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NANOMATERIALS IN ELECTROCHEMICAL BIOSENSORS FOR FOOD ANALYSIS – A REVIEW

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The interaction of nanotechnology and biosciences opens the possibility for a wide variety of biological research topics and day-to-day applications at the molecular and cellular level. In particular, nanotechnology has been revolutionizing the area of biosensor. Nanobiosensor, an integration of physical sciences, molecular engineering, biology, chemistry and biotechnology holds the possibility of detecting and manipulating atoms and molecules using nanodevices, which have the potential for a wide range of both industrial and domestic applications. The role of electrochemical nanobiosensor in food analysis is an important and interesting area. This review covers the basic principles and types of electrochemical biosensor formats, role of nanomaterials for biosensor and reported food-specific applications of electrochemical nanobiosensors.

INTRODUCTION

As agriculture and food technology have advanced and populations increase, the current global population is nearly 6 billion with 50% living in Asia, analytical and regulatory problems concerning food have become very complex. Food production and preservation has become very important due to the need to store food for long periods, to prevent chemical and microbiological deterioration, insect infestation and pathogenic contamination. Food quality control is essential both for consumer protection and also for the food industry. In the food industry, the quality of a product is evaluated through periodic chemical and microbiological analysis. These procedures conventionally use techniques as chromatography, spectrophotometry, electrophoresis, titration and others. These methods do not allow an easy, rapid monitoring, because they are complex analytical steps with expensive instrumentation, need well trained operators and in some cases, increasing the time of analysis. Nowadays food analysis needs rapid and affordable methods to determine compounds that have not previously been monitored and to replace existing ones [Wagner & Guilbault, 1994]. An alternative to ease the analysis in routine of industrial products is the biosensors development. Biosensors are a sub group of chemical sensors that integrate biological sensing elements with physical transducers where the interactions between biological sensing elements and target molecules are directly converted into an electronic signal. Biosensors represent a conceptually novel approach to real-time, on-site, and simultaneous detection of multiple biohazardous agents. Samples are minimally processed and they offer rapid testing in the field setting with the option for post-analysis culture in the laboratory. Real-time detection of pathogenic contaminants is critical to the prevention and control of widespread damage from natural or intentional contamination. It provides immediate interactive information about the sample being tested, enabling decision makers to take corrective measures and to quickly recognize impending threats. At the moment, no technology is available that provides field-based real-time diagnosis of pathogenic contamination. These devices represent a promising tool for food analysis due to the possibility to fulfill some demand that the classic methods of analysis do not attain [Venugopal, 2002].

According to the statement by the great scientist Arthur C. Clarke "Any sufficiently advanced technology is indistinguishable from magic". This statement is particularly true in molecular biosensing based on nanotechnology where the detection limits are 'magically' becoming smaller and smaller, even reaching zeptomolar concentrations in addition to opening up possibilities for ultra-sensitive multiplexed detection. The whole area of biosensor development continues to be an extremely dynamic and growing area for scientific research [Pearson *et al*., 2000]. Nanotechnology has recently become one of the most exciting forefront fields in biosensors fabrication. Nanotechnology is defined as the creation of functional materials, devices and systems through control of matter at the 1–100 nm scale. A wide variety of nanoscale materials of different sizes, shapes and compositions are now available. The huge interest in nanomaterials is driven by their many desirable properties. Use of nanomaterials in biosensors allows the use of many new signal transduction technologies in their manufacture. Because of their size, nanosensors, nanoprobes and other nanosystems are revolutionizing the fields of chemical and biological analysis. In particular, the ability to tailor the size and structure and hence the properties of nanomaterials offers excellent prospects for designing novel sensing

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systems and enhancing the performance of the bioanalytical assay. Here we review the recent advances in nanomaterials for electrochemical biosensors in food analysis.

ROLE OF BIORECEPTORS IN BIOSENSORS

A biosensor is generally defined as a measurement system that consists of a probe with biological recognition element, often called a bioreceptor, and a transducer (Figure 1). The interaction of the analyte with the bioreceptor is designed to produce an effect measured by the transducer, which converts the information into a measurable effect, for example, an electrical signal. Bioreceptors are used because they are important elements to specificity for biosensor technologies. They allow binding the specific analyte of interest to the sensor for the measurement with minimum interference from other components in complex mixtures. A bioreceptor is a biological molecular species (*e.g.*, an antibody, an enzyme, a protein, or a nucleic acid) or a living biological system (*e.g.*, cells, tissue, or whole organisms) that utilizes a biochemical mechanism for recognition. An example of this step is the use of an enzyme acting specifically to convert a reactant molecule into a product. Some enzymes show a specific sensitivity to a particular molecule (or substrate). Many enzymatic reactions involve cofactors. These cofactors are other molecules or ions that assist in the reaction. During the catalysis, the cofactors may be chemically changed, and as a consequence, the resulting physicochemical effects can monitor or detect the enzymatic process. Another example is that existing in immune systems in which antigens interact with antibodies. The antigen is recognized as a foreign body. A specific antibody is generated to act against it by binding to it and operating to remove the antigen. By this specific recognition and interaction performed on the molecular level, antibodies and antigens can be exploited as a means for diagnostic testing. Antibodies can be raised *in vitro* to detect specific molecules. In this way, antibodies may serve as the basis for the biosensor detection system. Each type of cell has within it a unique signature in its DNA. All of the information contained in the DNA appears encoded in a series of amino acids and, as such, forms the identifying backbone of that structure. The recognition of these sequences is of fundamental importance to the control, reading, and detection of these molecular structures. The basic principle of a DNA biosensor is to detect the molecular recognition provided by the DNA probes and to transform it into the signal using a transducer. Aptamers are small (*i.e.* 40 to 100 bases), synthetic oligonucleotides that can specifically recognize and bind to virtually any kind of target, including ions, whole cells, drugs, toxins, low-molecular-weight ligands, peptides, and proteins. Aptamers can function as the biorecognition elements in biosensor applications.

ELECTROCHEMICAL BIOSENSORS AND THEIR CLASSIFICATION

Among the various types of biosensors, the electrochemical biosensors are the most common as a result of numerous advances leading to their well understood biointeraction and detection process. In electrochemical biosensors the variation on electron fluxes leads to the generation of an electrochemical signal, which is measured by the electrochemical detector (Figure 1). The versatility of electrochemical biosensors is illustrated in the numerous applications of these sensors, from monitoring micro-organism levels in polluted environments to detecting glucose. As outlined by Chaubey and Malhotra, several key characteristics that make the electrochemical biosensors useful include their successful operation in turbid environments, satisfactory and consistent instrumental sensitivity, and the potential for miniaturization [Chen *et al*., 2004]. The two most important subclasses of electrochemical sensors include the voltammetric and potentiometric biosensors.

Voltammetric sensors investigate the concentration effect of the detecting species on the current potential characteristics of the reduction or oxidation of a specific reaction [Bakker, 2004]. Amperometric sensors are a subclass of the voltammetric sensors. The principle of functioning for the amperometric sensors is based on the application of a fixed potential to the electrochemical cell, resulting in a current because of an oxidation or reduction reaction. The current is, then, used to quantify the species involved in the reaction [Wang, 2005; Bakker, 2004]. The versatility of amperometric biosensors is also apparent from their direct or indirect measurement capability. As Chaubey and Malhotra describes, direct amperometry makes use of the intimate relationship between the products of the redox reaction and the measured current, whereas indirect amperometry uses conventional detectors to measure the metabolic substrate or product of the analyte of interest [Patel, 2002]. Potentiometric biosensors examine the potential difference measurement between the working electrode and the reference electrode as it relates to the redox reaction of the species of interest. The potentiometric biosensors monitor the accumulation of charge at zero current created by selective binding at the electrode surface [Bakker, 2004]. A disadvantage of these sensors compared with the amperometric counterparts is the extended time period required for the potentiometric sensor to reach equilibrium required for data collection.

A special case and a significant application of potentiometry is the development of the ion-selective electrodes. The original concept was the well-known glass electrode that monitors the pH of an electrolyte solution. Ion-selective field effect transistors (ISFETs) are basically FETs that incorporate an ion-sensitive surface [Castellarnau *et al*., 2007]. The surface electrical potential is dependent on the ions interacting with the semiconductor surface. This potential change can be monitored. The ISFET is assembled by coating the sensor electrode with an appropriately designed polymer layer. When the polymer layer is selectively permeable to analyte ions, these ions diffuse through the polymer layer. The consequence of this process is a change in the FET surface potential. In a similar manner, an enzyme-sensitive FET (ENFET) can be assembled. Recently the potentiometric sensors for neutral molecules were developed. [Radecki *et al*., 2004, 2006, 2007; Radecka *et al.,* 2007].

ROLE OF NANOTECHNOLOGY IN IMMOBILIZATION OF BIORECEPTORS

The sampling component of a biosensor contains a biosensitive layer that can either contain bioreceptors or be made of bioreceptors covalently attached to the transducer. For biosensing purposes, a layer of receptor molecules that are capable of binding the analyte molecules in a selective way must be previously immobilized on the transducer surface. The immobilisation of the receptor molecule on the sensor surface is a key point for the final performance of the sensor. The immobilization procedure must be stable and reproducible, and must retain the stability and activity of the receptor. One of the most promising strategies is immobilization based on nanotechnology [Niemeyer, 2001; Wang, 2001]. It is essential to create a biosensing surface in which the sensing mechanism is immobilized. The biosensing surface may contain enzymes, antibodies, antigens, microorganisms, mammalian cells, tissues, or receptors. Nature of biosensing surface is very important, namely the prolonged use of the sensor and an anticipated extended storage and working stability.

APPLICATIONS OF NANOMATERIAL IN BIOSENSOR FOR FOOD ANALYSIS

Electrochemical nanobiosensor construction and applications have been investigated to determine their properties and possible applications in immobilization of biomolecules, electrode design and signal transduction. These structures include nanotubes, nanofibers, nanorods, nanoparticles and thin films.

NANOPARTICLES

Nanoparticles have numerous possible applications in biosensors. For example, functional nanoparticles bound to biological molecules (*e.g.* peptides, proteins, nucleic acids) have been developed for use in biosensors to detect and amplify various signals. The electrochemical behavior and applications of nanoparticles have received increasing attention. Metal nanoparticles are generally defined as isolable particles between 1 and 50 nm in size, that are prevented from agglomerating by protecting shells. Owing to their small size such nanoparticles have physical, electronic and chemical properties that are different from those of bulk metals. Such properties strongly depend on the number and kind of atoms that make up the particle. Several reviews have addressed the synthesis and properties of nanoparticles [Bonnemann & Richards, 2001; Niemeyer, 2001]. Metal nanoparticles based electroanalysis has been reviewed by Hernandez-Santos *et al*. [2002]. Metal nanoparticles can be used to enhance the amount of immobilized biomolecules in construction of a sensor. Because of its ultrahigh surface area, colloidal Au has been used to enhance the DNA immobilization on a gold electrode, to ultimately lower the detection limit of the fabricated electrochemical DNA biosensor [Cai *et al*., 2001]. Self--assembly of approximately 16-nm diameter colloidal Au onto a cysteamine modified gold electrode resulted in an easier attachment of an oligonucleotide with a mercaptohexyl group at the 5'-phosphate end and increased the limit for nucleic acid detection. Nanoparticle-based amplification schemes have led to improved sensitivity of bioelectronic assays by several orders of magnitude (Figure 2). The use gold nanoparticle tags was reported for electronic detection of DNA hybridization [Wang *et al.*, 2001; Authier *et al*., 2001]. This protocol relies on capturing the nanoparticles to the hybridized target, followed by highly sensitive anodic stripping electrochemical measurement of the metal tracer. Analogous bioelectronic measurements of proteins based on sandwich immunoassays and gold nanoparticle tracers have also been reported [Dequaire *et al*., 2000; Liu *et al*., 2005]. Inorganic nanocrystals offer an electrodiverse population of electrical tags as needed for designing electronic coding. Different inorganic-nanocrystal tracers utilized for a multi-target electronic detection of DNA or proteins. Three encoding nanoparticles (zinc sulfide, cadmium sulfide and lead sulfide) have thus been used to differentiate the signals of three protein targets in connection with a sandwich immunoassay and stripping voltammetry of the corresponding metals. Magnetic nanoparticles are also a powerful and versatile diagnostic tool in biosensor. They usually can be prepared in the form of either single domain or superparamagnetic (Fe_3O_4), greigite (Fe_3S_4), maghemite (g-Fe_2O_3), and various types of ferrites (MeO-Fe_2O_3, where Me = Ni, Co, Mg, Zn, Mn, *etc*.). Bound to biorecognitive molecules, magnetic nanoparticles can be used to separate or enrich the analyte to be detected. The chemical modification of surfaces with functional monolayers or thin films attracts extensive recent research effort directed to the miniaturization of devices to nanoscale dimensions. Functionalization of electrodes with ordered arrays of redox active components has yielded assemblies revealing sensoric activities. In this way, the modification of electrode surfaces with redox active metal-nanoparticles has led to many electroanalytical sensors. They can be divided in two main groups: nonenzymatic sensors (metal nanoparticles or functionalized nanoparticles act as sensing phase) and enzymatic sensors (enzyme-modified metal nanoparticles act as sensing phase, where nanoparticles work as mediators). In addition, electrochemical devices are uniquely qualified for meeting the size, cost, low volume, and power requirements of decentralized testing and indicate great promise for a wide range of biomedical or environmental applications [Wang, 2002a, b]. Few interesting biosensor applications in food analysis are given in Table 1.

NANOTUBES AND NANOWIRES

One-dimensional (1-D) nanostructures, such as carbon nanotubes (CNT) and semiconductor- or conductingpolymer nanowires, are particularly attractive for bioelectronic detection. Because of the high surface-to-volume ratio and novel electron transport properties of these nanostructures, their electronic conductance is strongly influenced by minor surface perturbations (such as those associated with the binding of macromolecules). Such 1-D materials thus offer the prospect of rapid (realtime) and sensitive label-free bioelectronic detection, and massive redundancy in nanosensor arrays. These nanomaterials would allow packing a huge number of sensing elements onto a small footprint of an array device. Metal and

TABLE 1. Applications of elelctrochemical biosensor for common analytes in foods.

Analyte	Area of application	Reference
Organics: Amino acids, cholesterol, carbohydrates, pesticides, antibiotics, alcohols, vitamins, carboxylic acids, phenols, lipids, lecithin	Common constituents or contaminants in food products	[Lin *et al*., 2004; Deo & Wang, 2004; Malea, *et al*., 2004; Qua *et al*., 2007; Qiaocui *et al*., 2005; Schulze *et al*., 2002; Mello & Kubota, 2002; Patel, 2002; Peris, 2002; Radecki *et al*., 2004, 2006, 2007; Stobiecka *et al*., 2007; Radecka *et al.,* 2007]
L-alanine (with Balanine)	Flavor enhancer	
Citrate	Found in several fruits and in all animal and vegetable cells	
Catechins, catechols and tannin	Taste and function of green tea. Quality control in tea processing (substances of astringency)	
Polyphenols	Olive oils (taste and stability of the oil)	
Acetaldehyde	Wine, beer, yoghurts	
Malolactic acid	Wine quality	
Trimethylamine, putrescine, cadaverine and histamine	Meat spoilage and aging, histamine in red wine	
Nucleotides: hypoxanthine, inosine, inosine-5--monophosphate	Fish freshness, meat aging	
Salicylate	Antimicrobial agent, preservative (now forbidden in most countries)	
Benzoic acid, sorbic acid, tocopherol	Preservatives	
Glutamate	Flavour enhancer	
Lactate	Yoghurt, beer, fruit juices, wine	
Amygdalin	Cyanide-containing sugar in almonds	
Artificial sweeteners (aspartame, saccharin, cyclamate, acesulfame, *etc*.)	Soft drinks, desserts.	
Glucosinolates	Nitrile-and sulphur-containing heteroglycosides	
Drug and hormone residues Tetracyclines, Sulfonamides, Quinolones, b-lactams, Macrolides, Nitroimidazoles, Amphenicols, Miscellaneous	Poultry muscle, cattle muscle, fish, prawn, honey, milk	
Inorganics: Sulphites, sulphur dioxide	Used as food preservatives, oxidation prevention	
Potassium, sodium, calcium, magnesium, nitrate, nitrite, chloride, sulphate, fluoride, carbonate, and heavy metals	Vinegar, fruit juices, milk, soft drinks, mineral water	
Toxins: Saxitoxin, neosaxitoxin, gonyautoxins, domoic acid, brevetoxin, Protein A, hepatitis A virus, aflatoxin,Ciguatoxin, Ochratoxin A, Fumonisins (B1, B2), Patulin, Tetrodotoxin, choleratoxin	Protein A is a product of *Staphylococcus aureus*. Marine shellfish poisoning	[Min& Baeumner, 2004; Viswanathan *et al*., 2006; Venugopal, 2002; Zaytseva *et al*., 2005; Baeumner *et al*., 2003]
Pathogens: Salmonella, Escherichia coli, Listeria, Campylobacter,Staphylococcus, Yersinia, bacteriophages	In a variety of foodstuffs	
Food odorants: volatile metabolites, citrinin and ergosterol.	Bioelectronic nose, tongue for food quality	[Vidic *et al*., 2006; Falasconi *et al.*, 2005]

conducting polymer nanowires can be readily prepared by a template-directed electrochemical route [Siwy *et al*., 2005]. Carbon nanotubes (CNT) are particularly exciting 1-D nanomaterials that have generated a considerable interest owing to their unique structure-dependent electronic and mechanical properties [Baughma *et al*., 2001]. CNT can be divided into single-wall carbon nanotubes (SWCNT) and multi-wall carbon-nanotubes (MWCNT). SWCNT possess a cylindrical nanostructure (with a high aspect ratio), formed by rolling up a single graphite sheet into a tube. SWCNT can thus be viewed as molecular wires with every atom on the surface. MWCNT consist of an array of such nanotubes that are concentrically nested like rings of a tree trunk. The remarkable properties of CNT suggest the possibility of developing superior electrochemical sensing devices, ranging from amperometric enzyme electrodes to label-free DNA hybridization biosensors. The development of electrical DNA hybridization biosensors has attracted considerable research efforts [Palecek & Fojta, 2000; Gooding, 2002]. Such DNA sensing applications require high sensitivity through amplified transduction of the oligonucleotide interaction. An extremely important challenge in amperometric enzyme electrodes is the establishment of satisfactory electrical communication between the active site of the enzyme and the electrode surface. The redox center of most oxidoreductases is electrically insulated by a protein shell. Because of this shell, the enzyme cannot be oxidized or reduced at an electrode at any potential. The possibility of direct electron-transfer between enzymes and electrode sur-

faces could pave the way for superior reagentless biosensing devices, as it obviates the need for co-substrates or mediators and allows efficient transduction of the biorecognition event. "Trees" of aligned CNT in the nanoforest, prepared by self assembly, can act as molecular wires to allow electrical communication between the underlying electrode and redox proteins (covalently attached to the ends of the SWNT, Figure 3) [Gooding *et al*., 2003; Yu *et al*., 2003]. Willner's group demonstrated that aligned reconstituted glucose oxidase (GOx) on the edge of SWCNT can be linked to an electrode surface [Patolsky *et al*., 2004]. Such enzyme reconstitution on the end of CNT represents an extremely efficient approach for 'plugging' an electrode into GOx. Arrays of nanoscopic gold tubes or wires have been prepared by electroless deposition of the metal within the pores of polycarbonate particle track-etched membranes [Marc *et al*., 2003]. Glucose oxidase was immobilized onto the preformed self-assembled monolayers (SAMs) (mercaptoethylamine or mercaptopropionic acid) of gold tubes, *via* cross-linking with glutaraldehyde or covalent attachment by carbodiimide coupling. Glucose responses as large as 400 nA/mM cm^2 were obtained. Based on a slimier method of template synthesis, Miao *et al*. [1999] immobilized glucose oxidase in the polypyrrole nanotubes and produced a biosensor. Compared to conventional biosensor, immobilization on nanomaterials enhanced the amount of the enzyme loading, the retention of the immobilized activity and the sensitivity of the biosensor [Chen *et al*., 2004].

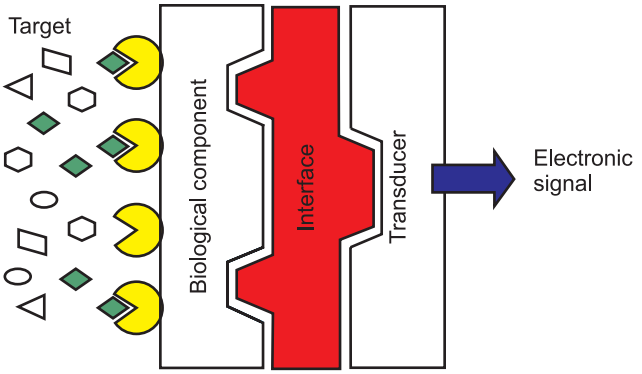

FIGURE 1. General schematic representation of biosensors.

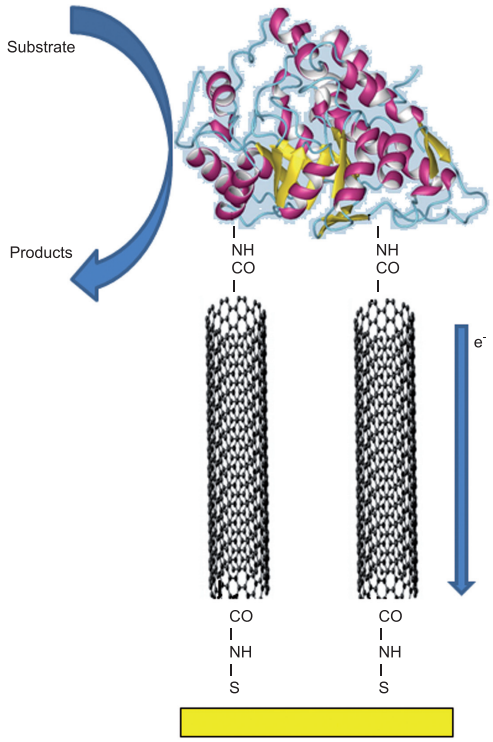

FIGURE 3. Carbon nanotube based enzyme biosensor.

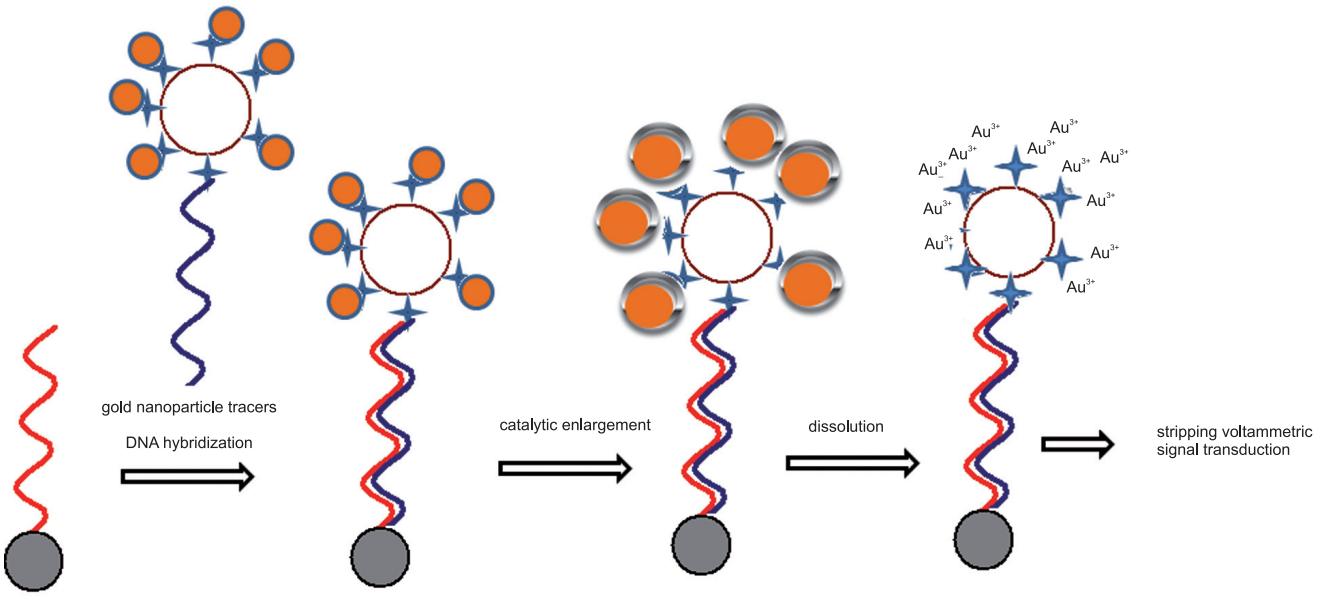

FIGURE 2. Amplified bioelectronic detection of DNA hybridization, using polymeric beads carrying multiple gold nanoparticle tracers, catalytic enlargement of the gold particles and a stripping voltammetric signal transduction.

SELF-ASSEMBLED NANOSTRUCTURE

The nanostructures explained thus far have been developed following the top-down approach, *i.e.* starting with large-scale objects and gradually reducing its dimensions. Self-assembling tries to develop the nano and microstructures following the bottom-up procedure, *i.e.* from simple molecules to more complicated systems [Riu *et al*., 2006]. Of the self-assembled structures, those using liposomes, polymerised lipid vesicles or pseudo-cellular membranes, are the most widely studied [Baeumner *et al*., 2003]. The use of self-assembled monolayers (SAMs) in various fields of research is rapidly growing. In particular, biosensors apply SAMs as an interface-layer between a metal surface and a solution or vapor. The most common compounds that are able to undergo the process of SAMs are alkanothiols, dialkyl disulfides or dialkyl sulfides on gold. The use of SAMs in the construction of biosensor is widespread to a large variety of biomolecules such as antibody, enzymes, DNA *etc.* A comprehensive review dealing with the application of alkanethiol selfassembled monolayers to enzyme electrodes has been published by Gooding & Hibbert [1999]. Molecular self-assembly mimics natural systems and is a key link between physics, chemistry and biology.

Molecular self-assembly can be used to create novel structures, materials, and devices for use in biosensors. The supported bilayer lipid membrane (BLM) provides a natural environment for embedding proteins, receptors, membrane/tissue fragments, and entire cells under nondenaturing conditions and in a well-defined orientation. This makes BLMs specially attractive for use in biosensors. A successful biomimetically engineered device based on BLMs was the ion channel switch biosensor reported by Cornell *et al*. [1997]. The basis of this 1.5 nm nanomachine was a self-assembled artificial membrane packed with gramicidin. Ion channels were formed in the membrane by two gramicidin molecules: one in the lower layer of the membrane attached to a gold electrode and one in the upper layer tethered to biological receptors such as antibodies or nucleotides. The detection mechanism operated by binding the target molecule to the receptor and thereby altering the population of conduction ion channel pairs within the tethered membrane. This resulted in a change in the membrane conduction. The device was capable of detecting picomolar concentrations of proteins [Wright & Harding, 2000; Cornell *et al*., 2001]. Fullerene C_{60} saturated BLMs applied for the design of an electrochemical sensor for detection of neutral odorant molecules was reported by Szymanska *et al*. [2001].

LIPOSOMES

Liposomes are microscopic, fluid-filled, pouches with endless walls that are made of layers of phospholipids identical to the phospholipids that make up cell membranes. Liposomes are typically used as the supporting substrate for immobilizing the biorecognition molecules. Liposomes are also used to amplify the electrochemical signals (Figure 4) [Baeumner *et al*., 2003; Subramanian *et al*., 2005]. Liposome-based nanobiosensors for very low level detection organophosphorus pesticides dichlorvos and paraoxon were reported by Vamvakaki & Chaniotakis [2007].

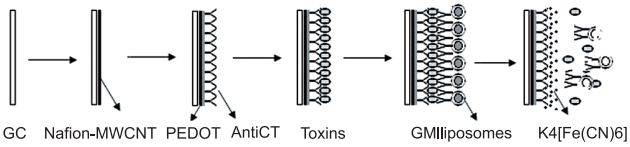

FIGURE 4. Scheme of electrochemical immunosensor for detection of cholera toxin in drinking water samples using potassium ferrocyanide encapsulated and ganglioside receptor functionalized liposomes and voltammetry.
GC-glassy carbon electrode; MWCNT-Multiwall carbon nanotube; Toxins- Cholera toxin; PEDOT; Polyethylenedioxythiophene, Anti CT- Cholera toxin antibody, GM1-ganglioside.

ELECTRONIC NOSE AND TONGUE

Electronic nose is a specific kind of sensor arrays. It is odor mapper that can discriminate several volatile compounds, according to the electronic response (*e.g*., voltage, resistance, conductivity) arising from the different gas sensors, usually metal-oxide chemosensors. Since some fungal species can produce volatile metabolites, this technology has been used in the mycotoxin field. Mycotoxins are secondary metabolites that moulds produce naturally. Due to their ubiquitous presence in foodstuffs and their potential risk for human health, prompt detection is important. Many researches have reported efficient nanobiosensors for mycotoxins analysis. After exposure of the volatile compounds to the sensor array, a signal pattern is collected and results are evaluated with multivariate analysis or processed by an artificial neural network. Electronic nose has been applied to classify cereal grains, to discriminate, mouldy, weakly musty and strongly musty oat samples [Jonsson *et al*., 1997], to predict ergosterol levels and fungal colony-forming units (CFU) in wheat [Jonsson *et al.,* 1997; Olsson *et al*., 2002], to predict deoxynivalenol and ochratoxin A levels in barley grains (the latter as below or above 5 lg/kg) [Olsson *et al*., 2002], to indicate ochratoxin A, citrinin and ergosterol production in wheat [Olsson *et al*., 2002], and to indicate mycotoxin formation by Fusarium strains [Falasconi *et al.*, 2005; Presicce *et al.,* 2006]. An electronic tongue comprising thirty potentiometric chemical sensors and pattern recognition tools for data processing was used for the analysis of mineral waters, coffee, soft drinks and flesh food, namely fish. The electronic tongue appeared to be capable of distinguishing between different sorts of beverages: natural and artificial mineral waters, individual and commercial brands of coffee, and commercial and experimental samples of soft drinks containing different sweeteners [Rudnitskaya *et al*., 2002]. Biosensor arrays can save time by detecting multiple target analytes simultaneously [Min & Baeumner, 2004].

SUMMARY

In this review, we have discussed about the existing nanomaterials based electrochemical biosensors and their application in the field of food analysis, highlighting the relationship between the property monitored and the type of nanomaterials used. Although fundamental developments in the nanoscience field are still appearing, the well known effects arising only when the size of the structures is reduced are being ap-

plied to develop new sensing devices. Among all the reviewed types of nanostructures, nanoparticles and carbon nanotubes probably stand out. Most of the reviewed nanostructures have successfully shown a great potential for being used in nanobiosensors, but the versatility and high applicability of nanoparticles and carbon nanotubes makes them clear candidates to be further used in nanosensors for food analysis.

REFERENCES

1. Abramson D., Hulasare R., York R.K., White N.D.G., Jayas D.S., Mycotoxins, ergosterol and odor volatiles in durum wheat during granary storage at 16% and 20% moisture content. J Stor. Prod. Res., 2005, 41, 67–76.
2. Authier L., Grossiord C., Brossier P., Limoges B., Gold nanoparticle-based quantitative electrochemical detection of amplified human cytomegalovirus DNA using disposable microband electrodes. Anal. Chem., 2001, 73, 4450–4456.
3. Baeumner A.J., Cohen R.N., Miksic V., Min J., RNA biosensor for the rapid detection of viable *Escherichia coli* in drinking water. Biosens. Bioelectron., 2003, 18, 405–413.
4. Bakker E., Electrochemical sensors. Anal. Chem., 2004, 76, 3285–3298.
5. Baughma R.H., Satishkumar C., Govindaraj A., Nath M., Nanotubes. Chem. Phys. Chem., 2001, 2, 79–105.
6. Bonnemann H., Richards F.J.M., Nanoscopic metal particles - synthetic methods and potential applications. Eur. J. Inorg. Chem., 2001, 2001, 2455–2480.
7. Cai H., Xu C., He P., Fang Y., Colloid Au-enhanced DNA immobilization for the electrochemical detection of sequence-specific DNA. J. Electroanal. Chem., 2001, 510, 78–85.
8. Castellarnau M., Zine N., Bausells J., Madrid C., Ju´arez A., Samitier J., Errachid A., Integrated cell positioning and cell-based ISFET biosensors. Sens. Actuators, B, 2007, 120, 615–620.
9. Chen J., Miao Y., He N., Wu X., Li., Nanotechnology and biosensor. Biotechnol. Adv., 2004, 22, 505–518.
10. Cornell B.A., Braach-Maksvytis V.L.B., King L.G., Osman P.D.J., Raguse B., Wieczorek L., A biosensor that uses ion-channel switches. Nature, 1997, 387, 580–583.
11. Cornell BA, Krishna G, Osman PD, Pace RD, Wieczorek L., Tethered bilayer lipid membranes as a support for membrane--active peptides. Biochem. Soc. Trans., 2001, 29, 613–617.
12. Presicce D.S., Fo´rrelo A., Taurino A.M., Zuppa M., Siciliano P., Laddomada B., Logrieco A., Visconti A., Response evaluation of an e-nose towards contaminated wheat by fusarium poae fungi. Sens. Actuators B, 2006, 118, 433–438.
13. Deo R.P., Wang J., Electrochemical detection of carbohydrates at carbon-nanotube modified glassy-carbon electrodes. Electrochem. Com., 2004, 6, 284–287.
14. Dequaire M., Degrand C., Limoges B., An electrochemical metallo immunoassay based on a colloidal gold label. Anal. Chem., 2000, 72, 5521–5528.
15. Falasconi M, Gobbi E., Pardo M., Torre M.D., Bresciani A., Sberveglieri G., Detection of toxigenic strains of fusarium verticillioides in corn by electronic olfactory system. Sens. Actuators B, 2005, 108, 250.
16. Wagner G., Guilbault G.G. (eds.), Food Biosensor Analysis. 1994, Marcel Dekker, New York, pp. viii + 257.
17. Gooding J.J., Electrochemical DNA hybridization biosensors. Electroanalysis, 2002, 14, 1149–1156.
18. Gooding J.J., Hibbert D.B., The application of alkanethiol self-assembled monolayers to enzyme electrodes. Trends Anal. Chem., 1999, 18, 525–533.
19. Gooding J.J., Wibowo R., Liu J.Q., 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.
20. Hernández-Santos D., González-García M.B., García A.C., Review: Metal-nanoparticles based electroanalysis. Electroanalysis, 2002, 14, 1225–1235.
21. Jonsson A., Winquist F., Schnürer J., Sundgren H., Lundström I., Electronic nose for microbial quality classification of grains. Int. J. Food Microbiol., 1997, 35, 187–193.
22. Kawde A-N, Wang J., Amplified electrical transduction of DNA hybridization based on polymeric beads loaded with multiple gold nanoparticle tags. Electroanalysis, 2004, 16, 101–107
23. Krajewska A, Lhotak, P. Radecka H., Potentiometric responses of ion-selective electrodes doped with diureidocalix[4]arene towards un-dissociated benzoic acid. Sensors, 2007, 7, 1655–1666.
24. Kurzątkowska K., Radecka H., Dehaen W., Wąsowicz M., Grzybowska I., Radecki J., Polymeric liquid membrane electrodes incorporated with undecylcalix[4]resorcinarene for screening of neutral forms of diaminobenzene isomers. Com. Chem. High T. Scr., 2007, 10, 604–610
25. Lin Y., Lu F., Tu Y., Ren Z., Glucose biosensors based on carbon nanotube nanoelectrode ensembles. Nano Lett., 2004, 4, 191–195.
26. Liu G., Lin Y., Ostatna V., Wang J., Enzyme nanoparticles-based electronic biosensor. Chem. Commun., 2005, 2727, 3481–3483.
27. Malea K.B., Hrapovica S., Liua Y., Wang D., Luong J.H.T., Electrochemical detection of carbohydrates using copper nanoparticles and carbon nanotubes. Anal. Chim. Acta, 2004, 516, 35–41.
28. Marc D, Sophie D.-C., Immobilisation of glucose oxidase within metallic nanotubes arrays for application to enzyme biosensors. Biosens. Bioelectron., 2003,18, 943–951
29. Mello L.D., Kubota L.T., Review of the use of biosensors as analytical tools in the food and drink industries. Food Chem., 2002, 77, 237–256.
30. Miao Y., Qi M., Zhan S., He N., Wang J., Yuan C., Construction of a glucose biosensor immobilized with glucose oxidase in the film of polypyrrole nanotubules. Anal Lett., 1999, 32, 1287–1299.
31. Min J.-H, Baeumner A.J., Characterization and optimization of interdigitated ultramicroelectrode arrays as electrochemical biosensor transducers. Electroanalysis, 2004, 16, 724–729.
32. Mir M., Vreeke M., Katakis I., Different strategies to develop an electrochemical thrombin aptasensor. Electrochem. Com., 2006, 8, 505–511.
33. Niemeyer C.M., Nanoparticles, Pproteins, and nucleic acids: Biotechnology meets materials science. Angew. Chem. Int. Ed., 2001, 40, 4129–4158.
34. Olsson J., Borjesson T., Lundstedt T., Schnurer J., Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Int. J. Food Microbiol., 2002, 72, 203–214.
35. Palecek E., Fojta M., Detecting DNA hybridization and damage. Anal. Chem., 2001, 73, 74A–83A.
36. Patel P.D., (Bio)sensors for measurement of analytes implicated in food safety: a review. Trends Anal. Chem., 2002, 21, 96–115.

37. Patolsky F., Weizmann Y., Willner I., Long-range electrical contacting of redox enzymes by SWCNT connectors. Angew. Chem. Int. Ed., 2004, 43, 2113–2117.
38. Pearson J.E., Gill A., Vadgama P., Analytical aspects of biosensors. Ann. Clin. Biochem., 2000, 37, 119–145.
39. Peris M., Present and future of expert systems in food analysis. Anal. Chim. Acta, 2002, 454, 1–11.
40. Qiaocui S., Tuzhi P., Yunu Z., Yang C.F., An electrochemical biosensor with cholesterol oxidase/ sol-gel film on a nanoplatinum/ carbon nanotube electrode. Electroanalysis, 2005, 17, 857–861.
41. Qua S., Wang J., Kong J., Yang P., Chen G., Magnetic loading of carbon nanotube/nano-Fe_3O_4 composite for electrochemical sensing. Talanta, 2007, 71, 1096–1102 .
42. Radecka H., Grzybowska I., Radecki J., Jakubowski P., Loteran S., Orlewska C., Maes W., Dehaen W., Salicylate determination in human plasma by ISEs incorporating Mn(III)-porphyrine and Zn(II)-dipyrromethene. Anal. Lett., 27, 4, 387–401.
43. Radecki J., Radecka H., Piotrowski T., Depraetere S., Dehaen W., Plavec. J., Interface host-guest interaction between caix[4] pyrrole and neutral derivatives of phenol as the base for their potentiometric discrimination. Electroanalysis, 2004, 16, 2073–2081.
44. Radecki J., Stenka I., Dolusic E., Dehaen W., Corroles as receptors in liquid membrane electrodes and their potentiometric response towards salicylic acid. Electrochim. Acta, 2006, 51, 2282–2288.
45. Radecki J., Dehaen W., Nitrogen-containing macrocycles as host molecules for the recognition of undissociated phenol derivatives: mechanism of potentiometric signal generation. Comb. Chem. High T. Scr., 2006, 9, 399–406.
46. Riu J., Maroto A., Rius F.X., Nanosensors in environmental analysis. Talanta, 2006, 69, 288–301
47. Rudnitskaya A.L.A., Boris S., Yuri1 V., Recognition of liquid and flesh food using an electronic tongue. Int. J. Food Sci. Tech., 2002, 37, 375–385.
48. Schulze H., Scherbaum E., Anastassiades M., Vorlov S., Schmid R.D., Bachmann T.T., Development, validation, and application of an acetylcholinesterasebiosensor test for the direct detection of insecticide residues in infant food. Biosens. Bioelectron., 2002, 17, 1095–1105.
49. Siwy Z., Troffin L., Kohli P., Baker L.A., Trautmann C., Martin C.R, Protein biosensors based on biofunctionalized conical gold nanotubes. J. Am. Chem. Soc, 2005, 127, 5000–5001.
50. Stobiecka A., Radecka H., Radeck J., Novel voltammetric biosensor for determining acrylamide in food samples. Biosens. Bioelectron, 2007, 22, 2165–2170
51. Szymanska I., Radecka H., Radecki J., Kikut-Ligaj D., Fullerene modified supported lipid membrane as sensitive element of sensor for odorants. Biosens. Bioelectron., 2001, 16, 911–915.
52. Vamvakaki V., Chaniotakis N.A., Pesticide detection with a liposome-based nanobiosensor. Biosens. Bioelectron. 2007, 22, 2848–2853.
53. Venugopal V., Biosensors in fish production and quality control. Biosens. Bioelectron., 2002, 17, 147–157.
54. Vidic J.M., Grosclaude J., Persuy M.A., Aioun J., Salesse R., Pajot-Augy E., Quantitative assessment of olfactory receptors activity in immobilized nanosomes: a novel concept for bioelectronic nose. Lab Chip, 2006, 6, 1026–1032.
55. Viswanathan S., Wu L.-C., Huang M.-R., Ho J.-A.A., Electrochemical immunosensor for cholera toxin using liposomes and poly(3,4-ethylenedioxythiophene)-coated carbon nanotubes. Anal. Chem., 2006, 78,1115–1121.
56. Wang J., Nanomaterial-based electrochemical Biosensors. Analyst, 2005, 130, 421–426.
57. Wang J., Portable electrochemical systems. Trends Anal. Chem., 2002, 21, 226.
58. Wang J., Real-time electrochemical monitoring: toward green analytical chemistry. Acc. Chem. Res., 2002, 35, 811–816.
59. Wang J., Xu D., Kawde A. N., Polsky R., Metal nanoparticle--based electrochemical stripping potentiometric detection of DNA hybridization. Anal. Chem., 2001, 73, 5576–5581.
60. 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. Commun., 2003, 5, 408–411.
61. Zaytsev N.V., Goral V.N., Montagn R.A., Baeumner A.J., Development of a microfluidic biosensor module for pathogen detection. Lab Chip, 2005, 5, 805–811.

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