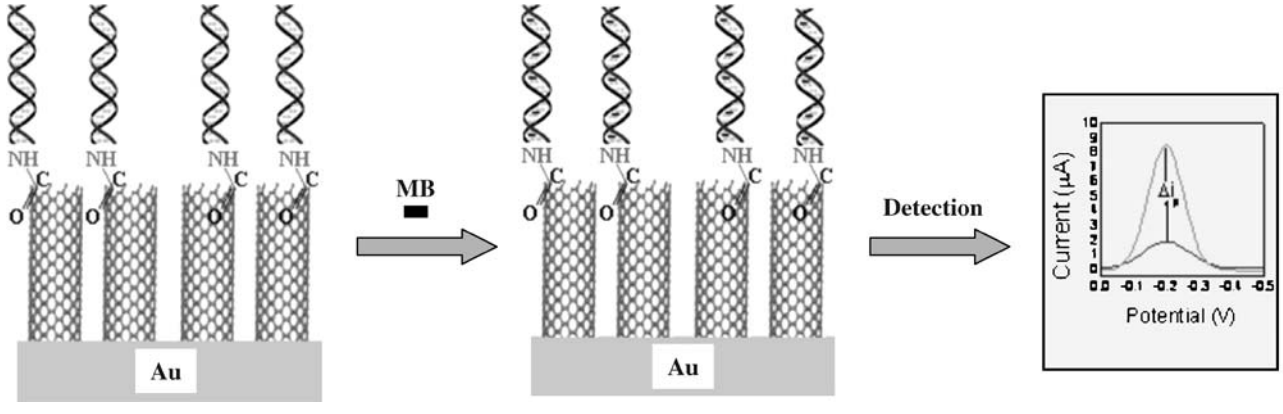

Fig. 11 Schematic illustration of DNA sensing with ssDNA/CNT-modified electrodes in the presence of an indicator (MB) such as methylene blue or duanomycin. Reprinted with permission from [26]

permitted the efficient detection of DNA hybridization in the presence of methylene blue as the redox indicator [26].

DNA can also be detected in the absence of an indicator by monitoring the electrochemical oxidation of guanine. A MWCNT paste electrode [62] and a SWCNT-modified GCE [63] are two examples involving the detection of short nucleotide sequences. A redox mediator can be used to improve the response of guanine oxidation. In this manner, attomoles of oligonucleotides could be detected at ssDNA-modified MWCNT arrays incorporated with $Ru(bby)_3^{2+}$ [22]. Furthermore, an improved label-free electrochemical DNA hybridization sensor based on AC voltammetric detection of guanine has been demonstrated by attaching MWCNTs onto a CPE using a hybridization assay [27]. The MWCNT-modified CPE exhibited a large improvement in signal in comparison to the bare CPE. A novel indicator-free AC impedance measurement technique could detect DNA at a MWCNT-GCE modified with a PPy matrix containing ssDNA [41]. Upon hybridization with the complementary nucleotide sequence, the impedance was found to decrease over a range of frequencies. Sensitive electrodes for the potentiometric detection of DNA have been demonstrated using an analytical protocol with ssDNA connected to enzyme-loaded CNTs immobilized on a GCE [64].

SWCNT-based field-effect transistors and chemiresistors

SWCNTs are prospective candidates for molecular scale electronic devices. FETs exhibiting high ON-state conductance have been realized from individual semiconducting SWCNTs [65]. In general, electron donors or electron acceptors can modulate the conductances of such tubes, making SWCNT-based FETs highly versatile components

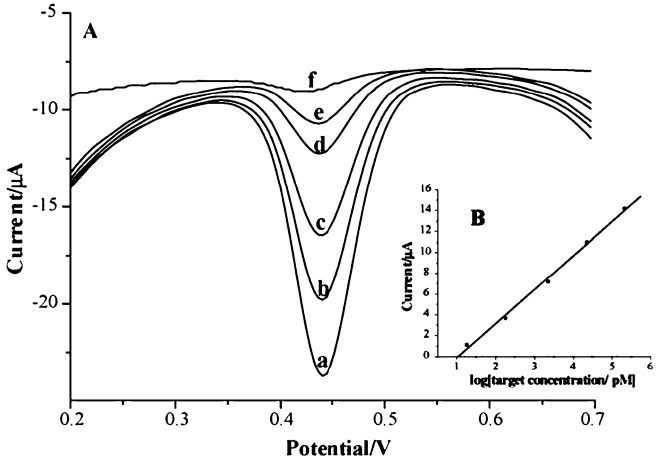

Fig. 12a–b **a** DPV signals at a Pt nanoparticle/CNT/ssDNA-modified GCE for different target DNA concentrations: (*a*) 2.25×10^5 pmol/L (*b*) 2.25×10^4 pmol/L (*c*) 2.25×10^3 pmol/L (*d*) 2.25×10^2 pmol/L (*e*) 2.25×10^1 pmol/L (*f*) 0 pmol/L. **b** The resulting logarithmic calibration plot. Reprinted with permission from [60]

of miniaturized sensors. Pronounced changes in the conductances of individual semiconducting SWCNTs have been observed upon exposure to water, NO_2, NH_3 and so on [47–49]. The sensitivity of such devices towards specific gases could be further improved by chemically modifying the tubes in a covalent or noncovalent manner. For instance, a humidity sensor has been fabricated using a CNTFET coated with a Nafion membrane loaded with cations (such as Na^+, K^+ or Ca^{2+}) through ion exchange [50]. Devices made with networks of SWCNTs are also well-suited to sensing. For instance, hydrogen sensors have been obtained by depositing palladium onto SWCNT networks [66].

Sensors based upon SWCNT-FETs can be operated in two different ways. One possibility is to monitor the conductance of an individual SWCNT or a network of SWCNTs during the introduction of the analyte solution. In this chemiresistor configuration, the resistance of the device is directly or inversely proportional to the concentration of the analyte. A second method is to measure the complete field-effect modulation of conductance after introduction of the test solution. This latter methodology is referred to as chemFET, where the threshold voltage shift provides information about the analyte concentration.

With the detection of glucose in mind, GOx has been immobilized onto a single semiconducting SWCNT acting as the channel of a liquid-gated FET [52]. The tube conductance decreased upon the adsorption of GOx, which was attributed to a change in surface potential of the tube. Since GOx carries groups with a pH-dependent charge, the GOx-modified SWCNTs displayed a different field-effect behavior at pH 4 and pH 5.5. Upon the introduction of 0.1 mmol/L glucose, with the tube immersed in water, a 10% increase in conductance was observed in the majority of the samples, although the detailed mechanism remained unexplained.

SWCNT-FETs are also promising devices for the specific recognition of proteins. Proteins bind nonspecifically on bare unmodified nanotubes [5, 54], which alters the electrical characteristics of the device. Biotin has been immobilized onto the tubes through an intermediate polymeric layer made of PEI/PEG, which greatly reduces nonspecific binding [5]. The controlled gate dependence characteristics of such a chemFET (Fig. 13) show that the docking of streptavidin onto biotin decreases the *p*-type current (where holes are the majority carriers). To explain this observation, structural deformations upon biotin–streptavidin binding were proposed that would create scattering sites for charge carriers in the tube. A similar sensor in a chemiresistor configuration was designed by Chen et al. [54], with the intermediate polymeric layer being composed of polyethylene oxide (PEO) chains instead of PEI/PEG. In this work, staphylococcal protein A (SpA) and the human autoantigen U1A were immobilized onto the PEO-modified nanotube network with the aim of detecting human immunoglobin (IgG) and various monoclonal antibodies, respectively. Here, only the conductance was monitored, which was found to decrease upon introduction of the analyte, although all of the

proteins investigated carried a different charge. It was proposed that the Schottky barrier at the interface between the metal electrode and the nanotubes is responsible for this unidirectional change [67]. However, in a different study including measurements of gate voltage dependence, it was demonstrated that the contacts have only a negligible effect on the resistance change upon adsorption of gases or chemicals [68]. Research into biosensors based on individual SWCNTs or networks of SWCNTs in a chemiresistor or chemFET configuration is thus still in its early stages, and much work remains to be done both from an application point of view as well as from a fundamental perspective.

Practical concerns

The superior electrocatalytic activity of CNT-based electrodes has sparked an explosive amount of research directed at using CNTs for electrochemical biosensing. In fact, a range of molecules can be easily oxidized at low potentials at CNT-based electrodes. Even if such electrodes are equipped with analyte-specific recognition units such as enzymes, they are still vulnerable to other electroactive compounds that can also be oxidized at these low potentials. Thus, for the assessment of a CNT-based biosensor, it is of utmost importance to carefully consider the interferents involved in the sample under consideration. Anti-interferent and permselective membranes such as Nafion and other charged polymers are therefore an essential component of CNT-based biosensing electrodes. An immediate consequence of the use of such membranes is that the sensor response is not controlled by the kinetics of the enzyme reaction anymore, but rather by mass transfer [2]. While this minimizes temperature effects, the price to be paid is reduced sensitivity and increased response time.

The performance of a biosensing electrode is strongly dependent on the type of raw nanotube material used and the deposition procedure. For example, chemical vapor deposition (CVD)-produced nanotubes combined with Nafion as the permselective membrane seem to offer the lowest oxidation potential towards NADH and H_2O_2. However, sensors prepared with CVD-grown CNTs dispersed in DMF exhibit the best sensitivity [69]. Figure 14 (i) presents amperometric responses obtained from a GCE modified with different kinds of tubes. CVD-grown tubes offer the best performance in this aspect [35]. The optimal composition of the biosensor is a trade-off between the various device parameters. A low amount of immobilized enzyme provides only a limited concentration range where the response is linear, whereas a large amount of enzyme could reduce the electrochemical activity of the CNTs (see Fig. 14ii). While direct immobilization of the enzyme without a matrix would be ideal for obtaining sensitive responses, such electrodes are prone to leaching of the enzyme. This loss and the subsequent reduction in sensitivity and reproducibility can be largely avoided by electropolymerized matrices.

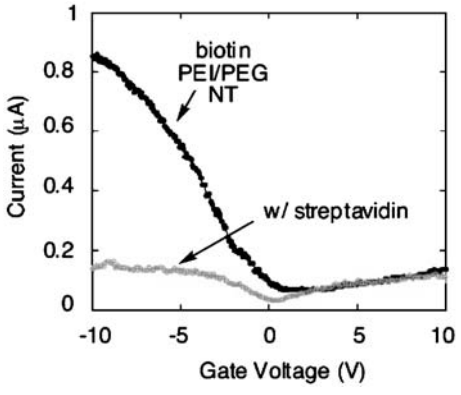

Fig. 13 Gate voltage characteristics of a biotinylated polymer-coated semiconducting SWCNT-FET with and without streptavidin. Reprinted in part with permission from [5]

Nanoscale sensors made from CNTs, in addition to having small size, exhibit higher signal-to-noise ratios in comparison to microelectrodes. However, the detection efficiency depends profoundly on the size and shape of the sensor electrode and the amount of analyte solution [70]. When working with very small amounts of solutions in the μL or nL range, the analyte transport is greatly restricted. Such mass transport limitations render the concentration of the analyte rather inhomogeneous. These problems can be overcome to a certain extent by the use of a microfluidic cell where the solution has a continuous flux, or by using a close-packed array of nanoelectrodes.

A wider application of SWCNT-FETs towards biosensing purposes is impeded by the still poor understanding of their sensing mechanism. The information provided by simple conductance measurements—without taking into account the effect of the gate voltage—is often quite ambiguous and susceptible to wrong conclusions. Due to the high sensitivity of the electronic properties of nanotubes to the environment, it is essential to perform complete gate dependence curves to obtain a clear idea of the sensing mechanism. While back-gate characteristics are complicated by the presence of hysteresis effects [71], the use of a liquid [72, 73] or polymer gate [74] has turned out to be more reliable for this purpose.

Another important aspect of designing biosensors is the range of analyte concentrations that is relevant for applications. For example, for a biosensor to be called a glucose sensor it must have the ability to sense in the clinically important range from a few μmol/L up to around 15 mmol/L.

Future perspectives

Beyond the applications outlined above, the field of CNT-based (bio)chemical sensors is currently experiencing a wealth of future developments. One promising research direction is the large-scale fabrication of nanoelectrode arrays made of CNTs [75]. For this purpose, CNTs are vertically grown at a low density using a plasma-enhanced

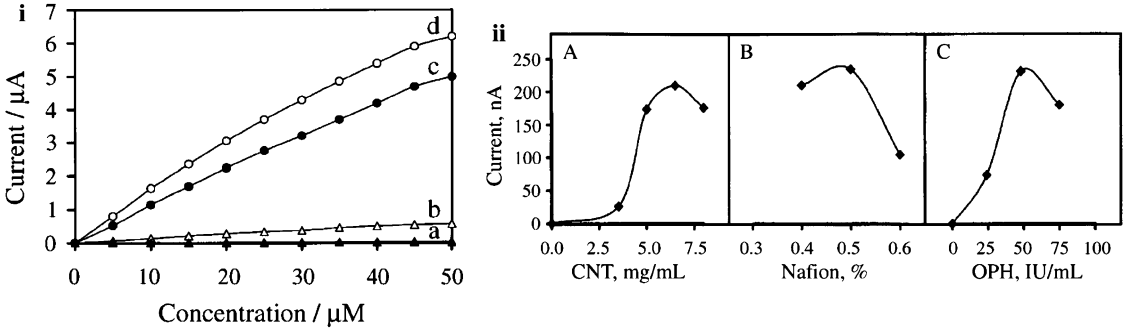

Fig. 14i–ii **i** Calibration plots derived from amperometric measurements of successive additions of 5 mmol/L *p*-nitrophenol at various electrodes: (*a*) bare glassy carbon (GCE); GCE modified by (*b*) arc discharge-produced MWCNTs, (*c*) chemical vapor deposition (CVD)-produced SWCNTs; (*d*) CVD-produced MWCNTs. Stirring rate: 300 rpm; operating potential: +0.85 V. **ii** Effect of the surface loading of CVD-grown MWCNT (*A*), Nafion (*B*) and OPH (*C*) upon the flow-injection response to 50 mmol/L paraoxon. Flow rate, 1 mL/min. Other conditions as in **i**. Reprinted with permission from [35]

chemical vapor deposition method, and then the interstitial spaces between the CNTs are filled with an electrically insulating epoxy resin, whereupon only the tips of the CNTs are exposed for the electrochemical detection. Such arrays can provide information about the ambient environment via specificity in the pattern of collected responses [76]. Significant progress has also been made in identifying the electrocatalytically active sites of MWCNTs. Specifically, the oxidation of NADH has been found to be preferred at the open tube ends and defects ("edge plane sites") along the tube axis [77]. Further to this, immuno-CNTs, which have recently been shown to be capable of recognizing pathogen cells via specific antibody–antigen interactions [78], may contribute to expanding the detection scope of CNT sensors.

Another intriguing development is the integration of biological cell membranes and CNT transistors, which opens the door to obtaining important information like the distribution of charges within the membrane [79]. Finally, recent advances in the implantation of CNT biosensors into living biological tissue [80], as well as the development of novel, fluorescence-based CNT nanosenors [81] are worth mentioning.

A very important issue related to the integration of CNTs into biological cells and tissues is the need to study their cytotoxicity towards biological species. Several experiments are underway to investigate the toxicity of CNTs when taken into cells and tissues. MWCNTs have been found to cause irritation in human keratinocytes [82]. SWCNTs have been reported to be toxic to mammalian cells beyond 10 µmol/L [83], while in another study they have been found to be nontoxic up to a concentration of 0.05 mg/mL [84]. On the other hand, most recent results [85] suggest that chemically modifying CNTs can reduce their cytotoxicity to a certain extent. However, in-depth systematic studies of the effect of CNTs on human cells and tissues as well as information related to safety issues are still lacking, and future work must concentrate on addressing these aspects.

Conclusion

Due to their small size and excellent electrochemical properties, CNTs continue to attract enormous interest as components in biosensors. Meanwhile, it is now well-established that CNT-based electrodes have electrochemical properties that are equal or superior to most other electrodes [86]. In particular, their high aspect ratios allow the tubes to be plugged into proteins, thus enabling direct electron transfer with enzymes like glucose oxidase whose redox centers would not normally be accessible [87]. Moreover, chemical modification of CNTs has proven to be an effective way to impart selectivity to the resulting biosensors, which has for instance been exploited for the highly sensitive detection of DNA [88].

In the future, efforts will need to be directed toward preventing nonspecific adsorption of biomolecules onto the tube walls, although promising advances have already been made in this direction [89]. Further improvements may be expected from extending the range of modifying molecules that can be attached to the tubes; enzymes, nucleic acids and metal nanocrystals have been mostly employed for this purpose so far. Particularly promising in this respect are electropolymerized coatings, which can be prepared with a broad range of compositions and with precisely controlled thicknesses [90, 91].

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