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Nanobiosensor for Health Care

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1. Introduction

A chemical sensor: is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal (IUPAC).

Biosensors: are analytical tools for the analysis of bio-material samples to gain an understanding of their bio-composition, structure and function by converting a biological response into an electrical signal (Figure 1). The analytical devices composed of a biological recognition element directly interfaced to a signal transducer which together relate the concentration of an analyte (or group of related analytes) to a measurable response. The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly.

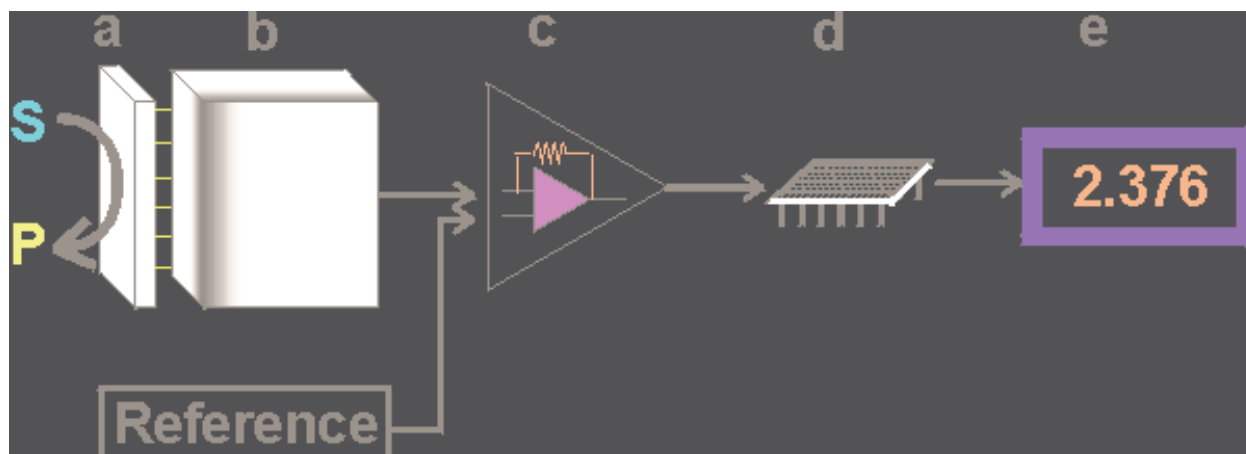


Fig. 1. Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e). (<http://www.lsbu.ac.uk/biology/enztech/biosensors.html>)

The key part of a biosensor is the transducer (shown as the 'black box' in Figure 1) which makes use of a physical change accompanying the reaction. This may be:

1. the heat output (or absorbed) by the reaction (calorimetric biosensors),
2. changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),

3. movement of electrons produced in a redox reaction (amperometric biosensors),
4. light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or
5. effects due to the mass of the reactants or products (piezo-electric biosensors).

There are three so-called 'generations' of biosensors; First generation biosensors where the normal product of the reaction diffuses to the transducer and causes the electrical response, second generation biosensors which involve specific 'mediators' between the reaction and the transducer in order to generate improved response, and third generation biosensors where the reaction itself causes the response and no product or mediator diffusion is directly involved.

The electrical signal from the transducer is often low and superimposed upon a relatively high and noisy (i.e. containing a high frequency signal component of an apparently random nature, due to electrical interference or generated within the electronic components of the transducer) baseline. The signal processing normally involves subtracting a 'reference' baseline signal, derived from a similar transducer without any biocatalytic membrane, from the sample signal, amplifying the resultant signal difference and electronically filtering (smoothing) out the unwanted signal noise. The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration. The analogue signal produced at this stage may be output directly but is usually converted to a digital signal and passed to a microprocessor stage where the data is processed, converted to concentration units and output to a display device or data store.

Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry (e.g. 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. The estimated world analytical market is about 12,000,000,000 year⁻¹ of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors.

2. The history of biosensor development

1916	First report on the immobilisation of proteins: adsorption of invertase on activated charcoal
1922	First glass pH electrode
1956	Invention of the oxygen electrode (Clark)
1962	First description of a biosensor: an amperometric enzyme electrode for glucose (Clark)
1969	First potentiometric biosensor: urease immobilised on an ammonia electrode to detect urea
1970	Invention of the Ion-Selective Field-Effect Transistor (ISFET) (Bergveld)
1972/5	First commercial biosensor: Yellow Springs Instruments glucose biosensor
1975	First microbe-based biosensor First immunosensor: ovalbumin on a platinum wire Invention of the pO ₂ / pCO ₂ optode

1976	First bedside artificial pancreas (Miles)
1980	First fibre optic pH sensor for <i>in vivo</i> blood gases (Peterson)
1982	First fibre optic-based biosensor for glucose
1983	First surface plasmon resonance (SPR) immunosensor
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for the detection of glucose
1987	Launch of the MediSense ExacTech™ blood glucose biosensor
1990	Launch of the Pharmacia BIACore SPR-based biosensor System
1992	i-STAT launches hand-held blood analyser
1996	Glucocard launched
1996	Abbott acquires MediSense for \$867 million
1998	Launch of LifeScan FastTake blood glucose biosensor
1998	Merger of Roche and Boehringer Mannheim to form Roche Diagnostics
2001	LifeScan purchases Inverness Medical's glucose testing business for \$1.3billion
1999-Current	BioNMES, Quantum dots, Nanoparticles, Nanocantilever, Nanowire and Nanotube

Table 1. Defining events in the history of biosensor development

3. Basic characteristics of a biosensor

A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks (see later), show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme.
3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
 - a. **Linearity:** Maximum linear value of the sensor calibration curve. Linearity of the sensor must be high for the detection of high substrate concentration.
 - b. **Sensitivity:** The value of the electrode response per substrate concentration.
 - c. **Selectivity:** Interference of chemicals must be minimised for obtaining the correct result.
 - d. **Response time:** The necessary time for having 95% of the response.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no

biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case, the biosensor should not be prone to fouling or proteolysis.

5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
6. There should be a market for the biosensor. There is clearly little purpose developing a biosensor if other factors (e.g. government subsidies, the continued employment of skilled analysts, or poor customer perception) encourage the use of traditional methods and discourage the decentralisation of laboratory testing.

4. Types of biosensors

1. **Resonant Biosensors:** in this type of biosensor, an acoustic wave transducer is coupled with an antibody (bio-element). When the analyte molecule (or antigen) gets attached to the membrane, the mass of the membrane changes. The resulting change in the mass subsequently changes the resonant frequency of the transducer. This frequency change is then measured.
2. **Optical-detection Biosensors:** the output transduced signal that is measured is light for this type of biosensor. The biosensor can be made based on optical diffraction or electrochemiluminescence. In optical diffraction based devices, a silicon wafer is coated with a protein via covalent bonds. The wafer is exposed to UV light through a photo-mask and the antibodies become inactive in the exposed regions. When the diced wafer chips are incubated in an analyte, antigen-antibody bindings are formed in the active regions, thus creating a diffraction grating. This grating produces a diffraction signal when illuminated with a light source such as laser. The resulting signal can be measured or can be further amplified before measuring for improved sensitivity.
3. **Thermal-detection Biosensors:** this type of biosensor is exploiting one of the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. They are constructed by combining immobilized enzyme molecules with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration. The total heat produced or absorbed is proportional to the molar enthalpy and the total number of molecules in the reaction. The measurement of the temperature is typically accomplished via a thermistor, and such devices are known as enzyme thermistors. Their high sensitivity to thermal changes makes thermistors ideal for such applications. Unlike other transducers, thermal biosensors do not need frequent recalibration and are insensitive to the optical and electrochemical properties of the sample. Common applications of this type of biosensor include the detection of pesticides and pathogenic bacteria.
4. **Ion-Sensitive Biosensors:** these are semiconductor FETs having an ion-sensitive surface. The surface electrical potential changes when the ions and the semiconductor interact. This change in the potential can be subsequently measured. The Ion Sensitive Field Effect Transistor (ISFET) can be constructed by covering the sensor electrode with a polymer layer. This polymer layer is selectively permeable to 4 analyte ions. The ions diffuse through the polymer layer and in turn cause a change in the FET surface potential. This type of biosensor is also called an ENFET (Enzyme Field Effect Transistor) and is primarily used for pH detection.

5. **Electrochemical Biosensors:** electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The underlying principle for this class of biosensors is that many chemical reactions produce or consume ions or electrons which in turn cause some change in the electrical properties of the solution which can be sensed out and used as measuring parameter. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) conductimetric, (2) amperometric and (3) potentiometric.
 - a. *Conductimetric:* the measured parameter is the electrical conductance / resistance of the solution. When electrochemical reactions produce ions or electrons, the overall conductivity or resistivity of the solution changes. This change is measured and calibrated to a proper scale. Conductance measurements have relatively low sensitivity. The electric field is generated using a sinusoidal voltage (AC) which helps in minimizing undesirable effects such as Faradaic processes, double layer charging and concentration polarization.
 - b. *Amperometric:* this high sensitivity biosensor can detect electroactive species present in biological test samples. Since the biological test samples may not be intrinsically electro-active, enzymes are needed to catalyze the production of radio-active species. In this case, the measured parameter is current.
 - c. *Potentiometric:* in this type of sensor the measured parameter is oxidation or reduction potential of an electrochemical reaction. The working principle relies on the fact that when a ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions. The voltage at which these reactions occur indicates a particular reaction and particular species.

5. Biosensors applications

Biosensors can have a variety of biomedical, industry, and military applications as shown in Figure 2. The major application so far is in blood glucose sensing because of its abundant market potential. However, biosensors have tremendous potential for commercialization in other fields of application as well. In spite of this potential, however, commercial adoption has been slow because of several technological difficulties. For example, due to the presence of biomolecules along with semiconductor materials, biosensor contamination is a major issue.

6. Nanobiosensors based on gold nanoparticles (GNPs)

Many interests have been directed to the biosensing of drugs and biological molecules. The nanotechnology of sol-gel based on molecular recognition and nanoparticles play a very important role in scientific researches (Atta et al., 2009a, 2009b, 2009c, 2010a, 2010b, 2010c, 2010d, 2011a, 2011b, 2011c). Due to extremely small size of nanomaterials they are more readily taken up by the human body. Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Nanoparticles are stable, solid colloidal particles and range in size from 10 to 1,000 nm. Drugs can be absorbed onto the particle surface, entrapped inside the particle, or dissolved within the particle matrix. Nanoparticles have benefits because of its size. Because of their size they can easily enter small places. Nanoparticles have attracted the attention of scientists because of their multifunctional character. Nanoparticles have large surface area to

volume ratio, that helps in diffusion also leading to special properties such as increased heat and chemical resistance.

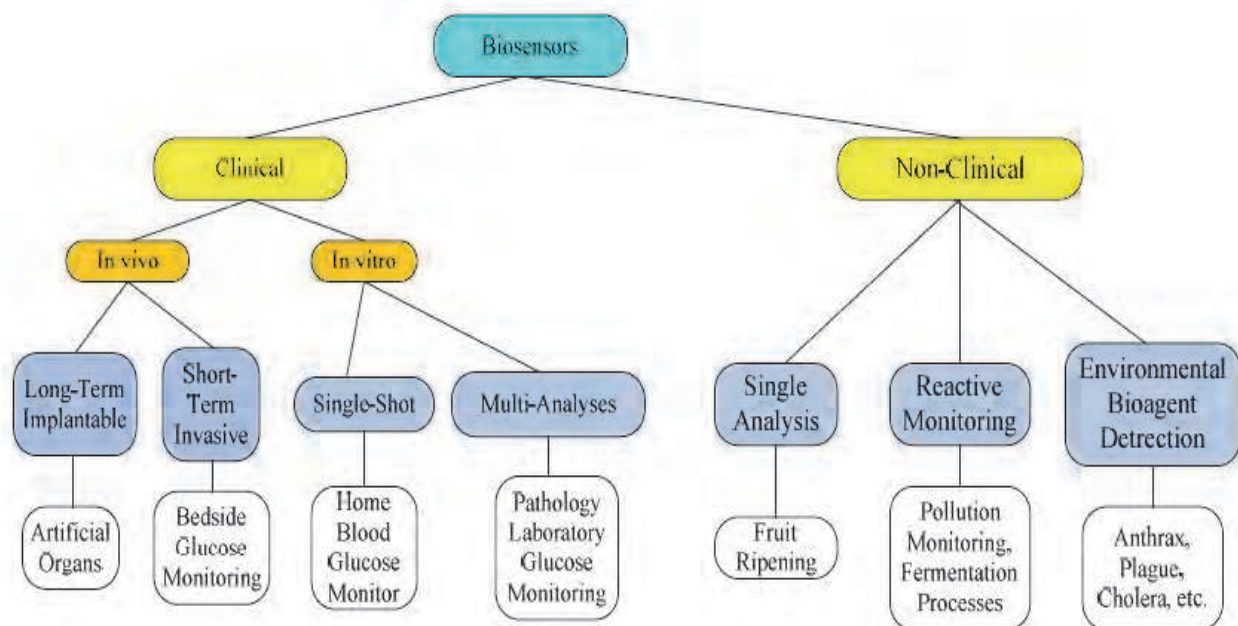


Fig. 2. Biosensors applications

The development of active nanostructures, capable of performing a function or executing a specific task, is currently a major focus of research efforts in bio-/nanotechnology. One already highly successful nanodevice paradigm is the nanosensor: a designed nanostructure, which can provide information about its local environment through its response. Nanomaterials are exquisitely sensitive chemical and biological sensors. Each sensor should be sensitive for one chemical or biological component of a substance. Thus, by having sensor arrays it is possible to tell the composition of an unknown substance. The application area will be wide, encompassing food industry, detection of pollution, medical sector, brewery etc. A nanobiosensor also referred to a nanosensor, is a biosensor with dimensions on the nanometer scale ($1 \text{ nm} = 10^{-9} \text{ m}$). So, Nanosensors are any biological, chemical, or physical sensory points used to convey information about nanoparticles to the macroscopic world. Though humans have not yet been able to synthesize nanosensors, predictions for their use mainly include various medicinal purposes and as gateways to building other nanoproducts, such as computer chips that work at the nanoscale and nanorobots. Presently, there are several ways proposed to make nanosensors, including top-down lithography, bottom-up assembly, and molecular self-assembly. Various kinds of nanomaterials have been being actively investigated for their applications in biosensors, such as gold nanoparticles (GNPs), carbon nanotubes (CNTs), magnetic nanoparticles and quantum dots which have been applied for the detection of DNA, RNA, proteins, glucose, pesticides and other small molecules from clinical samples, food industrial samples, as well as environmental monitoring.

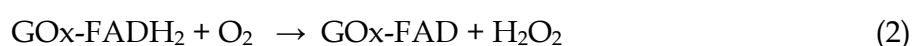
Metal nanoparticles, such as silver (Ag), gold (Au), platinum (Pt) and palladium (Pd) nanoparticles have attracted much interest in the construction of biosensors due to their

unique chemical and physical properties. Nanoparticles can offer many advantages, such as large surface-to-volume ratio, high surface reaction activity and strong adsorption ability to immobilize the desired biomolecules. Gold nanoparticles, in particular, have been widely used to construct biosensors because of their excellent ability to immobilize biomolecules. Many kinds of biosensors, such as enzyme sensor, immunosensor and DNA sensor, with improved analytical performances have been prepared based on the application of gold nanoparticles. Gold nanoparticles (GNPs) are not only better conductor but also offer good microenvironment for retaining the activity of enzyme. They can bind directly with enzymes without disrupting its biological recognition properties. Nowadays, it is revealed that GNPs also exhibit excellent catalytic effects on many important chemical reactions. In addition, GNPs are able to reduce the insulating effect of the protein shell and thus enhance electron transfer in the reaction processes. So far, the oxidation of carbon monoxide, electrochemical oxidation of methanol and hydrogenation of unsaturated substrates and many others are all based on the catalytic effect of GNPs. The most interesting point of GNPs is that their catalytic effect is highly size-dependent. The unique active sites and electronic states of GNPs can lead to their anomalous catalytic activity although the mechanism is still not fully understood.

6.1 Glucose biosensors

The maintenance of glucose level in human or animal blood is very important and any deviance from the normal glucose level may arouse sickness and disease. Thus, fast and accurate detection of the glucose level in blood is of significance to human health. The glucose biosensor has been also widely used as a clinical indicator of diabetes and in the food industry for quality control. Among the various detection methods, enzyme-based electrodes have been extensively studied because of their high selectivity and sensitivity. In 1962, Clark and Lyons developed the first enzyme electrode (Clark & Lyons, 1962). Clark used platinum (Pt) electrodes to detect oxygen. The enzyme glucose oxidase (GOx) was placed very close to the surface of platinum by physically trapping it against the electrodes with a piece of dialysis membrane. The enzyme activity changes depending on the surrounding oxygen concentration. Glucose reacts with glucose oxidase (GOx) to form gluconic acid while producing two electrons and two protons, thus reducing GOx. The reduced GOx, surrounding oxygen, electrons and protons (produced above) react to form hydrogen peroxide and oxidized GOx (the original form). This GOx can again react with more glucose. The higher the glucose content, more oxygen is consumed. On the other hand, lower glucose content results in more hydrogen peroxide. Hence, either the consumption of oxygen or the production of hydrogen peroxide can be detected by the help of platinum electrodes and this can serve as a measure for glucose concentration. Since then, a lot of interests have been put on enzyme-based biosensors. Various techniques such as spectrophotometric, electrochemical, chemiluminescence, fluorescence, and oxygen (O_2) sensor methods have been reported. Most of these methods are based on the immobilization of the enzyme glucose oxidase (GOx) on a solid substrate. Usually the immobilized enzyme can be reused for certain times. The performance of an enzyme-based biosensor relies heavily on the properties of the supporting materials. They should provide a good environment for enzyme immobilization and should be able to maintain their biological activity. Lots of materials have been used for enzyme immobilization including inorganic materials, organic materials and biomaterials. Biomaterials are considered to be more ideal

enzyme immobilization platform since they are more biocompatible with enzymes. Among these biomaterials, eggshell membrane (ESM) has been proved to be an effective and stable enzyme immobilization bio-platform because it not only maintains the enzyme activity but also extends the shelf-life of the immobilized enzyme. GNPs were in situ synthesized and deposited on an ESM and the GNPs-coated ESM was subsequently immobilized with GOx to form a GOx-GNPs/ESM which was positioned on the surface of an O₂ electrode to accomplish a glucose biosensor (Zhenga et al., 2010). GNPs are proposed to speed up the enzymatic reactions with the following reaction schemes:



GOx catalyzes the oxidation of β-d-glucose to gluconolactone and finally hydrolyzes to gluconic acid with a concomitant consumption of dissolved O₂. The depletion of dissolved O₂ can be simply monitored by an O₂ electrode. GOx can be easily immobilized with high loading and activity because of the large surface area of the membrane and the attachment of enzyme to the GNPs. Moreover, GNPs can facilitate the electron transfer of enzyme to oxygen acceptor and enhance the mediated bioelectrocatalytic oxidation of glucose, thus improving the sensitivity of detection. As a result, the GOx-GNPs/ESM biosensor should display higher sensitivity and possesses potential for clinical determination of glucose in human serum. GNPs on GOx/ESM can improve the calibration sensitivity (30 % higher than GOx/ESM without GNPs), stability (87.3% of its initial response to glucose after 10-week storage) and shortens the response time (<30 s) of the glucose biosensor. The linear working range for the GOx-GNPs/ESM glucose biosensor is 8.33 μM to 0.966 mM glucose with a detection limit of 3.50 μM (S/N=3). The biosensor has been successfully applied to determine the glucose in human blood serum samples and the results compared well to a standard spectrophotometric method commonly used in hospitals.

Blood serum sample	Concentration of glucose ^a (mM)	Concentration of glucose ^b (mM)	RSD ^c (%)	Glucose added (mM)	Glucose found (mM)	Recovery (%)	RSD ^c (%)
1	3.10	3.09	0.78	0.400	0.390	97.5	1.59
2	2.60	2.89	3.29	0.400	0.393	98.3	0.86
3	3.13	3.01	2.97	0.400	0.389	97.3	0.99
4	3.55	3.65	3.76	0.400	0.378	94.5	2.92
5	3.19	3.18	3.06	0.400	0.368	92.0	4.04
6	3.72	3.91	3.94	0.400	0.417	104	1.86
7	3.56	3.76	4.06	0.400	0.421	105	1.67

^a Determined by the spectrophotometric method in hospital.

^b Determined by the GOx-GNPs/ESM glucose biosensor.

^c Three replicates were performed.

Table 2. Determination and recovery of glucose in blood serum samples using the GOx-GNPs/ESM glucose biosensor (Zhenga et al., 2010).

Biopolymer chitosan is a polysaccharide derived by deacetylation of chitin. It has primary amino groups that have pKa values of about 6.3. At pH below the pKa, most of the amino groups are protonated, making chitosan a water-soluble polyelectrolyte. When the pH is raised above the pKa, the amino groups are deprotonated, and chitosan becomes insoluble. Chitosan is inexpensive and displays an excellent film-forming ability, biocompatibility, nontoxicity, high mechanical strength, and a susceptibility to chemical modifications. The stabilization of gold nanoparticles with chitosan has been extensively reported (Santos et al., 2004, Esumi et al., 2003). As chitosan in solution is protonated and positively charged, it can be adsorbed onto the surfaces of gold nanoparticles, stabilizing and protecting the nanoparticles, and further construct. Examples of biosensors based on the excellent properties of chitosan and gold nanoparticles were next described (Luo et al., 2005). Gold nanoparticles, which were prepared in advance through the reduction of HAuCl_4 with citrate, can be self-assembly onto electrodeposited chitosan films and then immobilize enzymes effectively. And also they can be mixed with chitosan and enzymes to construct biosensors through simple one-step electrodeposition. However, in both of these systems, gold nanoparticles need to be prepared previously, which prolongs the whole time of biosensor preparation and makes the procedure a bit complicated. Recently, several methods for the formation of gold nanoparticles on the surface of electrodes directly through the electrochemical reduction of HAuCl_4 have been reported. Mena et al. compared different strategies for the construction of amperometric enzyme biosensors using gold nanoparticle-modified electrodes (Mena et al., 2005). Compton et al. investigated electrochemical detection of As(III) at a gold nanoparticle-modified glassy carbon (GC) electrode which was fabricated by the electrochemical deposition of Au nanoparticles onto GC (Dai et al., 2004). By this means, one can synthesize gold nanoparticles on the surface of electrode directly in a short of time, and the sizes of the nanoparticles can be controlled by different conditions of electrochemical deposition with the advantageous properties being kept. Thus, a simple method for fabricating a chitosan film containing gold nanoparticles have been reported (Du et al., 2007b) in which HAuCl_4 solution is mixed with chitosan and electrochemically reduced to gold nanoparticles directly, and the produced gold nanoparticles were stabilized by chitosan and electrochemically deposited onto the glass carbon electrode under a certain voltage along with chitosan. The whole procedure cost only about 10 min. Then a model enzyme, glucose oxidase (GOx), was assembled on the chitosan gold nanoparticles modified electrode. The linear range of the glucose biosensor is from 5.0×10^{-5} to 1.30×10^{-3} M with a Michaelis–Menten constant of 3.5 mM and a detection limit of about 13 μM .

During the last years, numerous works have been published concerning the use of the silica sol-gel technology as a strategy to preserve the catalytic activity of enzymes after the immobilization step. In this sense, the polymeric network generated by sol-gel technology can be used as an adequate matrix in which several compounds, including biological material, can be encapsulated through physical entrapment rather than by covalent bonding leading to a non aggressive approach. Particularly, the sol-gel network provides a biocompatible environment for enzyme protection exhibiting additional advantages such as simplicity of preparation, chemical inertness, high stability, physical rigidity, renewable surface and tuneable properties. The final properties of the sol-gel matrix play a key role in the biosensor performance and can be easily controlled by varying some parameters such as the precursor or the preparation conditions (pH, ratio of compounds, etc). Various

precursors have been reported in the literature for the preparation of the sol-gel network, but among them the most used for enzyme encapsulation are oxysilanes such as methyltrimethoxy-silane (MTMOS), tetramethoxysilane (TMOS), 3-aminopropyltriethoxysilane (APTOS) and tetraethoxysilane (TEOS) (Wang et al., 1998, Walcarius, 2001, Salimi et al., 2004, Kumar et al., 2006, Pauliukaite et al., 2006, Singh et al., 2007). The preparation and characterization of a new organic-inorganic hybrid composite material from a three-dimensional silica polymer network, obtained by means of the sol-gel technology using tetraethoxysilane as precursor was reported (Barbadillo et al., 2009). This matrix provides an excellent network allowing the encapsulation of gold nanoparticles, conductive material (graphite powder, C) and a biosensing molecule such as glucose oxidase (GOx), chosen as a model since it is a stable, inexpensive and well-studied enzyme. This composite material combines the advantages induced from both the silica matrix, which enables the incorporation of the other elements while keeping the enzymatic activity of the assembly, and the presence of nanostructures, which enhances the electroactive area. As a consequence, the resulting biosensor TEOS/GNPs/GOx/C exhibits a wider linear range concentration, higher sensitivity and higher accuracy, when compared with a similar composite containing GOx but free of GNPs (TEOS/GOx/C). Taking into account the good performance of the resulting biosensor, this approach is a promising route for designing a wide range of biosensors.

Biosensor	Sensitivity ($\mu\text{A}\text{mM}^{-1}$)	Linear range (mM)	Applied potential (V)	Accuracy (R.S.D.)
TEOS/GOx/C (Barbadillo et al., 2009)	1.73	1-20	+0.25	1.1% (n=8)
TEOS/AuNPs/GOx/C (Barbadillo et al., 2009)	2.43	0.5-55	+0.25	0.5% (n=8)
Sol-gel/chitosan/GOx (Chen et al.2003)	0.27	Up to 14	+0.35	2% (n=7)
Fc/Ormosil/GOx/GP (Pandey et al., 2003)	1.76	Up to 35	+0.35	-
Sol-gel/CNT/GOx/Bppg (Salimi et al., 2004)	0.20	0.2-20	+0.3	1.8% (n=10)
Sol-gel/GOx/chitosan/PB/GC (Tan et al., 2005)	0.42	0.05-26	-0.05	3.8% (n=8)
Sol-gel/PNR/GOx (Pauliukaite et al.2006)	0.06	0.05-0.6	-0.25	-
Sol-gel/GOx/PtNPs-CNT (Yang et al., 2006)	0.28	1-25	+0.1	5.1% (n=10)
Sol-gel/PVA/GOx/SPE (Zuo et al., 2008)	0.44	0-4.1	-0.5	-
Sol-gel/GOx/PB/GC (Liang et al., 2008)	0.84	0.01-5.8	0	1.8% (n=8)
Solgel/GOx/CNT/chitosan/PtN P/GC (Kang et al., 2008)	2.08	0.001-6.0	+0.1	-

Table 3. A comparison between various biosensors based on sol-gel technology (Barbadillo et al., 2009).

The oxidase-based amperometric biosensors previously relied on the immobilization of oxidase enzymes on the surface of various electrodes. However, electron transfer efficiency of redox enzymes is poor in the absence of mediator, because enzyme active sites are deeply embedded inside the protein. The sensitivity of resulted biosensors can be significantly improved by the immobilization of mediators in the matrices. Among the different mediators described in the literature, ferrocene (Fc) and its derivatives, first reported by Cass et al. (Cass et al., 1984), have proved to be the most efficient electron transfers for the GOx enzymatic reaction. There are a lot of cases about ferrocene (Fc) and its derivatives introduced to enzyme biosensor as the mediator. However, leakage has been a main problem for the entrapment of mediators due to their low molecular weight in polymer matrices. In order to prevent the leakage of mediator, mediator can be linked covalently with polymer or with high molecular weight compounds before immobilization on the surface of electrode. Gorton et al. (Gorton et al., 1990) studied ferrocene-containing siloxane polymer modified electrode surface with a poly (ester-sulfuric acid) cation-exchanger to improve the stability of the mediator. Another alternative method is to synthesize a few Fc derivatives with specific functional groups (Jönsson et al., 1989, Foulds & Lowe, 1988), but the preparation methods are complicated. For instance, Jönsson et al. (Jönsson et al., 1989) used hydroxymethyl Fc and anthracene carboxylic acid to synthesize anthracene substituted ferrocene. The other alternative method to increase the stability of Fc and its derivatives is the formation of inclusion complex with cyclodextrin (CD), a class of torpidly shaped cycloamyloses with a hydrophilic outer surface and a hydrophobic inner cavity, which makes the dissolubility of Fc decrease. Several investigations have been made to study the characterization of interacting Fc-CD system and their roles. Liu et al. (Liu et al., 1998) developed the sensitive biosensor for glucose by immobilizing glucose oxidase in β -cyclodextrin via cross-linking and by including ferrocene in the cavities of dextrin polymer via host-guest reaction. Zhang et al. (Zhang et al., 2000) successfully used ferrocene with β -cyclodextrin to prepare β -CD/Fc inclusion complex modified carbon paste electrode. The water-soluble inclusion complex of 1,1-dimethylferrocene with (2-hydroxypropyl)- β -CD has been used in bioelectrocatalysis (Bersier et al., 1991). Gold nanoparticles were capped by inclusion complex between mono-6-thio- β -cyclodextrin and ferrocene through -SH, which resulted into stable fixation of ferrocene on the surface of gold nanoparticles (Chen & Diao, 2009). Then, the glucose biosensors were constructed by using GNPs/CD-Fc as the building block. The composite nanoparticles showed excellent efficiency of electron transfer between the GOx and the electrode for the electrocatalysis of glucose. The sensor (GNPs/CD-Fc/GOD) showed a relatively fast response time (5 s), low detection limit (15 μ M, S/N = 3), and high sensitivity (ca. 18.2 mA.M⁻¹.cm⁻²) with a linear range of 0.08–11.5 mM of glucose. The excellent sensitivity was possibly attributed to the presence of the GNPs/CD-Fc film that can provide a convenient electron tunneling between the protein and the electrode. In addition, the biosensor demonstrated high anti-interference ability, stability and natural life. The good stability and natural life can be attributed to the following two aspects: on the one hand, the fabrication process was mild and no damage was made on the enzyme molecule, on the other hand, the GNPs possessed good biocompatibility that could retain the bioactivity of the enzyme molecules immobilized on the electrode.

In comparison with spherical nanoparticles, one-dimensional (1-D) nanomaterials, especially nanowires, possess a number of unique physical and electronic properties that endow them with new and important activities. The excellent properties of nanowires are due to several beneficial features arising from their shape anisotropy on the electrochemical

reaction at electrodes: (i) facile pathways for the electron transfer by reducing the number of interfaces between the nanoparticle catalysts and (ii) effective surface exposure to work as active catalytic sites in the electrode–electrolyte interface. It has been reported that enzymes can be adsorbed onto these nanostructures, because these materials provide large surface area for enzyme loading and friendly microenvironment to stabilize the immobilized enzymes. Recent results suggest the possibility of incorporating large numbers of nanowires into large-scale arrays and complex hierarchical structures for high-density biosensors, electronics, and optoelectronics. Biosensors based on nanowires showed improved signal-to-noise ratios, high faradaic current density, fast electron-transfer rate, enhanced sensitivities, better detection limit. Recently, increasing research interest in biosensor field has been focused on composite materials based on 1-D materials and noble metal nanoparticles with a synergistic effect. Materials for such purposes include carbon nanotubes, carbon nanofibers, redox mediators and metal nanoparticles.

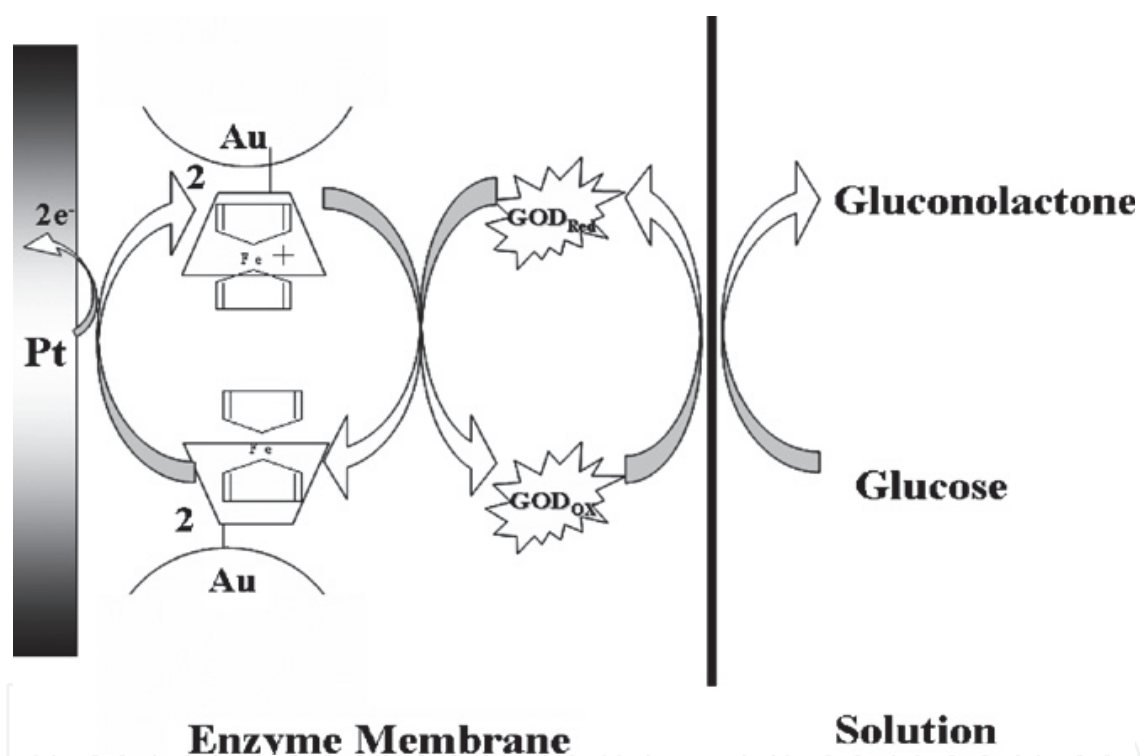


Fig. 3. Schematic illustration of sensing mechanism for electrocatalytic glucose on the GNPs/CD-Fc/GOD modified platinum electrode surface (Chen & Diao, 2009).

For example, coupling carbon nanofibers with palladium nanoparticles resulted in a remarkable improvement of the electroactivity of the composite materials towards reduction of H_2O_2 and oxidation of β -nicotinamide adenine dinucleotide in reduced form (NADH) (Huang et al., 2008). Zou et al. reported a glucose biosensor based on electrodeposition of platinum nanoparticles onto multiwalled carbon nanotubes (Zou et al., 2008). Wu et al. constructed a glucose biosensor based on multi-walled carbon nanotubes and GNPs by layer-by-layer self-assembly technique (Wu et al., 2007). Taking advantage of the nanowires and GNPs, a novel glucose biosensor was developed, based on the immobilization of glucose oxidase (GOx) with cross-linking in the matrix of bovine serum albumin (BSA) on a Pt electrode, which was modified with gold nanoparticles decorated Pb nanowires (GNPs-

PbNWs) (Wanga et al., 2009). Pb nanowires (PbNWs) were synthesized by an l-cysteine-assisted self-assembly route, and then gold nanoparticles (GNPs) were attached onto the nanowire surface through -SH-Au specific interaction. The synergistic effect of PbNWs and GNPs made the biosensor exhibit excellent electrocatalytic activity and good response performance to glucose. In pH 7.0, the biosensor showed the sensitivity of $135.5 \mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$, the detection limit of $2 \mu\text{M}$ ($S/N = 3$), and the response time $< 5 \text{ s}$ with a linear range of $5\text{--}2200 \mu\text{M}$. Furthermore, the biosensor exhibits good reproducibility, long-term stability and relative good anti-interference.

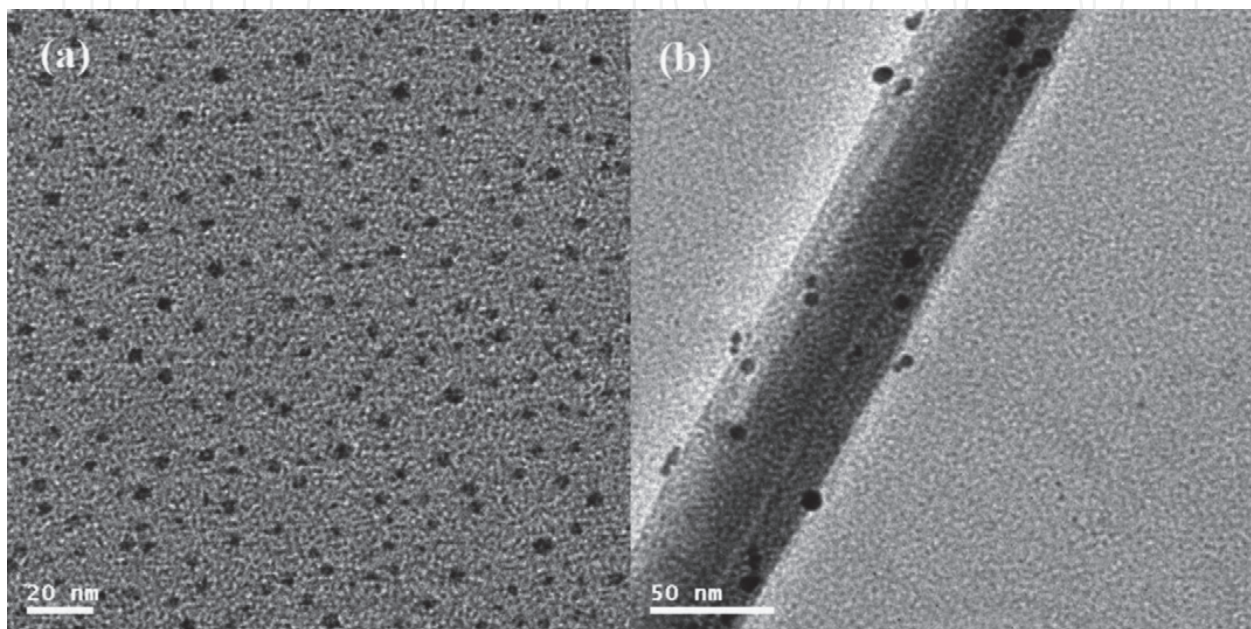


Fig. 4. TEM images of (a) GNPs, (b) GNPs-PbNWs (Wanga et al., 2009).

6.2 Cholesterol biosensors

Cholesterol is a fundamental parameter in the diagnosis of coronary heart disease, arteriosclerosis, and other clinical (lipid) disorders and in the assessment of the risks of thrombosis and myocardial infarction. The clinical analysis of cholesterol in serum samples is important in the diagnosis and prevention of a large number of clinical disorders such as hypertension, cerebral thrombosis and heart attack. Hence, it is important to develop a reliable and sensitive biosensor which can permit a suitable and rapid determination of cholesterol. Ideally, the total cholesterol concentration in a healthy person's blood should be less than 200 mg/dL ($< 5.17 \text{ mM}$). The borderline high is defined as $200\text{--}239 \text{ mg/dL}$ ($5.17\text{--}6.18 \text{ mM}$), and the high value is defined as above 240 mg/dL ($\geq 6.21 \text{ mM}$) (Shen & Liu, 2007). Different analytical methods have been used for the determination of cholesterol for instance colorimetric, spectrometric and electrochemical methods. Among these methods, electrochemical detection of cholesterol has achieved significant attention due to the rapid determination, simplicity, and low cost. Thus, amperometric biosensors are more attractive due to their low detection limit and enzyme stabilization can be easily achieved. Especially, the enzyme based cholesterol sensors have gained special focus taking the advantages of good stability, high sensitivity and wide linear range they hold a leading position among the presently available biosensor systems. Recently, many scientists and biologists focused on the preparation of newer nanocomposite with good biocompatibility that could be the

promising matrices for enzyme immobilization which can enhance the selectivity and sensitivity of the biosensors. Among the natural biocompatible macromolecules, chitosan (CS) is the biodegradable polymer obtained from marine versatile biopolymer-chitin. CS fibers situate apart from all other biodegradable natural fibers in several inherent properties such as outstanding biocompatibility, non-toxicity, biodegradability, high mechanical strength, fast metal complexation and hydrophilicity for enzyme immobilization. CS nanofibers (NFs) have remarkable characteristic such as exceptionally minute pore size with very outsized surface area-to-volume proportion, high porosity and diameters of the fiber was in nanometer scale. These properties of CSNFs hold fine enzyme immobilization scaffold and it was exploited for biosensor applications. These interesting matrices provide high surface area for high enzyme loading and compatible micro-environment helping enzyme stability. Besides, CS provides direct contact between enzyme active site and electrode. Enzyme immobilization is currently the gigantic increasing subject of considerable interest because the use of enzyme is frequently inadequate due to their availability in tiny quantity, instability, high cost and the limited possibility of economic recoveries of these bio-catalysts from an effective response unify. For a good enzyme immobilization, biocompatibility is the one of the most important key requisite that benefits the enzymatic bio-transformations to construct the biosensors. So, increase the biocompatibility of the support, various surface modification protocol have often been used such as adsorption, coating, self-assembly and graft polymerization. Among these techniques, it is relatively graceful and efficient to directly bind natural bio-macromolecules on the support surface to form a bio-mimetic compatible layer for enzyme immobilization. In the recent years, there is a trend to use nanostructured materials as supports for enzyme immobilization, since the large surface area to volume ratio of nanosize materials can effectively improve to the loading enzyme per unit to volume ratio of support and the excellent catalytic efficiency of the immobilized enzyme. Both nanofibers and nanoparticles were explored for this purpose. Recent developments in the field of nanobiotechnology, metal nanoparticles (MNPs) find numerous applications. Among the MNPs, GNPs be widely used for the catalytic and biological application. GNPs provides adequate micro-environment to enhance DET between biomolecule and electrode. In the fabrication of a cholesterol biosensor, cholesterol oxidase (ChOx) is most commonly used as the biosensing element. Cholesterol oxidase catalyzes the oxidation of cholesterol to H₂O₂ and cholest-4-en-3-one in the presence of oxygen. The enzymatic reaction in the use of cholesterol oxidase (ChOx) as a receptor can be described as follows:

ChOx



The electro-oxidation current of hydrogen peroxide is detected after application of a suitable potential to the system. The major problem for amperometric detection is the overestimation of the response current due to interferences such as ascorbic acid. This problem can be overcome by using a combination of two or three enzymes, which are more selective for the analyte of interest (Bongiovanni et al., 2001) or by devising techniques to eliminate or reduce the interference. A novel amperometric cholesterol biosensor was fabricated by the immobilization of ChOx (cholesterol oxidase) onto the chitosan nanofibers/gold nanoparticles (designated as CSNFs/AuNPs) composite network (NW) (Gomathia et al., 2010). The fabrication involves preparation of chitosan nanofibers (CSNFs) and subsequent electrochemical loading of gold nanoparticles. Field emission scanning electron microscopy

(FE-SEM) was used to investigate the morphology of CSNFs (sizes in the range of 50–100 nm) and spherical GNPs. The CSNF-GNPs/ChOx biosensor exhibited a wide linear response to cholesterol (concentration range of 1–45 μM), good sensitivity (1.02 $\mu\text{A}/\mu\text{M}$), low response time (5 s) and excellent long term stability. The combined existence of GNPs within CSNFs NW provides the excellent performance of the biosensor towards the electrochemical detection of cholesterol.

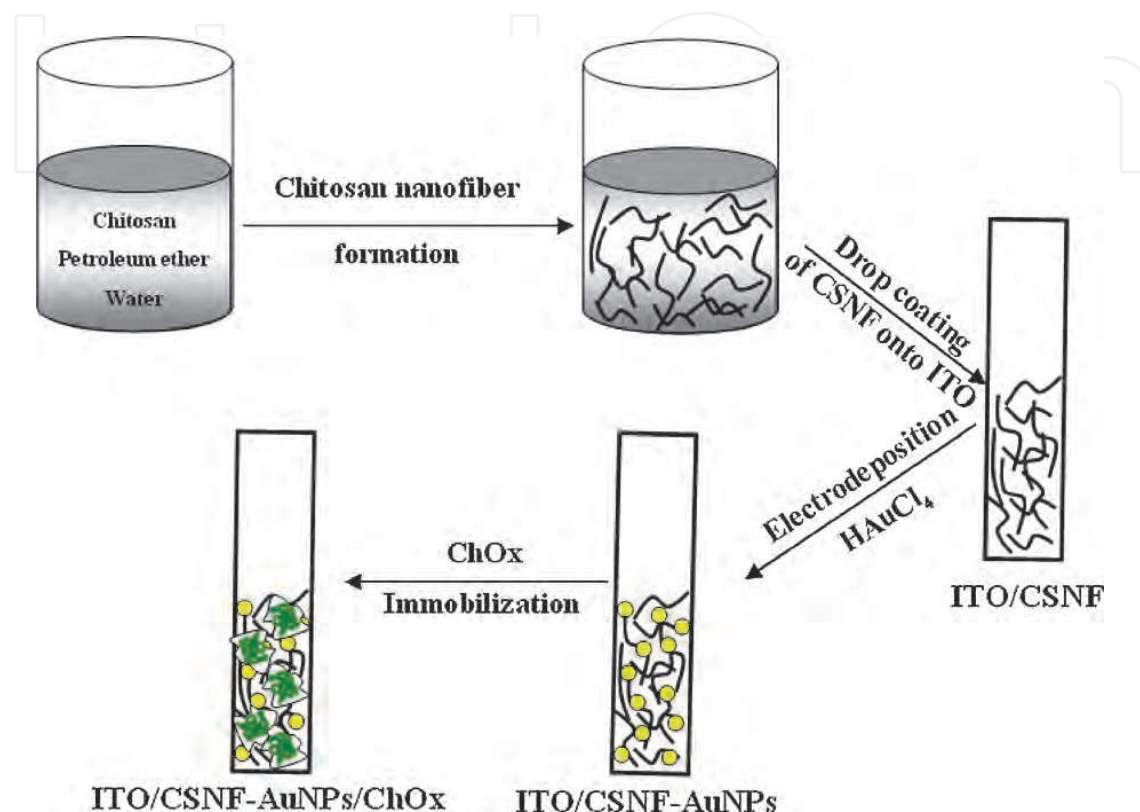


Fig. 5. Fabrication of CSNF-GNPs/ChOx biosensor electrode (Gomathia et al., 2010).

Many researchers have reported the inclusion of metal nanoparticles with a catalytic effect in polymer modified electrodes to decrease the overpotential applied to the amperometric biosensors (Safavi et al., 2009, Hrapovic et al., 2004, Ren et al., 2005, Huang et al., 2004). Amperometric cholesterol biosensors based on carbon nanotube-chitosan-platinum-cholesterol oxidase nanobiocomposite was fabricated for cholesterol determination at an applied potential of 0.4 V (Tsai et al., 2008). To improve the selectivity of the biosensor, Gopalana et al. reported the construction of a cholesterol biosensor by monitoring the reduction current of H_2O_2 at -0.05 V (Gopalana et al., 2009). Bimetallic alloys are widely used in catalysis and sensing fields. Owing to the interaction between two components in bimetallic alloys, they generally show many favorable properties in comparison with the corresponding monometallic counterparts, which include high catalytic activity, catalytic selectivity, and better resistance to deactivation. Among various bimetallic alloys, gold-platinum (AuPt) alloy is very attractive. It has excellent catalysis and resistance to deactivation due to the high synergistic action between gold and platinum (Xiao et al., 2009). Owing to these advantages of bimetallic nanoparticles, it becomes significant to develop AuPt nanoparticles for application in electrochemical sensors with appropriate characteristics such as high sensitivity, fast response time, wide linear range, better

selectivity, and reproducibility. An electrodeposition method was applied to form gold-platinum (AuPt) alloy nanoparticles on the glassy carbon electrode (GCE) modified with a mixture of an ionic liquid (IL) and chitosan (Ch) (AuPt-Ch-IL/GCE). AuPt-Ch-IL/GCE electrocatalyzed the reduction of H_2O_2 and thus was suitable for the preparation of biosensors. Cholesterol oxidase (ChOx) was then, immobilized on the surface of the electrode by cross-linking ChOx and chitosan through addition of glutaraldehyde (ChOx/AuPt-Ch-IL/GCE) (Safavia & Farjania, 2011). The fabricated biosensor exhibited two wide linear ranges of responses to cholesterol in the concentration ranges of 0.05–6.2 mM and 6.2–11.2 mM. The sensitivity of the biosensor was $90.7 \mu A \cdot mM^{-1} \cdot cm^{-2}$ and the limit of detection was $10 \mu M$ of cholesterol. The response time was less than 7 s. The Michaelis-Menten constant (K_m) was found as 0.24 mM. The effect of the addition of 1 mM ascorbic acid and glucose was tested on the amperometric response of 0.5 mM cholesterol and no change in response current of cholesterol was observed.

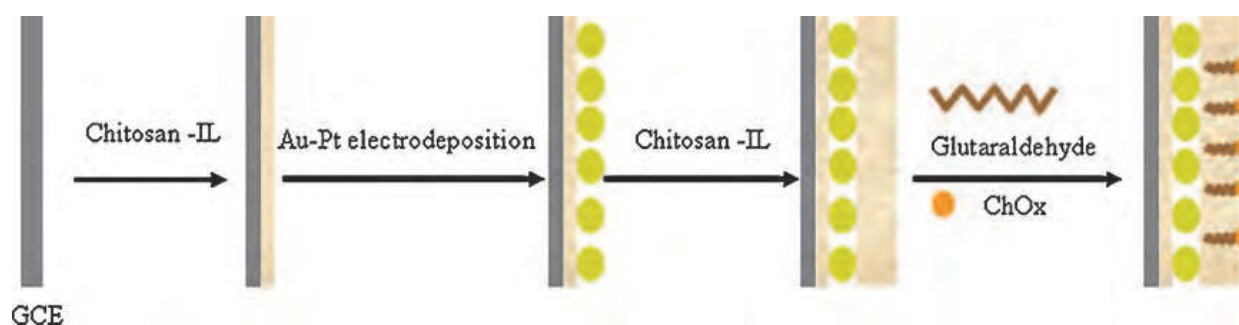


Fig. 6. Schematic illustration of preparation procedures of ChOx/AuPt-Ch-IL/GCE (Safavia & Farjania, 2011).

6.3 Tyrosinase biosensors

Phenolic compounds often exist in the wastewaters of many industries, causing problems for our living environment. Many of them are very toxic, showing adverse effects on animal and plants. Therefore, the identification and quantification of such compounds are very important for environment monitoring. Some methods are available for the phenolic compound assay, including gas or liquid chromatography and spectrophotometry (Chriswell et al. 1975, Poerschmann et al., 1997). However, demanding sample pretreatments, low sensitivities, and time-consuming manipulations limit their practical applications. A great amount of effort has been devoted to the development of simple and effective analytical methods for the determination of phenolic compounds. Among them, amperometric biosensor based on tyrosinase has been shown to be a very simple and convenient tool for phenol assay due to its high sensitivity, effectiveness, and simplicity (Wang et al., 2002, Dempsey et al., 2004, Rajesh et al., 2004, Xue & Shen, 2002, Zhang et al., 2003, Wang et al., 2000a, Yu et al. 2003, Campuzano et al., 2003, Tatsuma & Sato, 2004). The immobilization of tyrosinase is a crucial step in the fabrication of phenol biosensor. The earlier reports on the immobilization methods included polymer entrapment (Wang et al., 2002, Dempsey et al., 2004), electropolymerization (Dempsey et al., 2004, Rajesh et al., 2004), sol-gels (Rajesh et al., 2004, Yu et al. 2003), self-assembled monolayers (SAMs)¹ (Campuzano et al., 2003, Tatsuma et al., 2004), and covalent linking (Anh et al., 2002, Rajesh et al., 2004a). However, some of these immobilizations are relatively complex, requiring the use of solvents that are unattractive to the environment and result in relatively poor stability

and bioactivity of tyrosinase. Recent years have seen increased interest in searching for simple and reliable schemes to immobilize enzymes. The biocompatible nanomaterials have their unique advantages in enzyme immobilization. They could retain the activity of enzyme well due to the desirable microenvironment, and they could enhance the direct electron transfer between the enzyme's active sites and the electrode (Gorton et al., 1999, Jia et al., 2002). In spite of the big amount of literature on tyrosinase electrochemical biosensors, two general limitations need to be solved yet in order to improve their practical usefulness. One of them concerns the stability of the biosensors. Although many efforts have been made to improve the useful lifetime and reusability of tyrosinase electrodes, searching for appropriate microenvironments for retaining the biological activity of the enzyme, its inherent instability provokes that this useful lifetime is too short for practical applications in many cases. On the other hand, the low concentration levels of phenolic compounds that should be detected due to their classification as priority pollutants, requires that the tyrosinase biosensors are capable to achieve a high sensitivity. The aim of this work is the design of a new tyrosinase bioelectrode able to improve significantly these important analytical characteristics with respect to previous designs. The new bioelectrode design is based on the combination of the advantageous properties of a graphite-Teflon composite electrode matrix for the immobilization of enzymes, and the use of colloidal gold nanoparticles. In this new design, both the enzyme tyrosinase and gold nanoparticles are incorporated into the composite electrode matrix by simple physical inclusion. The use of graphite-Teflon composite pellets for the construction of enzyme electrodes has been extensively reported (Serra et al., 2002, GuzmanVazquez de Prada et al., 2003, Pena et al., 2001). The resulting bioelectrodes are easily renewable by polishing and allow incorporation of biomolecules and other modifiers with no covalent attachments, thus making the electrode fabrication procedure easy, fast and cheap. On the other hand, electrochemical biosensors created by coupling biological recognition elements with electrochemical transducers based on or modified with gold nanoparticles are playing an increasingly important role in biosensor research over the last few years (Yanez-Sedeno & Pingarron, 2005). So, colloidal gold allows proteins to retain their biological activity upon adsorption (Doron et al., 1995, Brown et al., 1996, Mena et al., 2005) and modification of electrodes with this type of nanoparticles provides a microenvironment similar to that of the redox proteins in native systems, reducing the insulating effect of the protein shell for the direct electron transfer through the conducting tunnels of gold nanocrystals (Liu et al., 2003a). Surface morphology of gold nanoparticles, and the interaction between the nanoparticles and the electrode surface, are significant factors which contribute to improve the electrical contact between the redox protein and the electrode material (Shipway et al., 2000). In this context, biosensors based on the immobilization of enzymes on gold nanoparticles for the determination of hydrogen peroxide, nitrite, glucose and phenols (Tang & Jiang, 1998, Xiao et al., 2000, Gu et al., 2001, Liu & Ju, 2002, Jia et al., 2002, Liu & Ju, 2003, Liu et al., 2003b, Xiao et al., 2003, Carralero-Sanz et al., 2005) have been recently reported.

The preparation of a tyrosinase biosensor based on the immobilization of the enzyme onto a glassy carbon electrode modified with electrodeposited gold nanoparticles (Tyr-nAu-GCE) was reported (Carralero-Sanz et al., 2005). The enzyme immobilized by cross-linking with glutaraldehyde retains a high bioactivity on this electrode material. Under the optimized working variables (a Au electrodeposition potential of -200mV for 60 s, an enzyme loading of 457 U, a detection potential of -0.10V and a 0.1 mol. L^{-1} phosphate buffer solution of pH 7.4 as working medium) the biosensor exhibited a rapid response to the changes in the

substrate concentration for all the phenolic compounds tested: phenol, catechol, caffeic acid, chlorogenic acid, gallic acid and protocatechualdehyde. A R.S.D. of 3.6% ($n = 6$) was obtained from the slope values of successive calibration plots for catechol with the same Tyr-nAu-GCE with no need to apply a cleaning procedure to the biosensor. The useful lifetime of one single biosensor was of at least 18 days, and a R.S.D. of 4.8% was obtained for the slope values of catechol calibration plots obtained with five different biosensors. The Tyr-nAu-GCE was applied for the estimation of the phenolic compounds content in red and white wines. A good correlation of the results ($r = 0.990$) was found when they were plotted versus those obtained by using the spectrophotometric method involving the Folin-Ciocalteu reagent.

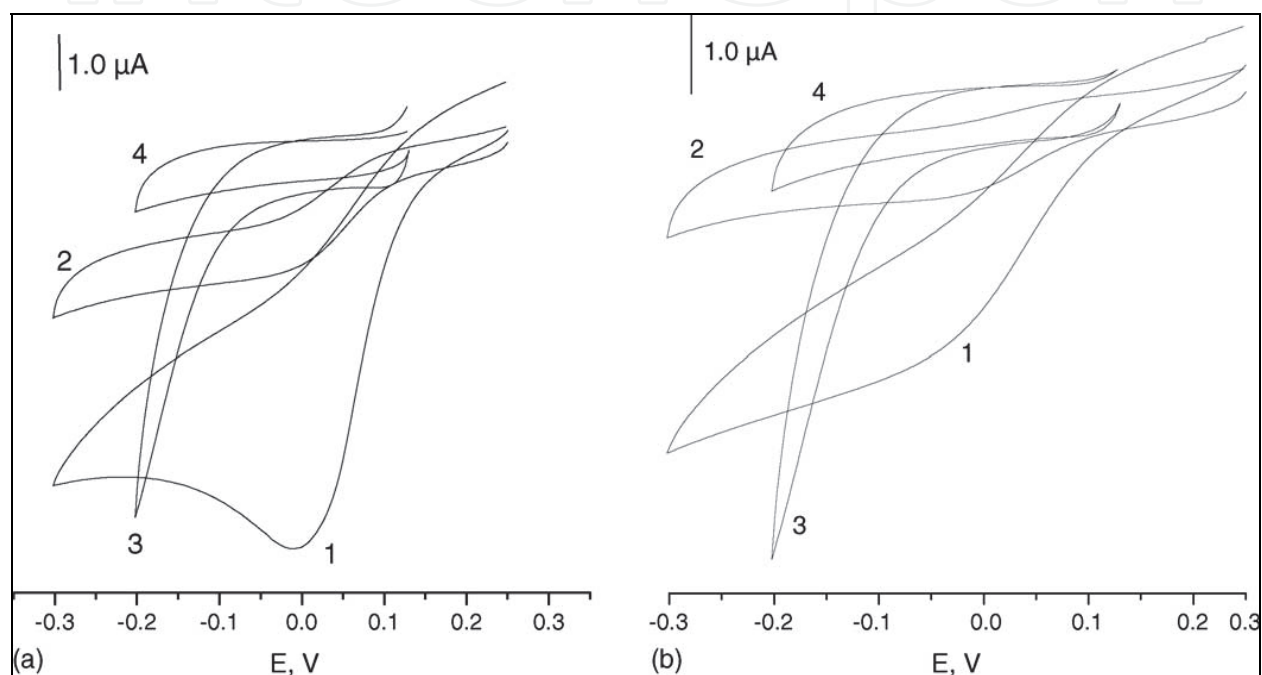


Fig. 7. Cyclic voltammograms for 2.0×10^{-4} mol.L⁻¹ solutions of catechol (a) and caffeic acid (b), at: (1) Tyr-nAu-GCE; (2) Tyr-GCE; (3) Au-GCE; (4) GCE; $v = 25 \text{ mVs}^{-1}$. Supporting electrolyte: 0.05 mol.L⁻¹ phosphate buffer (pH 7.4) (Carralero-Sanz et al., 2005).

The design of a new tyrosinase biosensor with improved stability and sensitivity was reported (Carralero-Sanz et al., 2006). The biosensor design is based on the construction of a graphite-Teflon composite electrode matrix in which the enzyme and colloidal gold nanoparticles are incorporated by simple physical inclusion. The Tyr-Au_{coll}-graphite-Teflon biosensor exhibited suitable amperometric responses at -0.10 V for the different phenolic compounds tested (catechol; phenol; 3,4-dimethylphenol; 4-chloro-3-methylphenol; 4-chlorophenol; 4-chloro-2-methylphenol; 3-methylphenol and 4-methylphenol). The limits of detection obtained were 3 nM for catechol, 3.3 μM for 4-chloro-2-methylphenol, and approximately 20 nM for the rest of phenolic compounds. The presence of colloidal gold into the composite matrix gives rise to enhanced kinetics of both the enzyme reaction and the electrochemical reduction of the corresponding *o*-quinones at the electrode surface, thus allowing the achievement of a high sensitivity. The biosensor exhibited an excellent renewability by simple polishing, with a lifetime of at least 39 days without apparent loss of the immobilized enzyme activity. The usefulness of the biosensor for the analysis of real

samples was evaluated by performing the estimation of the content of phenolic compounds in water samples of different characteristics.

A highly efficient enzyme-based screen printed electrode (SPE) was obtained by using covalent attachment between 1-pyrenebutanoic acid, succinimidyl ester (PASE) adsorbing on the graphene oxide (GO) sheets and amines of tyrosinase-protected gold nanoparticles (Tyr-Au) (Song et al., 2010). Herein, the bi-functional molecule PASE was assembled onto GO sheets. Subsequently, the Tyr-Au was immobilized on the PASE-GO sheets forming a biocompatible nanocomposite, which was further coated onto the working electrode surface of the SPE. Attributing to the synergistic effect of GO-Au integration and the good biocompatibility of the hybrid-material, the fabricated disposable biosensor (Tyr-Au/PASE-GO/SPE) exhibited a rapid amperometric response (less than 6 s) with a high sensitivity and good storage stability for monitoring catechol. This method shows a good linearity in the range from 8.3×10^{-8} to 2.3×10^{-5} M for catechol with a squared correlation coefficient of 0.9980, a quantitation limit of 8.2×10^{-8} M ($S/N = 10$) and a detection limit of 2.4×10^{-8} M ($S/N = 3$). The Michaelis-Menten constant was measured to be 0.027 mM. This disposable tyrosinase biosensor could offer a great potential for rapid, cost-effective and on-field analysis of phenolic compounds.

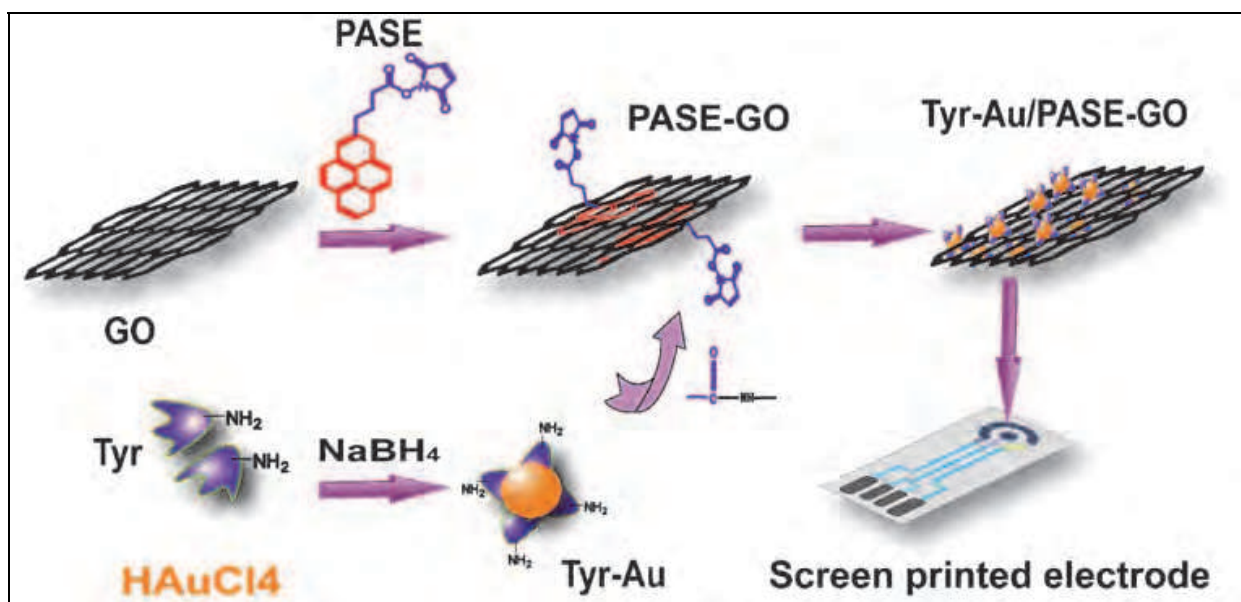


Fig. 8. Assembling process of Tyr-Au/PASE-GO on SPE (Song et al., 2010).

6.4 Urease biosensors

Kidneys perform key roles in various body functions, including excreting metabolic waste products such as urea from the bloodstream, regulating the hydrolytic balance of the body, and maintaining the pH of body fluids. The level of urea in blood serum is the best measurement of kidney function and staging of kidney diseases. The normal urea level in serum ranges from 15 to 40 mg/dL (i.e., 2.5–7.5 mM). An increase in urea concentration causes renal failure such as acute or chronic urinary tract obstruction with shock, burns, dehydration, and gastrointestinal bleeding, whereas a decrease in urea concentration causes hepatic failure, nephritic syndrome, and cachexia. Therefore, there is an urgent need to develop a device that rapidly monitors urea concentration in the body. Most existing urea

biosensors utilize urease (Urs) as the sensing element. The available Urs on the electrode surface hydrolyzes urea into NH_4^+ and HCO_3^- ions. The concentration of urea is measured by monitoring the liberated ions using a transducer such as amperometric, potentiometric, optical, thermal, or piezoelectric. Although various urea biosensors that use a range of transducers have been studied extensively, the Urs-based amperometric urea biosensor is considered one of the most promising approaches because it offers fast, simple, and low-cost detection. The response time of such a biosensor is directly associated with the hydrolysis rate of urea on the electrode surface; therefore, rapid production of NH_4^+ ions on the electrode will lead to a highly sensitive biosensor. It is well established that the performance of biosensors greatly depends on the physicochemical properties of the electrode materials, enzyme immobilization procedure, and enzyme concentration on the electrode surface. Many electrode materials have been used to fabricate urea biosensors. However, there is an ongoing demand for new types of electrode materials that can provide the Urs enzyme with better stability and performance for *in vitro* urea measurement. In this context, the use of nanomaterials to fabricate biosensors is one of the most exciting approaches because nanomaterials have a unique structure and high surface-to-volume ratio. The surfaces of nanomaterials can also be tailored in the molecular scale in order to achieve various desirable properties. Many attempts have been made to fabricate a third-generation biosensor with self-assembly technology; however, these approaches were based on planar self-assembly that may only offer limited available surface area on the electrode, which can compromise the performance of the biosensor. Meanwhile, gold nanoparticles have played an increasingly important role for biosensor applications over the last decade.

Gold nanoparticles can (1) provide a stable surface for the immobilization of biomolecules without compromising their biological activities and (2) permit direct electron transfer from the redox biomolecules to the bulk electrode materials, thereby enhancing the electrochemical sensing ability. For example, Shipway et al. systematically studied the new electronic, photoelectronic, and sensing systems that used gold nanoparticle superstructures on the electrode surface (Shipway et al., 2000). In addition, previous studies indicated that biological macromolecules such as enzymes can generally retain their enzymatic and electrochemical activity after being immobilized onto the gold nanoparticles (Brown et al., 1996, Xiao et al., 1999). An amperometric biosensor was fabricated for the quantitative determination of urea in aqueous medium using hematein, a pH-sensitive natural dye (Tiwari et al., 2009). The urease (Urs) covalently immobilized onto an electrode made of gold nanoparticles functionalized with hyperbranched polyester-BoltronR H40 (H40-Au) coated onto an indium-tin oxide (ITO) covered glass substrate. The covalent linkage between the Urs enzyme and H40-Au nanoparticles provided the resulting enzyme electrode (Urs/H40-Au/ITO) with a high level of enzyme immobilization and excellent lifetime stability. The biosensor based on Urs/H40-Au/ITO as the working electrode showed a linear current response to the urea concentration ranging from 0.01 to 35 mM. The urea biosensor exhibited a sensitivity of 7.48 nA/mM with a response time of 3 s. The Michaelis-Menten constant for the Urs/H40-Au/ITO biosensor was calculated to be 0.96 mM, indicating the Urs enzyme immobilized on the electrode surface had a high affinity to urea.

A renewable potentiometric urease inhibition biosensor based on self-assembled gold nanoparticles has been developed for the determination of mercury ions (Yang et al., 2006a).

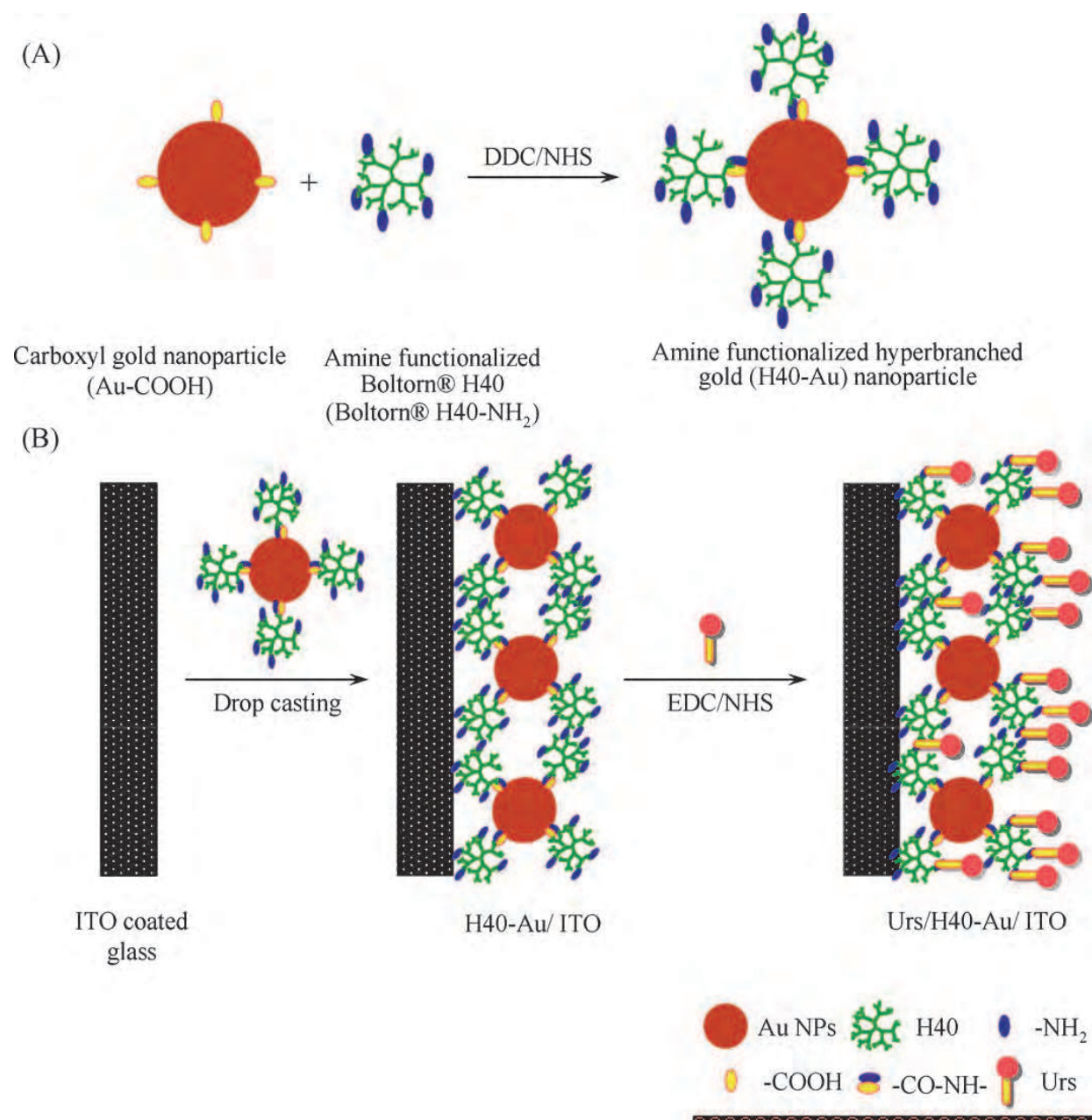


Fig. 9. Schematic presentation of the [A] preparation of hyperbranched gold (H40-Au) nanoparticles and [B] fabrication of H40-Au/ITO and Urs/H40-Au/ITO electrodes (Tiwari et al., 2009).

Gold nanoparticles were chemically adsorbed on the PVC-NH₂ matrix membrane pH electrode surface containing *N,N*-didecylaminomethylbenzene (DAMAB) as a neutral carrier and urease was then immobilized on the gold nanoparticles. The linear range of determination of Hg²⁺ was 0.09–1.99 $\mu\text{mol.L}^{-1}$ with a detection limit of 0.05 $\mu\text{mol.L}^{-1}$. The advantages of self-assembled immobilization are low detection limit, fast response and ease regeneration. The assembled gold nanoparticles and inactive enzyme layers denatured by Hg²⁺ can be rinsed out via a saline solution with acid and alkali successively. This sensor is generally of great significance for inhibitor determination, especially in comparison with expensive base transducers.

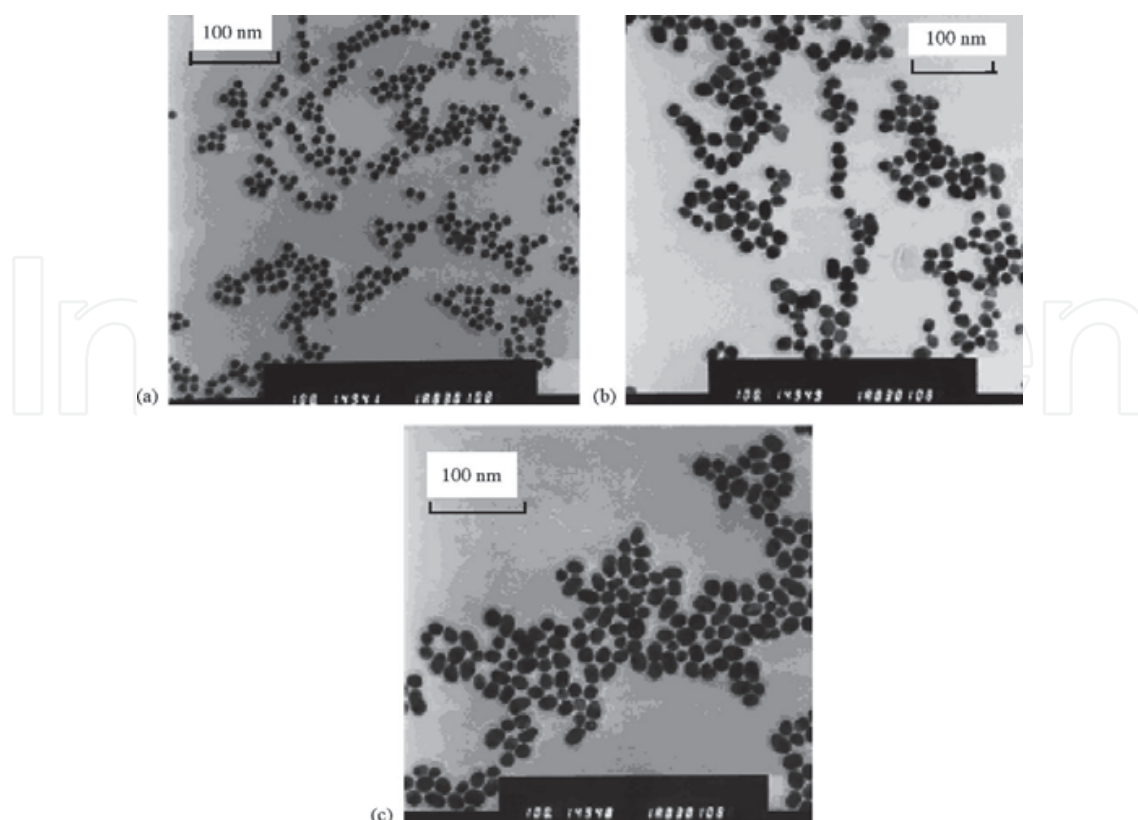


Fig. 10. TEM of gold nanoparticles with different size: 12 nm (a), 20 nm (b) and 35 nm (c) (Yang et al., 2006a).

6.5 Acetylcholinesterase biosensors

Carbamate and organophosphate pesticides have come into widespread use in agriculture because of their high insecticidal activity and relatively low environmental persistence. However, overuse of these pesticides results in pesticide residues in food, water and environment, and leads to a severe threat to human health due to their high toxicity to acetylcholinesterase (AChE), which is essential for the functioning of the central nervous system in humans. For these reasons, it has great significance to develop a fast, reliable and inexpensive analytical method for determination of trace amounts of these pesticides. Common analytical techniques for determination of these compounds, such as gas and liquid chromatography are sensitive, reliable and precise. However, these methods require expensive instrumentation, complicated pretreatment procedure and professional operators, which limit their application for real-time detection of these compounds. In order to simplify procedure and decrease cost, enzyme based biosensors could be a reliable and promising alternative to classical methods because of their simple fabrication, easy operation, high sensitivity and selectivity. It is well known that acetylthiocholine chloride (ATCl) can be catalytically hydrolyzed by AChE to thiocholine (TCh), which could be electrochemically oxidized at special potential. The hydrolysis reaction of ATCl would be inhibited by carbamate and organophosphorous pesticides, because AChE could irreversibly combine with these pesticides, which results in AChE inactivation to give low TCh concentration and low oxidation current. Therefore, based on the inhibition of carbamate and organophosphate pesticides on the AChE activity, the concentrations of

pesticides would be monitored by measuring the electrochemical oxidation peak current of TCh. The key aspect in construction of this kind of biosensor is the immobilization of AChE on the solid electrode surface with high electron transfer rate and bioactivity. In order to settle it, a variety of matrix materials have been employed, among them, GNPs have attracted enormous interest in the fabrication of electrochemical biosensors for possessing conductive sensing interface, catalytic properties and conductivity properties. Moreover, GNPs can provide an environment similar to that of proteins in a native system and allow protein molecules more freedom in orientation, which will reduce the insulating property of protein shell and facilitate the electron transfer through the conducting tunnel of GNPs. Gold nanoparticles were synthesized *in situ* and electrodeposited onto Au substrate (Dua et al., 2008). The GNPs modified interface facilitates electron transfer across self-assembled monolayers of 11-mercaptoundecanoic acid (MUA). After activation of surface carboxyl groups with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide, the interface displayed good stability for immobilization of biomolecules. The immobilized acetylcholinesterase (AChE) showed excellent activity to its substrate, leading to a stable AChE biosensor. Under the optimal experimental conditions, the inhibition of malathion on AChE biosensor was proportional to its concentration in two ranges, from 0.001 to 0.1 $\mu\text{g}\cdot\text{mL}^{-1}$ and from 0.1 to 25 $\mu\text{g}\cdot\text{mL}^{-1}$, with detection limit of 0.001 $\mu\text{g}\cdot\text{mL}^{-1}$. The simple method showed good reproducibility and acceptable stability, which had potential application in biosensor design.

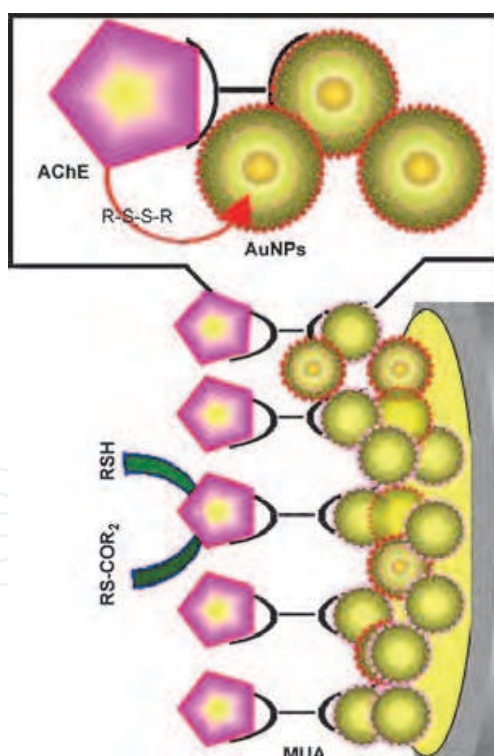


Fig. 11. Principle of GNPs served as mediator for electron transfer across SAMs for AChE biosensor design (Dua et al., 2008).

GNPs are particularly attractive for fabricating electrochemical sensors and biosensor. However, GNPs are inherently instable and apt to agglomerate. In order to settle this problem, it is necessary to use protective agents. SF is a natural protein, which can be

extracted from silkworm cocoon. Due to the unique properties of SF with thermal stability, nontoxicity, low cost and biocompatibility, it is widely used as a substrate for enzyme immobilization. Furthermore, GNPs could be in situ produced by the reduction of SF at room temperature, in which SF acts as both reducing agent and protector. It has been demonstrated that GNPs and SF could interact to form a bioconjugate, and this kind of GNPs-SF colloid possessed a stable and highly dispersed nature. A sensitive and stable amperometric biosensor for the detection of methyl paraoxon, carbofuran and phoxim had been developed based on immobilization of acetylcholinesterase (AChE) on gold nanoparticles and silk fibroin (SF) modified platinum electrode (Yin et al., 2009). The SF provided a biocompatible microenvironment around the enzyme molecule to stabilize its biological activity and effectively prevented it from leaking out of platinum electrode surface. In the presence of acetylthiocholine chloride (ATCl) as a substrate, GNPs promoted electron transfer reaction at a lower potential and catalyzed the electrochemical oxidation of thiocholine (TCh), thus increasing detection sensitivity. Under optimum conditions, the inhibition percentages of methyl paraoxon, carbofuran and phoxim were proportional to their concentrations in the range of 6×10^{-11} – 5×10^{-8} M, 2×10^{-10} – 1×10^{-7} M and 5×10^{-9} – 2×10^{-7} M, respectively. The detection limits were found to be 2×10^{-11} M for methyl paraoxon, 1×10^{-10} M for carbofuran and 2×10^{-9} M for phoxim. Moreover, the fabricated biosensor had good reproducibility and acceptable stability. The biosensor is a promising new tool for pesticide analysis.

A novel interface embedded in situ gold nanoparticles (GNPs) in chitosan hydrogel was constructed by one-step electrochemical deposition in solution containing tetrachloroauric (III) acid and chitosan (Du et al., 2007a). This deposited interface possessed excellent biocompatibility and good stability. The immobilized AChE, as a model, showed excellent activity to its substrate and provided a quantitative measurement of organophosphate pesticides involved in the inhibition action. Operational parameters, including the deposition time, tetrachloroauric (III) acid concentration have been optimized. Under the optimal electrodeposition, an amperometric sensor for the fast determination of malathion and monocrotophos, respectively was developed with detection limit of $0.001 \mu\text{g}\cdot\text{mL}^{-1}$. The simple method showed good fabrication reproducibility and acceptable stability, which provided a new avenue for electrochemical biosensor design.

6.6 Horseradish peroxidase

Over the last years, considerable efforts have been devoted to the development of horseradish peroxidase (HRP, EC 1.11.1.7, H_2O_2 oxidoreductase)-based mediatorless electrochemical biosensors for the fast, simple, selective and accurate quantification of H_2O_2 . This interest is justified by the industrial, chemical and biomedical applications of this oxidant compound. In addition, H_2O_2 constitutes a relevant biochemical mediator in many cellular processes, as well as a by-product of several oxidases with analytical applications. Different strategies has been described for connecting the catalytic active site of HRP with electrode surfaces, in order to construct such kind of third generation H_2O_2 biosensors in which the direct electron transfer between the enzyme and the electrode is allowed without the use of any natural or artificial redox mediator. Among these methods, it should be highlighted the use of electroconductive polymers (Zhaoyang et al., 2006, Luo et al., 2006, Mala Ekanayake et al., 2009), metal nanoparticles (Zhaoyang et al., 2006, Luo et al., 2006,

Mala Ekanayake et al., 2009, Jeykumari et al., 2008, Schumb et al., 1995, Ferreira et al., 2004, Alonso Lomillo et al., 2005, Pingarron et al., 2008), redox polymers and sol-gel materials (Wang et al., 2000, Jia et al., 2005, Garca et al., 2007), DNA (Song et al., 2006) and carbon nanotubes (Jeykumari et al., 2008) as wiring materials for HRP. On the other hand, the immobilization strategy to be employed is another key factor to consider in the design of an enzyme biosensor. This approach should favor the maintenance of the active enzyme conformation as well as provide a favorable hydrophilic microenvironment around the biocatalyst in order to contribute to the best catalytic performance of the enzyme (Song et al., 2006, Villalonga et al., 2007). In this regard, it has been previously reported the preparation of highly active and stable biocatalysts by the polyelectrostatic immobilization of enzymes in polysaccharide-coated supports (Gomez et al., 2006). In addition, several ionic polysaccharides such as sodium alginate (Camacho et al., 2007, Ionescu et al., 2006, Cosnier et al., 2004) and chitosan and its derivatives (Qin et al., 2006, Li et al., 2008), have been successfully used as coating materials for preparing robust enzyme biosensors. Horseradish peroxidase was cross-linked with cysteamine-capped Au nanoparticles and further immobilized on sodium alginate-coated Au electrode through polyelectrostatic interactions (Chico et al., 2009). The electrode was employed for constructing a reagentless amperometric biosensor for H₂O₂. The electrode showed linear response (poised at -400 mV vs. Ag/AgCl) toward H₂O₂ concentration between 20 μM and 13.7 mM at pH 7.0. The biosensor reached 95% of steady-state current in about 15 s, and its sensitivity was 40.1 mA/M.cm². The detection limit of the enzyme-based electrode was determined as 3 μM, at a signal-to-noise ratio of three. The electrode retained 97% of its initial analytical response after 1 month of storage at 4 °C in 50 mM sodium phosphate buffer, pH 7.0. The stability of the biosensor was significantly reduced when it was incubated in high ionic strength solutions, retaining only 44% of its initial response after 1 month of storage at 4 °C in 1 M NaCl ionic strength in 50 mM sodium phosphate buffer, pH 7.0.

The preparation of horseradish peroxidase (HRP)-GNPs-silk fibroin (SF) modified glassy carbon electrode (GCE) by one step procedure was reported (Yina et al., 2009). The enzyme electrode showed a quasi-reversible electrochemical redox behavior with a formal potential of -210mV (vs. SCE) in 0.1M phosphate buffer solution at pH 7.1. The response of the biosensor showed a surface-controlled electrochemical process with one electron transfer accompanying with one proton. The cathodic transfer coefficient was 0.42, the electron transfer rate constant was 1.84 s⁻¹ and the surface coverage of HRP was 1.8×10⁻⁹ mol.cm⁻². The experimental results indicated that GNPs-SF composite matrix could not only steadily immobilize HRP, but also efficiently retain its bioactivity. The biosensor displayed an excellent and quick electrocatalytic response to the reduction of H₂O₂.

A novel method for fabrication of horseradish peroxidase (HRP) biosensor has been developed by self-assembling gold nanoparticles on thiol-functionalized poly(styrene-co-acrylic acid) (St-co-AA) nanospheres (Xu et al., 2004). At first, a cleaned gold electrode was immersed in thiol-functionalized poly(St-co-AA) nanosphere latex prepared by emulsifier-free emulsion polymerization of St with AA and function with dithioglycol to assemble the nanospheres, then gold nanoparticles were chemisorbed onto the thiol groups. Finally, horseradish peroxidase was immobilized on the surface of the gold nanoparticles. The sensor displayed an excellent electrocatalytic response to reduction of H₂O₂ without the aid of an electron mediator. The sensor was highly sensitive to hydrogen peroxide with a detection limit of 4.0 μmol.L⁻¹, and the linear range was from 10.0 μmol.L⁻¹ to 7.0 mmol.L⁻¹.

The biosensor retained more than 97.8% of its original activity after 60 days of use. Moreover, the studied biosensor exhibited good current repeatability and good fabrication reproducibility.

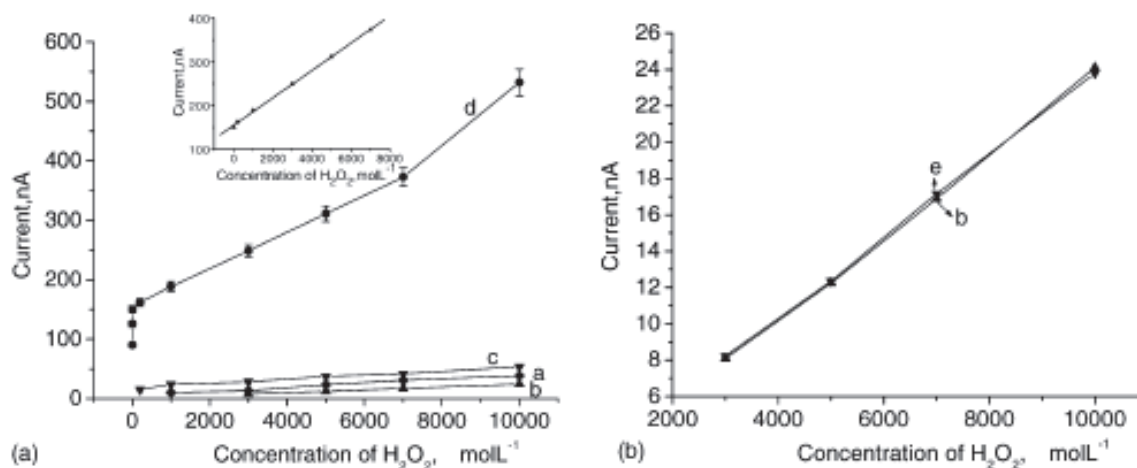


Fig. 12. Steady-state amperometric responses of electrodes to the reduction of H₂O₂ in the stirring PB under elimination of oxygen: (a) the non-modified gold electrode; (b) the latex modified electrode; (c) the gold nanoparticle modified electrode before HRP addition; (d) the gold nanoparticle modified electrode after HRP addition; (e) the latex modified electrode after HRP addition; Applied potential, -200mV ; supporting electrolyte, 100 mmol.L^{-1} pH 7 (Xu et al., 2004).

A one-step method for fabrication of horseradish peroxidase (HRP) biosensor has been developed (Di et al., 2005). The gold nanoparticles and HRP were simultaneously embedded in silica sol-gel network on gold electrode surface in the presence of cysteine. The immobilized HRP exhibited direct electrochemical behavior toward the reduction of hydrogen peroxide. The heterogeneous electron transfer rate constant was evaluated to be 7.8 s^{-1} . The biosensor displayed an excellent electrocatalytic response to the reduction of H₂O₂ without any mediator. The calibration range of H₂O₂ was from $1.6\text{ }\mu\text{mol.L}^{-1}$ to 3.2 mmol.L^{-1} and a detection limit of $0.5\text{ }\mu\text{mol.L}^{-1}$ at a signal-to-noise ratio of 3. The biosensor exhibited high sensitivity, rapid response and long-term stability.

The design and development of a screen printed carbon electrode (SPCE) on a polyvinyl chloride substrate as a disposable sensor is described (Tangkuaram et al., 2007). Six configurations were designed on silk screen frames. The SPCEs were printed with four inks: silver ink as the conducting track, carbon ink as the working and counter electrodes, silver/silver chloride ink as the reference electrode and insulating ink as the insulator layer. Selection of the best configuration was done by comparing slopes from the calibration plots generated by the cyclic voltammograms at 10, 20 and 30mM K₃Fe(CN)₆ for each configuration. The electrodes with similar configurations gave similar slopes. The 5th configuration was the best electrode that gave the highest slope. Modifying the best SPCE configuration for use as a biosensor, horseradish peroxidase (HRP) was selected as a biomaterial bound with gold nanoparticles in the matrix of chitosan (HRP/GNP/CHIT). Biosensors of HRP/SPCE, HRP/CHIT/SPCE and HRP/GNP/CHIT/SPCE were used in the amperometric detection of H₂O₂ in a solution of 0.1M citrate buffer, pH 6.5, by applying a potential of -0.4 V at the working electrode. All the biosensors showed an immediate

response to H_2O_2 . The effect of HRP/GNP incorporated with CHIT (HRP/GNP/CHIT/SPCE) yielded the highest performance. The amperometric response of HRP/GNP/CHIT/SPCE retained over 95% of the initial current of the 1st day up to 30 days of storage at 4 °C. The biosensor showed a linear range of 0.01–11.3 mM H_2O_2 , with a detection limit of 0.65 μM H_2O_2 (S/N = 3). The low detection limit, long storage life and wide linear range of this biosensor make it advantageous in many applications, including bioreactors and biosensors.

6.7 DNA biosensors

DNA biosensors for the detection of nucleic acid sequences have attracted ever increasing interests in connection with highly demanding research efforts directed to gene analysis, clinical disease diagnosis, or even forensic applications (Service, 1998, Butler, 2006, Staudt, 2001, Farace et al., 2002, Reisberga et al., 2006). Various techniques including optical, electrochemistry, surface plasmon resonance spectroscopy, and quartz crystal microbalance, etc have been well developed for DNA detection (Rosi & Mirkin, 2005, Gerion et al., 2003, Drummond et al., 2003, He et al., 2000). Among them, electrochemistry offer great advantages such as simple, rapid, low-cost and high sensitivity (Lao et al., 2005). A key issue faced with any DNA hybridization biosensor is the immobilization amount and accessibility of probe DNA for hybridization recognition (Moses et al., 2004, Lowe et al., 2003, Ding et al., 2008, Ostatná et al., 2005). Increasing the immobilization amount and controlling over the molecular orientation of probe DNA would markedly improve the performance properties of DNA biosensor. It has been well elaborated that the immobilization amount and the molecular orientation of probe single-stranded DNA could remarkably influence the operational performance of DNA electrochemical biosensor (Liu et al., 2008, Basuray et al., 2009). Therefore, numerous different immobilization strategies have been proposed and employed aimed at improving the link stability between DNA and transducer surface (Cederquist et al., 2008), or increasing the amount of immobilized DNA (Liu et al., 2005), and sometimes simplifying the immobilization procedure (Kjllman et al., 2008). In order to achieve this goal, nanomaterials could be used as an elegant solution for the control of DNA immobilization and hybridization. For a decade, metal nanoparticles have shown huge potential in the fields of biosensing, diagnostics and molecular therapeutics because of its excellent optical and electrical properties (Brown et al., 1996, Bao et al., 2003, Ma et al., 2004, Kidambi et al., 2004). Owing to the large surface area and biocompatibility with biosystem, gold nanoparticles have been shown as a good candidate for enhancement of DNA immobilization and hybridization and they have been directly linked onto the biosensor surface via various strategies such as covalent linking, electrodeposition, electroless deposition, sol-gel, etc (Li et al., 2007, Yamada et al., 2003, Zhao et al., 2007, Jena & Raj, 2007). The self-assembly of GNPs on the electrode surface could be easily achieved via the use of a bi-functional chemical linking agent such as 1,6-hexanedithiol, cysteamine. Although these self-assembly methods are very simple and rapid, the formed monolayer on the electrode surface are usually insulated or could not offer a good electrical conductivity between GNPs and electrode surface, which is not especially favorable for the fabrication of electrochemical sensor or biosensor.

A novel protocol for development of DNA electrochemical biosensor based on GNPs modified glassy carbon electrode (GCE) was proposed (Li et al., 2011), which was carried out by the self-assembly of GNPs on the mercaptophenyl film (MPF) via simple

electrografting of in situ generated mercaptophenyl diazonium cations. The resulting MPF was covalently immobilized on GCE surface via C-C bond with high stability, which was desirable in fabrication of excellent performance biosensors. Probe DNA was self-assembled on GNPs through the well-known Au-thiol binding. The recognition of fabricated DNA electrochemical biosensor toward complementary single-stranded DNA was determined by differential pulse voltammetry with the use of Co(phen)_3^{3+} as the electrochemical indicator. Taking advantage of amplification effects of GNPs and stability of MPF, the developed biosensor could detect target DNA with the detection limit of 7.2×10^{-11} M, which also exhibits good selectivity, stability and regeneration ability for DNA detection. DNA biosensor which was based on the self-assembly of GNPs on the mercapto-diazoaminobenzene monolayer modified electrode was also reported (Liet al., 2010a). The mercapto-diazoaminobenzene monolayer was obtained by covalent immobilization of 4-aminothiophenol (4-ATP) molecules onto another 4-ATP monolayer functionalized gold electrode by diazotization-coupling reaction. The DNA immobilization and hybridization on the GNPs modified electrode was further investigated. The prepared GNPs-ATP-diazo-ATP film demonstrated efficient electron transfer ability for the electroactive species toward the electrode surface due to a large conjugated structure of the mercapto-diazoaminobenzene monolayer. The recognition of fabricated electrochemical DNA biosensor toward complementary single-stranded DNA was determined by differential pulse voltammetry with the use of Co(phen)_3^{3+} as an electrochemical indicator. A linear detection range for the complementary target DNA was obtained from 3.01×10^{-10} to 1.32×10^{-8} M with a detection limit of 9.10×10^{-11} M. The fabricated biosensor also possessed good selectivity and could be regenerated easily.

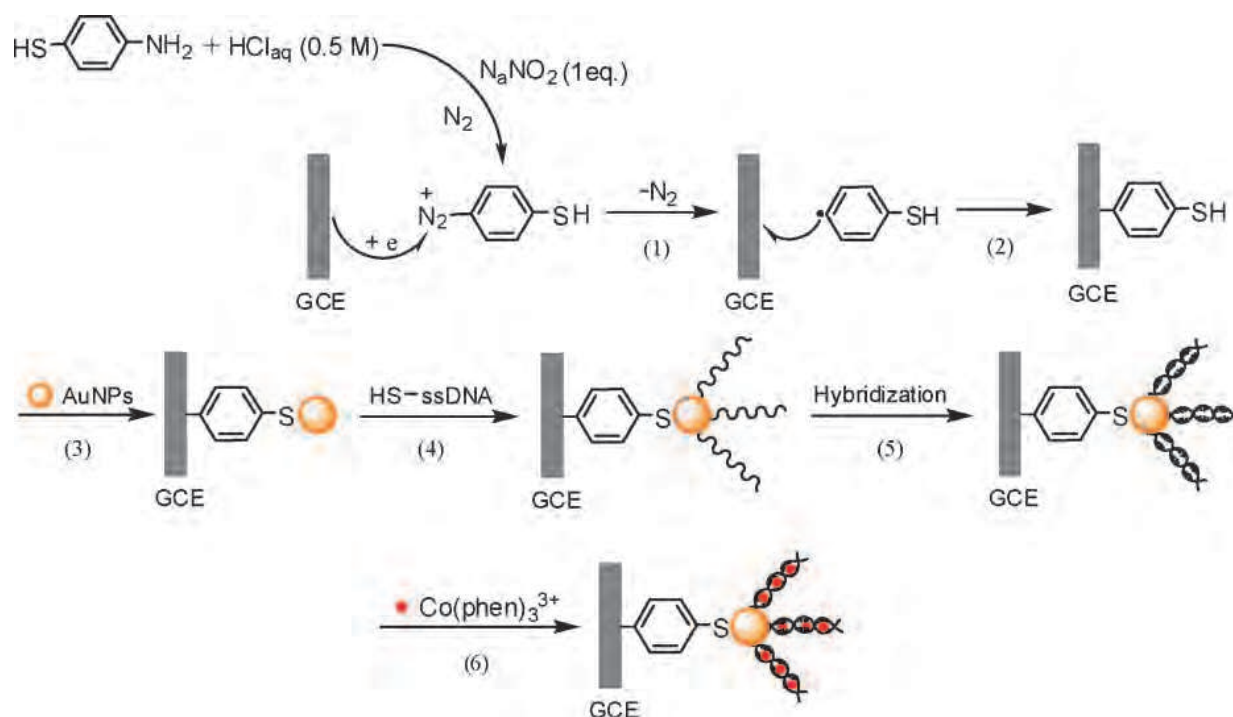


Fig. 13. Schematic representation of the fabrication of DNA biosensor (Li et al., 2011).

Colloidal gold nanoparticles and carboxyl group-functionalized CdS Nanoparticles (CdS NPs) were immobilized on the Au electrode surface to fabricate a novel electrochemical

DNA biosensor (Du et al., 2009). Both GNPs and CdS NPs, well known to be good biocompatible and conductive materials, could provide larger surface area and sufficient amount of binding points for DNA immobilization. DNA immobilization and hybridization were characterized with differential pulse voltammetry (DPV) by using $[\text{Co}(\text{phen})_2(\text{Cl})(\text{H}_2\text{O})]\text{Cl} \cdot 2\text{H}_2\text{O}$ as an electrochemical hybridization indicator. With this approach, the target DNA could be quantified at a linear range from 2.0×10^{-10} to 1.0×10^{-8} M, with a detection limit of 2.0×10^{-11} M by 3σ . In addition, the biosensor exhibited a good repeatability and stability for the determination of DNA sequences.

Layers for DNA immobilization	Detection limit (mol.L ⁻¹)
Au and CdS NPs (Du et al., 2009)	2.0×10^{-11}
LBL Au NPs and MWCNTs (Ma et al., 2008)	7.5×10^{-12}
CNTs (Niu et al., 2011)	1.4×10^{-10}
Pt NPs and CNTs (Zhu et al., 2005)	1.0×10^{-11}
ZrO ₂ /SWNTs/PDC/GCE (Yang et al., 2007)	1.38×10^{-12}
Multilayer gold nanoparticles (Tsai et al., 2005)	1×10^{-11}
Conducting polyaniline nanotube (Chang et al., 2007)	3.759×10^{-14}
CdS nanoparticles and polypyrrole (Peng et al., 2006)	1×10^{-9}

Table 4. The performance comparison of various fabricated DNA biosensors (Du et al., 2009).

7. Conclusion

Nanotechnology has been widely and successively applied in the field of sensing of drugs and biological molecules. The most important example of nanosensors are gold nanoparticles (GNPs) which offer many advantages, such as large surface-to-volume ratio, high surface reaction activity and strong adsorption ability to immobilize the desired biomolecules, good microenvironment for retaining the activity of enzyme, excellent catalytic effects on many important chemical reactions, their catalytic effect is highly size-dependent thus, the unique active sites and electronic states of GNPs can lead to their anomalous catalytic activity.

The biomaterials to be sensed include a large variety of materials such as:

1. Glucose and cholesterol which are largely attributed to the human health and the food industry.
2. Phenolic compounds whose identification and quantification are very important for environment monitoring.
3. Some carbamate and organophosphate pesticides which affect food, water and environment, and leads to a severe threat to human health.
4. H₂O₂ whose quantification is justified by the industrial, chemical and biomedical applications of this oxidant compound. In addition, H₂O₂ constitutes a relevant biochemical mediator in many cellular processes, as well as a by-product of several oxidases with analytical applications.
5. DNA and nucleic acids sequences detection which are directed to gene analysis, clinical disease diagnosis, or even forensic applications.

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Fig1	(http://www.lsbu.ac.uk/biology/enztech/biosensors.html)
Table1	Not published
Fig2	Not published
Table2	2634100245226
Table3	2634101412860
Fig3	2633701365374
Fig4	2633701475869
Fig5	2634161086239
Fig6	2633710689524
Fig7	2633710837251
Fig8	2634150357126
Fig9	2633710969058
Fig10	2633711095490
Fig11	2633711408445
Fig12	2633720091311
Fig13	2633720380564
Table4	2634110691438

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Biosensors for Health, Environment and Biosecurity

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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 16 different countries. The book consists of 24 chapters written by 76 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

How to reference

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