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Nanostructured Metal Oxides Based Enzymatic Electrochemical Biosensors

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1. Introduction

Studies of nanobiosensors based on semiconductor nanostructured metal oxides are of practical and theoretical importance in biological science, environmental science and analytical chemistry (Wang et al., 2005; Luo et al., 2006; Valentini & Palleschi (2008); Chopra et al., 2007). These one-dimensional nanostructured metal oxides have profound applications in optics, optoelectronics, sensors, and actuators duo to their semiconducting, piezoelectric, and pyroelectric properties (Wang et al., 2005; Chopra et al., 2007; Kerman et al., 2008; Chow et al., 2005). Nanostructured metal oxides not only possesses high surface area, nontoxicity, good biocompatibility and chemical stability, but also shows fast electron communication features made the materials to be able to function as biomimetic membrane material to fix and modify proteins (Wang et al., 2005; Chopra et al., 2007; Valentini & Palleschi (2008)). These biomimetic and high electron communication features, high surface to volume ratio and electro-catalytic activity of the nanosized materials make them ideal as immobilization matrices, as transduction platform and/or mediators. Stability, sensitivity, selectivity and other analytical characteristics of biosensors are essential features to design desirable microenviroment for the direct electron transfer between the enzyme's active sites and the electrode. To improve these characteristics various conventional materials matrices have been proposed. Among them nanostructured metal oxides matrices not only retain the bioactivity of the immobilized enzyme but also enhanced the sensing characteristics such as sensitivity, selectivity and low detection limit of the fabricated amperomatric enzymatic biosensors. Morphology of the nanosized material is one of the most ideally suited important factor to determine the properties for biosensor applications since they are conductive, biocompatible, easily functionalized while they have very large surface area. Nanosized metal oxides based electrochemical enzymatic biosensors have active surfaces that can easily be modified for immobilization of biomolecules. However, this advantage may not apply to many non-oxide semiconductor nanomaterials because their surfaces are not stable in an air environment, which leads to formation of an insulating native oxide layer and may degrade device reliability and sensitivity.

Whereas, nanostructured metal oxides based electrochemical transducer surfaces promote the direct electron transfer reactions, amplify and orient the analytic signal of the biorecognition events. When a redox protein is immobilized on a biocompatible metal oxide electrode surface, it will exhibit reasonably fast electron transfer kinetic and permit the

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electrochemical measurement of its substrate without addition of a mediator to the analyzed solution. Since the direct contact of redox protein with metal oxide surfaces usually leads to significant changes in protein structure, function and increased the bioactivity of the electrochemical enzymatic transducer. These electrochemical transducers are regarded as particularly suitable for direct and fast biosensing since they can convert the biological recognition event into a direct electrical signal. This means, that there is no need for complex signal transduction equipment and the detection can be accomplished with an inexpensive electrochemical analyzer. Electrochemical biosensing approaches include the intrinsic electroactivity of redox enzyme, electrochemistry of nanoparticles and metal oxide nanoparticles. Recently electrochemical enzymatic transducers have received considerable popularity in connection to the simple detection of analytes (proteins and enzymes), because of their rapid, high sensitivity, portability, low cost (disposability), simpler and minimal power requirements make them excellent candidate for analyte detection.The high sensitivity and wide linear range of such devices have opened new door in widespread biomedical applications such as clinical diagnosis (for cancer diagnostics and detection of infectious organisms), in environmental monitoring (for pesticide detection, heavy metal–trace pollutant quantification, and genotoxic molecules), and finally in food quality control (for genetically modified organisms-GMOs-and several food toxins). In this article, we describe the importance of nano-structured metal oxides for fabrication of amperomatric of amperometric and voltammetric electrochemical enzymatic biosensors.

2. Synthesis and properties of nanostructured metal oxides

For the synthesis of nanostructured metal oxides various physical and chemical routes have been developed to determine their morphological properties and possible applications in biosensors (see in Table 1). Physical methods, which often involve vapor deposition, or ball milling process require subdivision of bulk precursors to nanoparticles. Chemical procedures start from reduction of metal ions to metal atoms, followed by controlled aggregation and separation of atoms from the bulk. The chemical methods or solution based chemistry methods are quit more suitable to obtaining small, uniform nanoparticles such as nanorods, nanowires, nanofibers, nanobelt, nanocomb, nanotubes and porous materials than the former for the particular biosensor applications of the nanomaterials.

The properties of the nanoparticles generally depend on size, shape and stabilizing agents, which are controlled by the preparation conditions. Moreover, the size and the uniformity of the nanoparticles depend on the kind and the amount of reducing agent employed in synthesis. Recently, the use of MnO_2 nanoparticles to construct an interface for direct electron transfer of redox proteins and retention of bioactivity is being extensively reported. One dimensional (1D) nanostructures (including rods, wires, belts, and tubes) provide a better model system for investigating the dependence of electronic transport, optical and mechanical properties on size confinement and dimensionality. They are also expected to play an important role as both interconnects and functional units in fabricating electronic, optoelectronic, electrochemical, and electromechanical devices with nanoscale dimensions. Considering the importance of metal oxides in catalysis, electrochemistry, functional ceramics, and sensors, their fabrication in nanostructured form with anisotropic morphology appears to be a particularly attractive goal and has been pursued in a number

Nanostructured Processing methods			
SN	**Physical vapour deposition Methods**	**Chemical vapor deposition methods**	**Solution based chemistry methods**
1	Pulse laser deposition	Thermal and Low Pressure Chemical Vapor Deposition	Sonochemical method
2	Thermal Evaporation	Plasma-Enhanced Chemical Vapor Deposition	Sol-gel technique
3	Rf magnetron sputtering	Metal-Organic Chemical Vapor Deposition	Microemulsion process
4	Flame pyrolysis	Molecular Beam Epitaxy	Hydrothermal/solvothermal methods
5	Laser ablation	Atomic Layer Deposition	Supercritical fluid precipitation process
7	Mechanical alloying techniques		Homogeneous/heterogeneous precipitation
8	Mechanical milling		Electrochemical deposition process

Table 1. General applied synthesis methods of nanostructured materials

of laboratories. Much effort has been devoted to synthesized, characterized the novel properties and application of metal oxides such as nanofibers, nanorods, nanowires, and nanotubes. When developing a synthetic method for generating nanostructures, the most important issue that one need to address is the simultaneous control over dimensions, morphology (or shape), and mono-dispersbility (or uniformity).

3. Electrocatalytic behavior of nanostructured metal oxides in the development of biosensor

Numerous papers have been published in the literature indicating nanostructured metal oxides as a convenient component, forming an appropriate environment for the immobilization of enzyme at the electrode surface and its interaction with metallic or conducting electrode surface. Stable immobilization of macromolecular biomolecules on semiconducting metal oxide nano-surface with complete retention of their biological recognition properties is a crucial problem for the commercial development of miniaturized biosensor. Owing to large specific surface area and high surface free energy of nanoparticles can absorb enzymes strongly and play an important role in the immobilization of enzymes in construction of biosensor devices. Generally, the adsorption of enzymes directly onto naked surfaces of bulk materials may frequently result in their denaturation and loss of bioactivity. However, the adsorption of such enzymes onto the surfaces of nanoparticles can retain their bioactivity because of biocompatibile nature of metal oxides nanoparticles. Since most of the metal oxide nanoparticles carry high isoelectric point (>IEP), they can

electrostatically adsorb enzymes with different charges with the low isoelectric point enzymes or protiens. Wang et al., (2006) have applied ZnO nanocomb as a glucose sensing matrix that is most frequently used nanomaterial because they have high isoelectric point (IEP~9.5). Therefore, low isoelectric point enzymes and protein strongly adsorbed on the nanostructured metal oxide surface and provides direct electron communication between the enzymes and conducting electrode. For example, it is reported that ZnO nanorods (Wei et al., 2006) have high isoelectric point (IEP~9.5), it is suitable for immobilization of low isoelectric point enzymes such as glucose oxidase (GOx) or (IEP~4.2), cholesterol oxidase etc. The positively charged ZnO nanorods matrix not only provides a friendly microenvironment for immobilization of negatively charged GOx and retain its bioactivity, but also promotes electron transfer between GOx and the electrode to a large extent. Ansari et al., (2008) have immobilized GOx and cholesterol on sol-gel derived nanostructured CeO_2 film deposited on indium-tin-oxide (ITO) glass plate for fabrication of glucose and cholesterol biosensors, respectively. Topoglidis et al.,(2000) firstly employed titanium nanoparticles for successfully immobilization of horseradish peroxidase to construct the H_2O_2 biosensor. Several investigators have studied the influence of nanoparticle size on the performance of the prepared biosensors and nanoparticles with smaller size were found to be more suitable for enzyme immobilization. Many similar studies have been reported for the construction of biosensors based on the immobilization of different enzymes with nanostructured metal oxides, such as glucose oxidase, cholesterol oxidase, urease, HRP, myoglobin, hemoglobin, cytochrome C, and tyrosinase etc. Other nanostructured metal oxides, such as CeO_2, SnO_2, Fe_3O_4, MnO_2, Pr_6O_{11}, Sb_2O_3, TiO_2, ZnO and ZrO_2 nanoparticles have been used for the immobilization of enzymes for sensitive bioelectronic device development.

Electrical contacting of redox-enzymes with electrodes is a key process in the construction of third-generation enzyme electrodes. While enzymes usually lack direct electrical communication with electrodes due to the fact that the active centers of enzymes are surrounded by considerably thick insulating protein shells, and the electron transfer between electrodes and the active centers are blocked, the electro-catalytic properties of nanoparticles, mostly metal oxide nanoparticles at nanoscale dimensions made them suitable for enhancing the electron transfer between the active centers of enzymes and electrodes acting as electron transfer “mediators” or “electrical wires”. The conductance of metal oxides nanoparticles depend on their crystalline structure. It has been reported that the conductances of nanowires, nanotubes, nanoribbons, nanorods and nanofibers was superior in respect to other morphological nanomaterials. Due to the high electrical conductivity of the resulting dimension nanostructured metal oxides, these materials constructed sensors amplify the signal-to-noise ratio and the sensitivity by more than one order of magnitude compared to that observed at bulk materials electrodes. This improved analytic performance was due to both factors, such as the high enzyme loading and a better electrical communication ability of the nano-morphological structure and the active center of the biomolecules. This is known as the direct electron transfer ability of nanowires, nanotubes, nanorodes and nanofibers demonstrated that both flavin adenine dinucleotide(FAD) and glucose oxidase (GOx) were found to spontaneously adsorb (physical adsorption) to metal oxides nanoparticles (deposited on the conducting glass

electrodes by different casting process) and to display quasi-reversible one electron transfer reaction.

Several electroanalytical techniques such as cyclic voltammetry, pulse and square-wave voltammetry and electrochemical impedance spectroscopy have been employed to analyze the electron transport phenomena such as charge transfer, diffusional mass transport, adsorption, chemisorption, chemical reaction, and convection. Electroanalytical techniques can provide information on chemical, biochemical, and physical systems, which can supplement spectroscopic characterization and perhaps theoretical calculations. Since last decade, it was interesting to find that direct electron transfer of some redox proteins can also take place with the help of metal oxides nanoparticles without need for additional mediators. Modification of electrode surfaces with nanoparticles provides a microenvironment similar to that of the redox proteins in native systems and gives the protein molecules more freedom in orientation, thereby reducing the insulating effect of the protein shell for direct electron transfer through the conducting tunnels of metal oxide nanoparticles.

4. Performance of nanostructured metal oxide based enzymatic electrochemical biosensors

Because of novel electrocatalytic behavior of nanostructured metal oxide, which combines characteristics of electrochemistry (e.g., simplicity, fast, high sensitivity & selectivity and low detection limit). Metal oxide nanoparticles exhibit higher ratios of surface area to volume than their bulk counterparts, so metal oxide nanoparticle modified electrochemical interfaces will provide larger electrochemically active areas and therefore probably lead to higher detection sensitivity for target molecules. Some novel nanoparticles, particularly manganese oxide (MnO_2) nanoparticles, can easily act as enhancing agents for effective acceleration of electron transfer between electrode and detection molecules, so leading to more rapid current response for target molecules. Superparamagnetic iron oxide nanoparticles act as a supramolecular assembling unit with advanced functional properties for constructing a variety of architectures on the surface of electrodes and further tailoring of an electrochemical-sensing interface. These metal oxide nanoparticles conjugate with some important biomolecules (e.g., redox enzyme) and act as nano-connectors that activate redox enzymes or electrical labels for biorecognition events. Furthermore, metal oxide nanoparticles modified electrochemical interfaces behave as nanoelectrode ensembles; in principle, the electroanalytical limit of detection at a nanoelectrode ensemble can be much lower than that at an analogous macrosized electrode because, there is a bigger ratio between Faradaic and capacitive currents. Inspired by the important features of nanostructured metal oxide has been in full flourish recently and a great deal of literature has reported that metal oxide-modified interfaces have enhanced electrochemical signaling. Nanostructured metal oxide based enzymatic biosensors have excellent prospects for interfacing biological recognition events with electronic signal transduction so as to design a new generation of bioelectronic devices with high sensitivity. Indeed, there has been substantial progress in the past decade on enzymatic electrochemical biosensors. In this article, we have collected the recent research activities on nanostructured metal oxide based enzymatic electrochemical biosensors.

4.1 Glucose biosensors

For determination of glucose, the reaction with glucose oxidase (GOX) enzyme electrode is shown with the following equations:

$$\beta\text{-glucose} + O_2 \rightarrow \beta\text{-gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H+ + 2e^- \quad (2)$$

The amount of H_2O_2 produced in equation (1) is usually determined by amperometric method by oxidation at the working electrode according to equation (2). This is referred as an amperometric biosensor. Equation (2) shows that protons are produced in the follow-up oxidation reaction. The production amount of H_2O_2 is usually detected by measuring the current during the oxidation reaction in equation (2). GOx carried a negative charge normally immobilized by physically onto nanostructured metal oxide films fabricated by solution based chemistry or physical methods. These metal oxides with nanostructure could provide large surface to volume ratio and increased surface activity, making their unique advantages over other conventional materials for enzym immobilization and signal transduction. They could keep activity of enzyme due to the desirable microenvironment and enhance the direct electron transfer between the enzyme's active sites and the electrode. It has been reported that the glucose biosensor based on nanostructured metal oxides has a high sensitivity, selectivity and fast response with low detection limit for hydrogen peroxide as compared to the other conventional materials, as discussed in the following reports. Kumar et al., (2008) have collected the applications of nanostructured ZnO for construction of electrochemical glucose biosensors, which are successfully applied in the development of a new glucose sensing platform for electrochemical glucose detection. As discussed earlier, nanostructured metal oxide platform possess several unique advantages such as high specific surface area, nontoxicity, chemical stability, electrocatalytic activity, and high electron communication features. The large specific surface energy and high electron communication features of the nanosized ZnO enhanced the electrochemical sensitivity of biomolecular reaction and rapid response with low detection limit. Nanosize metal oxide enhanced electrochemical sensitivity is based on the discovery that immobilized metal membranes either as a continuous film, particles, colloid or monolayer significantly amplifies the electrochemical signals following molecular recognition at enzyme electrodes. Wang and his co-workers (2006) synthesized ZnO nanocomb by vapor phase transport method to immobilization of GOx for sensitive detection of glucose. The electron transfer pathways between immobilized enzyme (GOx) molecules which are integrated with nanocomb ZnO. They suggetsted that, electrons are directly transferring from electrode to redox enzyme *via* nanosized ZnO nanocomb. The nanozied nanocomb provides a channel to communicate the electrons from enzyme to electrode surface. To understand of these pathways are very important to construct the nanostructured metal oxide based electrochemical enzymatic biosensors. The developed ZnO nanocomb glucose biosensor shows high sensitivity (15.33 $\mu A/cm_2$ mM) for glucose detection and high affinity of GOx to glucose (the apparent Michaelis-Menten constant Km^{app} = 2.19 mM) with low detection limit measured upto 0.02 mM. In another report, Wei et al., (2006) grows ZnO nanorods on standard gold electrode for use as an electrochemical biosensing interface at low potential

(+0.8V). Electrochemical characterization of the ZnO nanorod-GOx-modified system retains its bioactivity and can specifically catalyze the oxidation of glucose. Interestingly, it was found that the ZnO nanorods have high affinity with enzyme (GOx, the apparent Michaelis-Menten constant *Km* = 2.9 mM) for higher loading of enzyme. This biosensor was highly reproducible sensitive (23.1 μA cm^{-2} mM^{-1}), selective and shows rapid response (5 s) toward glucose with a linear range covered from 0.01 to 3.45 mM of glucose and an experiment limit of detection of 0.01 mM. Zang et al., (2007) have immobilized GOx by physically and chemically onto ZnO nanowires for construction of high performance glucose sensor. The extended bioaffinity of enzyme with ZnO nanowires could be achieved by selecting the right metal oxide film thickness on the electrode surface, because in the presence of a thicker metallic layer, a diffusion barrier toward the glucose oxidation was observed. This effect produces the ease bioaffinity of glucose for the low loading of enzyme and avoiding the high detection limit of analyte. Moreover, this glucose sensor showed long term stability with the incorporation of the inorganic zinc oxide nanowire. Zhao et al., (2007) proposed new strategy for co-adsoprtion of GOx onto porous Co doped ZnO nanoclusters with an averaged particle size of 5 nm to construct a novel amperometric glucose sensing. Electrochemical characterization of the developed ZnO-based nanoclusters electrode shows nanoporous network structure, making them differ from undoped ZnO nanostructures and other low dimensional nanostructures. ZnO:Co nanoclusters-GOx system exhibited high electrocatalytic activity to impart high sensitivity(13.3 μA/mA cm^2), selectivity and low detection limit (20 μM).

Based on these electrocatalytic properties of the metal oxides, Li et al., (2001) applied porous nanocrystalline TiO_2 for immobilization of GOx to electrochemically detection of glucose. Transmission electron microscopy, Infrared and Raman spectroscopy were employed to analyze the morphology of the material and interaction of the enzyme with nanocrystalline TiO_2. The resulting biosensor shows rapid, stable and linear response in the concentration range of 0±3mM with apparent Michaelis-Menten constant *Km* of 6.08 mM.

Umar et al., (2009) synthesized flower-shaped copper oxide nanostructured by simple low-temperature hydrothermal process and used to fabricate highly sensitive amperometric glucose biosensor. They found the linear dynamic range from 0.01 to 10.0 mM with detection limit upto 1.37 μM. The proposed glucose sensor exhibits reproducible sensitivity (47.19 $\mu AmM^{-1} cm^{-2}$) and fast response time less than 5 s.

Ansari et al., (2008) have developed a simple, highly reproducible sensitive and selective method for electrochemical detection of glucose using by sol-gel derived nanostructured CeO_2 film deposited onto gold electrode surface. The fabricated biosensor show linear amperomatric response (50–400 mg/dL) with low detection limit (12.0 μM). Nanoporous sol-gel film provides higher loading and strong bioaffinity (*Km* = 13.55 μM) of the enzyme to enhance the detection limit as well as shelf-life (12 weeks) of the bioelectrode. In another approach (Saha et al., 2009), similar group has developed nanoporous CeO_2 film onto platinum electrode by pulse laser deposition method for immobilization of GOx to sensitive detection of glucose. Atomic force microscopy images were used to investigate the surface texture of the electrode surface before and after enzyme immobilization, which is porous in nature. The biosensor has linearity within the concentration range from 25–300 mg/dl.

In order to explore applications of nanosized metal oxides for construction of electrochemical biosensors Yang et al., (2004) employed ZrO_2/Chitosan nanoporous matrix

for adsorption of GOx to detect the glucose concentration. Electrochemical characterization demonstrated that chitosan membrane enhanced the adhesive ability to the electrode, which is increasing the enzyme affinity to the biometallic surface. The linearity of this biosensor was within the concentration range from 1.25×10^{-5} to 9.5×10^{-3}M. This novel biosensor exhibited quite high response sensitivity (0.028 μAmM^{-1}) and low detection limit (1.0×10^{-5}M) useful for potential applications. In order to overcome the insolubility of the nonconducting biopolymer chitosan Kim et al., (2006) had employed Nafion to improve the adhesive ability as well higher enzyme loading. This bioelectrode show better stability for enzyme immobilization and detect the glucose concentration within the range of 0.03-15.08 mM with a sensitivity of 3.40 μA/mM and the detection limit of 0.037 mM**.**

Ansari et al., (2009) prepared sol–gel derived nanostructured tin oxide film onto indium-tin-oxide (ITO) for glucose sensing. High bioaffinity of the enzyme (GOx) was observed to the sol-gel derived nanostructured tin oxide electrode which can be attributed to favorable conformation of GOx and higher GOx loading due to microenvironment of nanoporous sol–gel derived Nano-SnO_2 film. The proposed sensor was highly sensitive (2.687 $\mu Amg/dLcm^2$) and selective toward glucose sensing, which exhibits wide linearity concentration rage (10–300 mg/dL) and low detection limit (0.169 mg/dL).

Another strategy was proposed to employed nanomaterials for construction of enzymatic biosensors for rapid, selective and highly reproducible sensitive detection of glucose concentrations from serum samples. Chen et al., (2008) have coupled the MnO_2 nanoparticles with MWCNTs resulted in remarkable improvement of the electrocatalytic activity of the nanocomposite materials toward the electrode surface through synergistic effect. The proposed biosensor permits effective low potential amperometric detection. The biosensor exhibits excellent response performance to glucose with the linear range upto 28 mM with a sensitivity of 33.19 μAmM^{-1}. In addition, chitosan was introduced into the MnO_2 nanoparticles and electrochemically deposited on the electrod to immobilization of GOx for sensing glucose (Xu et al., 2004). Furthermore, the biosensor shows rapid response, high sensitivity, good reproducibility, long-term stability, and freedom of interference from other coexisting electroactive species.

Another approach in the electrochemical sensor field concerns the assembling of a sensing platform based on the magnetic loading of ferrocene modified $Fe_3O_4@SiO_2$ nanocomposite on electrode surface. The resulting magnetic nanocomposite brings new capabilities for electrochemical devices by combining the advantages of ferrocene and $Fe_3O_4@SiO_2$ nanocomposite provides an alternative way for loading ferrocene on electrodes. Electrochemical measurements Indicated that ferrocene incorporated electrod exhibited higher electrocatalytic activity toward the redox processes of H_2O_2. The response for glucose was improve because of the presence of ferrocene in the nanocomposite electrode, higher sensitivity and extended linear range of response from 1.0×10^{-5} to 4.0×10^{-3} M with a detection limit of 3.2 μM observed. Other glucose sensing applications of superparamagnetic iron oxide nanoparticles have been reported in literature (Wang et al., 2006), such biosensor based on carbon nanotube (CNT)/Fe_3O_4 dispersed into chitosan, successfully used to detect glucose (Qu et al., 2007). It has been found that, CNTs enhanced the electroactive surface area for higher enzyme loading. Another report this group proposed polyaniline coated CNT/Fe_3O_4 nanocomposite for glucose sensing (Liu et al., 2008). Due to the ability of carbon nanotubes to promote electron-transfer reactions, the high

conducting properties of polyaniline and the sensitivity of presented electrochemical glucose biosensors were remarkably improved. It was found that CNTs can promote the electroactive surface area and can accelerate the rate of electron transfer between the redox-active enzyme and the electrode.

A novel approach have introduced for glucose sensing by Salimi et al., (2007). They found the excellent electrochemical performance of electrochemically co-deposited nickel oxide nanoparticles and GOx onto the glassy carbon electrode surface. The resulting biosensor showed strong adsorption of GOx to the nickel nanoparticles (the apparent Michaelis–Menten constant of 2.7 mM), which improved the enzyme loading and detect the glucose concentration with wide concentration range from 30 μM to 5 mM. Nickel nanoparticles facilitating the direct electron transfer between the active site of enzymes and surface of the electrodes. The corresponding detection sensitivity was 446.2 nA/mM.

4.2 Cholesterol biosensors

Cholesterol oxidase (ChOx) is most commonly used enzyme in the construction of cholesterol biosensor. Cholesterol oxidase is a flavin-enzyme (flavin-adenine-dinucleotide) that produces hydrogen peroxide according to the reaction.

$$\text{Cholesterol} + O_2 \rightarrow \text{4-Cholesten-3-one} + H_2O_2 \quad (3)$$

The structure of cholesterol oxidase reveals deeply buried active sites occupied by water molecules in the absence of its substrate steroids. Cholesterol oxidase is industrially and commercially important for application in bioconversions for clinical determination of total or free serum cholesterol in the serum samples. More recently, cholesterol biosensor based on physically cholesterol oxidase onto metallic oxide nanoparticle have been applied to determine the cholesterol concentration from the serum samples some of them are summarized here. Singh et al., (2007) revealed the effective applications of nanoporous ZnO film fabricated by rf sputtering method on gold electrode for cholesterol detection. The biosensor showed excellent linear sensing response to cholesterol with in concentration range from 25–400 mg/dl. In another report nanostructured ZnO film deposited onto ITO electrode via sol-gel chemical rout represented reproducible, highly linear response in a wide concentration range (5–400 mg/dl) and fast response time (10 s) to cholesterol(Solanki et al., 2009). The high sensitivity (0.059 μA/mg dl^{-1} cm^{-2}) and moderate detection potential (+0.8 V) led to cholesterol low detection limit of 0.5 mg/dl. In another approach (Khan et al., 2008) these authors dispersed ZnO nanoparticles in chitosan matrix for film fabrication onto conducting electrode by selective immobilization of cholesterol oxidase to construct the cholesterol biosensor. ZnO-chitosan nanocomposite films deposited on ITO electrode surface were mechanically stable due to excellent film forming ability of chitosan. The resulting biocompatible nanocomposite of ZnO/chitosan has been applied as matrix to covalently immobilize cholesterol oxidase, considering that chitosan has abundant amino groups, exhibits excellent film-forming ability for the solubility in slightly acidic solution due to the protonation and insolubility in solution with pH above pKa 6.3. High sensitivity (1.41×10^{-4} Amgdl^{-1}) with a wide linear concentration range from 5-300 mgdl^{-1} of cholesterol with a detection limit as 5mg dl^{-1} was achieved. This cholesterol biosensor can be used to estimate cholesterol in serum samples. An attractive electrochemical protocol for measuring cholesterol based on ZnO nanoparticles grow at low temperature has been proposed by Umar et al., (2009). The novelty of this work concerns the observed electro-catalytic activity

of the ZnO-ChOx based nanocomposite bioelectrode demonstrating the ultra sensitivity for cholesterol sensing at a low potential and at higher current values. These optimized nanocomposite electrodes showed significantly better performance than those obtainable from flower shaped ZnO nanostructured (used here for comparison) in terms of linear range of concentration (0.001–0.5 μM), low detection limit (0.00037μM), higher sensitivity (23.7 μA μM cm^{-2})) and response time of 5 s. Finally, an excellent amperomatric response was measured at low temperature synthesized ZnO nanoparticles bioelectrode might be due to synthesized procedure as well as particle size effect on the matrix performance.

Sol-gel derived nanostructured CeO_2 film had been applied to immobilization ChOx for cholesterol sensing. Electrochemical signal of cholesterol concentration was linear in a wide range from 10–400 mg/dL and high sensitivity 2.08 mA ($mgdL^{-1}$ cm^{-2}). A low value of Michaelis–Menten constant (*Km*) was achieved 2.08 mM indicates strong ChOx affinity to cholesterol and no interference was found in the presence of cholesterol (Ansari et al., 2008).

Chitosan as a biocompatible, nontoxic and nonconducting natural biopolymer applied in biosensor. Chitosan has strong adhesive ability to the substrate to improve the enzyme adsorption on the substrate surface. Tin oxide nanoparticles introduced into the chitosan matrix enhanced the electrocatalytic activity of the biopolymer for sensitive cholesterol detection. High linear response was measured over the concentration range of 10 – 400 mg/dL with low detection limit of 5 mg/dL. Good stability and reproducibile sensitivity (34.7 μA/mg dL^{-1} cm^{-2}) to cholesterol detection within response time 5 s (Ansari et al., 2009).

Kouassi et al.,(2005) have studied the activity of cholesterol oxidase immobilized on magnetic nanoparticles via carbodiimde chemistry. The enzyme activity was well preserved upon binding onto the nanoparticle when subjected to thermal and various pH conditions. Kinetic studies showed a significant improvement in magnetic nanoparticles bound cholesterol oxidase owing to the large surface area and high chemical and thermal stability of magnetic nanoparticles, which enhanced the electrocatalytic activity of the nanocomposite.

4.3 Hydrogen peroxide biosensors

Sensitive and accurate determination of a small quantity of H_2O_2 is of great importance, because H_2O_2 is not only a byproduct of several highly selective oxidases, but also an essential mediator in biology, medicine, industry, and many other fields. Different configurations for the design of the H_2O_2 biosensors have been reviewed. In fabrication of H_2O_2 biosensor, there are two major issues to realize the application of electrochemical technique. First, the key process is to retain the biologic activity of the redox protiens (HRP, myoglobin, hemoglobin, cytochrom C etc.) immobilized on the electrode surface in its native status. Another key factor is to electrically connect redox protiens with the electrode surface, which can provide a pathway of electron transfer between the redox center of protienes and the electrode surface. The simplest method is direct adsorption of native enzyme on the electrode surface for direct transferring of electron to the electrode. In order to fabricate sensitive and selective biosensor a variety of materials have been employed to modify the electrodes as a bridge of electron transfer between the redox center of enzymes and the electrode surface.

Some metal oxide nanoparticles have been extensively used in bioaffinity sensors for immobilized heme proteins to detect the H_2O_2. Because, nanometer size materials gives rise

to high reactivity and other beneficial physical properties (electrical, electrochemical, optical and magnetic). Furthermore, direct electron transfer ability of the nanosized metal oxide made them able to function as biomimic membrane material to fix and modify proteins. The direct electrochemical responses of heme-protien have been achieved on the nanostructured metal oxide film. Many proteins have been employed to construct the potential H_2O_2 biosensors, such as HRP, cytochrome c, myoglobin, and hemoglobin, are capable of reducing H_2O_2 electrocatalytically. (Zhu et al., 2007; Liu et al., 2005; Zhao et al., 2006; Lu et al., 2008; Duan et al., 2008; Xiang et al., 2009). The improved electro-catalytic ability of the microperoxidase/ZnO nanoparticles co-modified electrode was measured, which greatly promote the direct electron transfer between the protein and electrode. The fast electron transfer between them beneficial to developing more sensitive H_2O_2 biosensors. Zhao et al., (2006) have employed nanoporous ZnO film deposited on graphite electrode to measured the electrocatalytic response of NO_2^- and H_2O_2 by immobilization of myoglobin.The entrapped myoglobin realized fast direct electron transfer with the electrode and displayed an elegant catalytic activity toward the reduction of hydrogen peroxide, nitrite, and trichloroacetic acid. This mediator-free biosensor showed linear response within the concentration range from 1.0 x10^{-5} to 1.8 x 10^{-4} mol L^{-1} and 4.8 x 10^{-6} to 2.0 x 10^{-4} mol L^{-1} and detection limit 4.0 x 10^{-6} mol L^{-1}, 2.0 x 10^{-6} mol L^{-1} for NO_2^- and H_2O_2, respectively. Liu et al., (2005) used hydroquinone as a mediator for covalent immobilization of HRP enzyme for sensing of H_2O_2. The linearity of this fabricated biosensor was achieved of1.0 x10^{-5} to 1.8 x 10^{-3}M with a low detection limit 2.0 μM. Good reproducibility sensitivity and stability was found retained about 78% upto 40 days. An enhanced sensitivity and selectivity of the Nafion mixed flower like ZnO-gold nanocomposite was measured, due to negatively charged gold nanoparticles provides a tunnel to transfer the electron from surface to electrode. On the other hand Nafion is a proton-conductive and biocompatible perfluorosulfonate linear polymer that exhibits excellent film-forming ability, which improves the proteins loading as well as communication of the electron on the matrix. Therefore, the immobilized protein on the substrate represented strong affinity (the apparent Michaelis–Menten constant K_m =1.76mM). The biosensor have linear response in a wide concentration rang 1.5×10^{-5} to 1.1×10^{-3} M and detection limit is 9.0×10^{-6}M (Xiang et al., 2009). Lu et al., (2008) have developed porous nanosheet-based ZnO microspheres for the construction of direct electrochemical H_2O_2 biosensor. In this context, porous ZnO nanosheet permit a direct electron transfer between redox proteins and bulk electrode materials, allowing electrochemical sensing to be performed without using electron-transfer mediator. ZnO nanosheet efficiently improved the electron transfer rate between the analyte and the electrode surface with an apparent Michaelis-Menten constant (K_m) of 143 μM for the peroxide sensing. The developed biosensor displayed good performance for the detection of H_2O_2 and $NaNO_2$ with a wide linear range of 1–410 and 10–2700 μM, respectively. Duan et al., (2008) prepared complex film containing of hemoglobin, nano ZnO, chitosan and nano Au to immobilized onto glassy carbon electrode for H_2O_2 sensing. Chitosan improved the film forming ability to the substrate and presence of gold nanoparticles enhanced electrocatalytic performance such as reproducible high sensitivity, linearity (1.94 x 10^{-7} - 1.73 x 10^{-3} mol L^{-1}) and detection limit (9.7x 10^{-8} mol L^{-1}) of the fabricated bioelectrode.

To fabricate a highly reproducible sensitive and selective biosensor based on direct electron transfer from enzymes to the electrode surface. Recently, research activities have been directed toward combining the advantageous feature of sol-gel derived nanostructured TiO_2

film and the glassy carbon electrode fabrication. So-gel network is helpful to retain the biological activity on the matrix. These matrixes preserve the native stabilities and reactivities of biological macromolecules for sensing. Amperomatric biosensor provid a wide linear calibration range from 4.0×10^{-6} M to 1.0×10^{-3} M, with a detection limit of 8.0×10^{-7} M (Xu et al., 2002; Yu & Ju et al., 2002). A similar strategy has been followed by Yu and Ju (2002) to immobilize HRP onto sol-gel modified porous titania matrix. Sol-gel network provide long term stability of enzyme in storage and showed strong bioaffinity to the substrate (Km = 1.89 ± 0.21 mM). The biosensor provided a wide linear calibration range (0.08 to 0.56 mM) with a detection limit of 1.5 μM and high sensitivity (61.5 μAmM^{-1}) for monitoring of H_2O_2. The biosensor retained 80% of its original activity after two months of operation. The developed sol-gel TiO_2 films represent good sensitivity and long term stability for enzymes immobilization, owing to its high surface area for enzyme loading and good biocompatibility. Some investigators have been fabricated titania nanoparticles to immobilize heme protein for H_2O_2 detection (Kumar et al., 2008; Zhao et al., 2008; Lo et al., 2008; Curulli et al., 2007). They suggested that the nanoparticles promote the electron transfer rate efficiently from enzyme to the electrode surface. Titania nanotubes exhibited superior biosensing capabilities towards H_2O_2. Owing to their large surface area, titania nanotubes provides friendly microenvironment for enzyme loading and improve the stability of the entrapped enzymes (the apparent Michaelis-Menten constant Km = 140 μM). Heme proteins (myoglobin) in these films facilitated relatively faster electron transfer (k_{ET} = 86 ± 7 s^{-1}) and catalytic activity than that between myoglobin in the solution and bare electrodes ((Liu et al., 2005). Most studies reported (Topoglidis et al., 2000) in literature are based on immobilization of protein which were focused on layered or mesopore structured materials due to their higher specific surface area. They found that titania nanotubes based biosensor exhibited high sensitivity to H_2O_2 and low detection limit (0.6 μM). Xiao et al., (2007) have analyzed the electrocatalytic ability of HRP-TiO_2 nanotube arrays, they possessed appreciably different sensitivities to H_2O_2 due to their different conductivity. They suggested that TiO_2 nanotube arrays demonstrated the best sensitivity for H_2O_2 in the range of 10^{-5}–$3x10^{-3}$ M at pH 6.7 and at a potential of -600 mV. Titania nanotubes containing thionine were electrochemically deposited on Ti substrate to immobilization of HRP for H_2O_2 sensing. The constructed biosensor has linear response to the H_2O_2 concentration from $1.1x\ 10^{-5}$ to $1.1\ x\ 10^{-3}$ M and detection limit was optimized as $1.2\ x\ 10^{-6}$ M (Liu et al., 2005). The sensor was fabricated by electrochemical deposition of nanotubular TiO_2 and platinum nanoparticles at very low potential (+ 0.3 V) for amperomatrically detection of H_2O_2. They found amperomatric signals were linear that is proportional to hydrogen peroxide concentration in the range $4\ x\ 10^{-6}$ to $1.25\ x\ 10^{-3}$ M and detection limit of the sensor was 4.0 μM (Cui et al., 2008). Zhang et al., (2004) have presented that TiO_2 nanoparticles exhibited better biosensing capabilities towards H_2O_2. The sensor was fabricated by casting the mixture of HRP solution and aqueous titanium oxide nanoparticles dispersed onto pyrolytic graphite electrode. They found that the constructed biosensor presented a better amperomatric response at 0 V with a larger sensitivity and a wider linear range. Viticoli and his co-workers (2006) have developed third-generation biosensors based on TiO_2 nano-structured films. They found that, functionalised TiO_2 thin films deposited on Si substrates exhibited high sensitivity, low detection limit (~10^{-6} M) and fast response time to H_2O_2.

Liu et al., (2003) have reported amperomatric biosensor based on sol-gel derived nanoporous ZrO_2 for H_2O_2 sensing. The resulting biosensor exhibited high sensitivity (111 μAmM^{-1}) for H_2O_2 over a wide concentration range from 2.5×10^{-7} to 1.5×10^{-4} mol l^{-1}, fast

response (10 s). This biosensor was stabile over 3 months. Liu et al., (2004) measured the direct electrochemistry and thermal stability of hemoglobin immobilized onto nanometer-sized ZrO_2 modified pyrolytic graphite electrode. They suggested that nanometer-sized ZrO_2 and dimethyl sulfoxide accelerate the electron transfer rate between hemoglobin and the electrode. The modified electrode showed a high thermal stability up to 74^{o}C. The response was linear for H_2O_2 over the concentration ranging from 1.5 to 30.2 μM with a detection limit of 0.14 μM. Zong et al., (2007) reported reagent less biosensor based on hemoglobin modified zirconia nanoparticles for H_2O_2 detection. They examined the enzymatic activity with the zirconia nanoparticles that is strongly co-adsorbed to the surface nanoparticles and showed an excellent electrocatalytic activity to the reduction of H_2O_2. They found fast electron transfer (electron transfer rate constant = 6.46 s^{-1}) between the surfaces adsorbed enzyme (hemoglobin) and electrode. A wide linear concentration range for H_2O_2 was achieved 0.8 to 132 μM and low detection limit 0.12 μM. This research group proposed another strategy for H_2O_2 sensing by co-adsorption of myoglobin on zirconia nanoparticles enhanced grafted collagen hybrid composite (Zong et al., 2007). Grafted collagen provided a good matrix for protein immobilization and biosensing preparation. This method was useful for monitoring H_2O_2 in practical samples with the satisfactory results. They examined that ZrO_2- grafted collagen matrix accelerate the electron transfer between Mb and the electrode with a surface-controlled process and an electron transfer rate constant of 3.58±0.35 s^{-1} at 10–500mVs^{-1}. The linearity of the biosensor to H_2O_2 concentration was from 1.0 to 85.0 μMwith the limit of detection of 0.63 μM. Tong et al., (2007) applied HRP-ZrO_2 nanocomposite deposited by electrochemical method on gold electrode for fabrication of sensitive electrochemical biosensor. Under optimized conditions such as pH and potential, biosensor exhibited linear amperomatric response for H_2O_2 in a wide concentration range from 0.02 - 9.45mM with a detection limit of 2 μM. In another approach HRP was covalently immobilized on DNA/electrodeposited ZrO_2/modified, gold electrode. They suggetsted that sandwich DNA provides a microenvironment for the immobilization of enzyme or protiens and promotes electron transfer between HRP and the electrode surface. The resulting biosensor (HRP–ZrO_2/Au electrode) showed a linear response to H_2O_2 over a concentration range from 3.5 μM -10 mM with a detection limit 0.8 μM. The optimized bioelectrode represent excellent electrochemical performance such as sensitivity, reproducibility and response time for sensitive and selective detection of H_2O_2 in clinical samples (Tong et al., 2007).

Magnetic nanoparticles are promising materials in fabrication of biosensors and bioreactors. Among magnetic nanoparticles, Fe_3O_4 nanoparticles are the most commonly used magnetic materials because of their good biocompatibility, strong superparamagnetic property, low toxicity and easy preparation. Iron oxide nanocomposites matrices have applied for covalent immobilization of heme proteins for sensitive biosensor device development. The sensitivity of the fabricated biosensors depends on the proteins immobilization procedure on the conducting electrode. Several immobilization procedures have been used for the enzyme immobilization on the nanomaterials electrode surface, through them can examine the electron transfer features of bioelectrode (Cao et al., 2006; Cao et al., 2003; Zhao et al., 2006; Zhang et al., 2007). The direct immobilization of redox protein or enzyme without mediator onto nanomaterials electrode surface has an advantageous procedure among them due to sensitive and selective detection of analyte. Studies of direct electrochemistry of protein or enzymes at electrodes serve as a basis for building electrochemical biosensors, enzymatic

bioreactors, and biomedical devices. Cao et al., (2006) investigated the direct electrochemistry of heme protein immobilized on Fe_3O_4 nanoparticles for highly reproducible sensitive H_2O_2 sensing. This approach simplified such devices without using mediators and is of particular significance for fabrication the third generation biosensors. Direct electrochemical study can also establish a model for mechanistic studies of electron transfer between enzymes in biological systems. The direct electron transfer of haemoglobin by immobilizing it on Fe_3O_4 nanoparticle multilayer films was investigated (Zhao et al., 2006). The good sensitivity and long-term stability of the biosensor was measured indicating that the direct immobilization of heme proteins on Fe_3O_4 matrix is a promising substrate for hydrogen peroxide sensing. These films were constructed on several conductive bases (glassy carbon electrode, ITO glass, and Al foil) by first electrodeposition of chitosan/Fe_3O_4 thin films and then a layer-by-layer assembly using phytic acid and chitosan/Fe_3O_4. To enhance the sensitivity of the biosensor and minimize the problems of mediators, Zhao et al., (2005) fabricated a H_2O_2 biosensor based on Prussian blue modified magnetic nanoparticles. The synthesized nanoparticles catalyze the reduction of H_2O_2 without mediator. Crosslinked glutaraldehyde chemistry have used to covalent immobilization of hemoglobin onto carbon-coated iron nanoparticles for sensitive amperomatric hydrogen peroxide sensing. The resulting nanocomposite provides a shelter for the enzyme to retain its bioactivity under considerably extreme conditions, and the iron nanoparticles in the biocomposite offer excellent affinity to enzyme. The electrocatalytic response exhibited a linear dependence on H_2O_2 concentration in a wide range from 3.1 mM to 4.0 mM with a detection limit of 1.2 mM (Zhang et al., 2007). Hrbac et al., (2007) have reported amperomatrically H_2O_2 detection by adsorption of heme protein on iron oxide nanoparticles (hematite, maghemite, amorphous Fe_2O_3, β-Fe_2O_3 and ferrihydrite) deposited carbon past electrode. Prussian blue modified iron oxide nanopartilces based electrode exhibited excellent amperomatric response towards detection of hydrogen peroxide such as linearity (8.5 mM) and reproducible sensitivity, response time (<3 s) with detection limit was 2×10^{-5} M. HRP was immobilized on gold modified nanoporous silica based magnetic microparticles matrix for H_2O_2 sensing. The analytical performance of the resulting biosensor was characterized by electrochemical techniques. The resulting bioelectrode provided a linear response to H_2O_2 over a concentration range comprised between 5×10^{-7}-1.3×10^{-4} M with a detection limit of 4×10^{-7} M (Elyacoubia et al., 2006). Lin et al., (2005) have fabricated a CH-Fe_3O_4 nanocomposite modified glassy carbon electrode for determination of H_2O_2. The linearity range was obtained as 4-5 mM with detection limit 7.6 and 7.4 μmol/L and biosensor was stable up to 9 months.

Attempts have been made to applied sol-gel tin oxide matrix to heme proteins immobilization for rapid detection of H_2O_2 (Jia et al., 2005a&b; Topoglidis et al., 2003). Sol-gel derived bioelectrodes showed better analytical performance in respect to other conventional method prepared bioelectrodes, such as faster response, wider detection range, lower detection limit, better affinity, higher enzyme loading, simpler operation and better storage stability for electrochemical detection of hydrogen peroxide due to the introduction of the collagen to form well-distributed porous three-dimensional structure. The linear concentration range of the biosensor was from 0.01-0.25 mM. (Jia et al., 2005a). The smaller *Km* (the apparent Michaelis-Menten constant value *Km* = 0.345 mM) value was measured means that the immobilized HRP onto SnO_2 film possesses higher enzymatic activity, and exhibits higher affinity to H_2O_2. (Jia et al., 2005b)

Ansari et al., (2009) have applied nanostructured CeO_2 film deposited onto indium-tin-oxide (ITO) glass substrate by solution casting process to immobilization of HRP via physiosorption technique for H_2O_2 reduction. The designed biosensor exhibited acceptable stability (5 weeks), wide linearity range (1.0–170 μM) for H_2O_2 concentration, long-term shelf life and good reproducibility. A similar approach has been used to fabricate an amperometric hydrogen peroxide biosensor based on electrochemically deposited PANI/CeO_2 nanocomposite films onto ITO electrode (Ansari et al., 2009). Polyaniline is used to modify the electrocatalytic activity of the CeO_2. Polyaniline CeO_2 nanoparticles served as a linker for rapid detection of H_2O_2. This property results in an electrocatalytic activity of the immobilized HRP to the reduction of H_2O_2, used for preparation of H_2O_2 sensors. The proposed biosensor showed excellent performance for H_2O_2 sensing such as sensitivity (159.6 nA/mM), detection limit (50 mM), long time stability (8 weeks) and fast response time.

MnO_2 nanoparticles and dihexadecyl hydrogen phosphate composite film have been used as an enzyme (HRP) immobilization matrix with enhanced activity and stability (Yao et al., 2006). Manganese dioxide (MnO_2) nanoparticles have been proved to be a catalytic substance to promote the decomposition of H_2O_2 to O_2. The MnO_2nanoparticles can retain the bioactivity of proteins to a large extent and accelerate the electron transfer between proteins and electrodes. The biosensor was optimized under optimal conditions which showed high sensitivity (2.66×105 $\mu AM^{-1}cm^{-2}$) wide linear concentration range of 1.2×10^{-7}–2.0×10^{-3} M with a substantially low detection limit of 8.0×10^{-8} M.

Antimony oxide bromide (AOB) nanorods dispersed in chitosan have been applied for H_2O_2 biosensor (Lu et al., 2006). Chitosan is a biopolymer has an excellent film-forming ability and biocompatibility. The solubility of antimony oxide bromide nanorods in these biopolymers facilitates the construction of electrochemical biosensing platform. AOB nanorods mixed with chitosan were placed on the surface of a glassy carbon electrode results in a robust AOB-chitosan film, which facilitates the electrooxidation of NADH. The fabricated biosensor has a linear response from 1–121 μM with a correlation coefficient of 0.993. They found the bioelectrode displayed good sensitivity ($1.44mAcm^{-2}M^{-1}$) and reproducibility, wide linear range, low detection limit, fast response and excellent long-term stability.

4.4 Urea biosensor

Zinc oxide nanoparticles have been introduced into the chitosan solution to immobilization of urease enzyme for urea sensing. Chitosan film containing ZnO have been deposited onto ITO electrode for covalent immobilization of urease. The possibility to combine both properties, such as the higher surface area and electrocatalytic effect exhibited from ZnO, with the surface adhesive properties of chitosan can be used to manipulate and control the analytic signal in the presence of a specific substrate. The marked electrocatalytic activity toward urea permits effective low-potential amperometric biosensing. The optimum configuration for biosensors has allowed highly sensitive (0.13 $\mu A/mM\ cm^{-2}$), fast response time (10 s), and highly selective (0% interference of at glucose (5 mM), ascorbic acid (0.05 mM), uric acid (0.1 mM), cholesterol (5 mM), and lactic acid (5 mM) at maximum physiological levels) analytes quantification with lower detection limit (3 mg/dl), higher reproducibility and long shelf life upto 3 month (Pratima et al., 2009). Hubalek et al., (2007)

have developed nickel nanoelectrode to determined the urease by electrochemical and votammetry methods. Ansari el al. (2009) prepared sol-gel-derived titanium oxide–cerium oxide (TiO_2–CeO_2) nanocomposite film deposited onto ITO-coated glass substrate for urea sensing. A biosensor was fabricated by casting the mixture of enzymes (urease and glutamate dehydrogenase) on nanobiocomposite for determination of urea concentration. The performance of the sensor showed sensitive determination of urea with a linear range from10–700 mg/dL, response time of 10 s, sensitivity as 0.9165 $\mu Acm^{-2}mM^{-1}$, detection limit of 0.166 mM with negligible interference from physiological levels of uric acid, cholesterol, glucose, and ascorbic acid **(**Ansari et al., 2009). Recently, Zhang et al., (2004) reported the use of uricase immobilized ZnO nanorods dispersed into Nafion placed onto glassy carbon electrodes (GCE) as a new platform for developing enzymatic biosensors. They found that, ZnO nanorods derived electrode retained the enzyme bioactivity and enhance the electron transfer between the enzyme and the electrode. In the presence of an electron mediator electrode showed high affinity (*Km* = 0.238 mM) to the electrode and excellent electrocatalytic response such as linearity dependence on the uric acid concentration ranging from 5.0×10^{-6} to 1.0×10^{-3} mol L^{-1} with a detection limit of 2.0×10^{-6} mol L^{-1}. Functionalized multiwalled carbon nanotubes (MWCNTs) mixed with tin oxide has applied for the fabrication of reagentless amperomatric uric acid biosensor. MWCNTs have used as an electron promoter which efficiently enhanced the electron transfer rate between the enzyme and electrode surface. This biosensor exhibited a linear dependence on the uric acid concentration over the range from 1.0 x 10^{-7} to 5.0 x 10^{-4} mol L^{-1} with little ascorbic acid interference in physiological level (Zhang et al., 2005). In a novel strategy based on titanate nanotubes have developed by Liu et al., (2006) for measuring the biosensing properties amperomatrically. Titania nanotubes have deposited onto glassy carbon electrode for selective detection of dopamine in the presence of ascorbic acid and uric acid. The linear calibration curve for dopamine was observed over the concentration range 0.1–30 lM in a physiological solution that contains 0.1 mM AA and 0.3 mM uric acid.

4.5 Choline biosensor

Choline is also an important component of phospholipids (lecithin and sphingomyelin), which is required for the synthesis of the neurotransmitter acetylcholine precursor. Choline found in central and peripheral nervous systems of mammals. Therefor, direct monitoring of choline from the serum samples by enzyme-based biosensors have emerged as most promising field. A number of enzyme electrodes, with immobilized ChOx, were reported for choline determination based on the detection of liberated H_2O_2. Amperometric biosensors based on the immobilization of bi-enzymes HRP and ChOx for the determination of choline were also reported. In the single enzyme system as following Eq.,

$$\text{choline} + O_2 \rightarrow \text{betaine aldehyde} + H_2O_2 \quad (4)$$

In the presence of oxygen, choline is converted by ChOx, producing H_2O_2. Then H_2O_2 can be subsequently detected with the help of conducting materials electrodes. Bai et al., (2007) have used MnO_2 nanoparticles based electrod for sensitive determination of choline concentration. MnO_2 nanoparticles modified electrode in the presence of H_2O_2 exhibited bi-direction electrocatalytic ability toward the reduction/oxidation of H_2O_2. The amperomatric signal was linear of choline chloride in the concentration range 1.0 x 10^{-5} – 2.1 x 10^{-3} M and

no obvious interference from ascorbic acid and uric acid was observed in physiological level (Bai et al., 2007). Similar nanocomposite material–based on electrochemical H_2O_2/choline biosensors have been developed by modifying a glassy carbon electrode with chitosan and three different dimensionality (amorphous MnO_2 nanoparticles, α-MnO_2 nanoparticles, and *β*-MnO_2 nanowires)/GCE film **(**Bai et al., 2008**)**. They found the biocatalytic activity of the three different nanostructures for the determination of choline amperomatrically in the following order: amorphous MnO_2 > α-MnO_2 > β-MnO_2. Due to the different specific surface areas of the nanomaterials, the amount of enzyme entrapped on MnO_2 electrode surface is different that is depend on crystalline structures, and dimensionality of the materials. Therefor, the biosensors based on α-MnO_2 nanoparticles and β-MnO_2 nanowires represent excellent electrochemical response for amperometric detections of choline chloride in the linear ranges of 2.0×10^{-6}-5.8×10^{-4} M and 1.0×10^{-6}-7.9×10^{-4} M with the detection limits of 1.0 and 0.3 *μ*M, respectively. For sensitive determination of choline and acetylcholine, bovine serum albumin and choline oxidase co-adsorbed electropolymerized poly (*N*-acetylaniline) film/sol–gel ZnO membrane prepared onto Pt electrode. They suggested that BSA improved the stability and activity of the enzymes on sol-gel derived electrode. The resulting biosensors at an applied potential of +0.6V exhibited fast response, expanded linear response in the concentration range of 1.0×10^{-6} to 1.5×10^{-3}M to acetylcholine with a detection limit of 6.0×10^{-7} M and a linear response range up to 1.6×10^{-3} M to choline with a detection limit of 5.0×10^{-7} M (Yang et al., 2005**)**.

4.6 Phenol biosensors

Phenolic compounds are very toxic, showing adverse effects on animal and plants which are often exist in the wastewaters of many industries. Therefore, the identification and quantification of such compounds are very important for environment monitoring. Li et al., have developed phenolic biosensor based on ZnO nanoparticles. ZnO nanoparticles dispersed chitosan nanocomposite provid an advantageous microenvironment in terms of its favorable isoelectric point for tyrosinase loading and the immobilization of tyrosinase enzyme. They suggested that nanocomposite electrode retained its activity to a large extent and facilitated the electron communication on the electrode surface at a large extent.The resulting biosensor has 95% of steady-state current within 10 s, and the sensitivity was 182 μA $mmol^{-1}L$. The linear range for phenol was determined from 1.5×10^{-7} to 6.5×10^{-5} mol L^{-1} with a detection limit of 5.0×10^{-8} mol L^{-1}**(**Li et al.,). Another approach mediator free sol-gel derived ZnO matrix has used for phenol sensing. The performance of the developed biosensor was such as linearity (1.5×10^{-7} to 4.0×10^{-5} mol L^{-1}), sensitivity (168 μAmmol L^{-1}), detection limit (8.0×10^{-8} mol L^{-1}) fast response time long term stability (2 weeks) (Liu et al., 2005**)**. Zhang et al., (2003) have applied hybrid sol-gel titania matrix for the construction of sensitive mediator free tyrosinase biosensor. Under optimum pH titania retained the tyrosinase activity and stability attached on to the surface of a glassy carbon electrode. The reproducible high sensitivity $15.78\mu A\mu M^{-1}cm^{-2}$ and low detection limit 1×10^{-8} M was achieved for monitoring phenols.

To determination of pesticides in vegetable samples Yang et al., (2005) have proposed an acetylcholinesterase biosensor based on nanoparticles ZrO_2/chitosan. Acetylcholinesterase was covalently immobilized onto ZrO_2/chitosan nanocomposite matrix. The experimental conditions were optimized showed that pesticides inhibit the activity of enzyme with an effect of decreasing of oxidation current. The linear response was achieved from 9.90×10^{-6}

to 2.03 x 10^{-3} M, 6.6 x 10^{-6} to 4.4 x 10^{-4} M, 1.0 x 10^{-8} to 5.9 x 10^{-7} M and 8.6 x 10^{-6} to 5.2 x 10^{-4} M acetylcholinesterase, phoxim, malathion and dimethoate, respectively with the detection limit of 1.3 x 10^{-6} M for phoxim. The proposed samples have used to determine pesticides in real vegetable samples.

A chitosan film containing MnO_2 nanoparticles was electrodeposited on glassy carbon electrode to covalent immobilization of lactate oxidase for the determination of lactate concentrations. Due to their excellent film-forming ability and strong adsorbability of enzyme on the nanocomposite electrode surface. They found linear response to lactate in the range of 0.020–4.0 mM, with reproducibile high sensitivity (3.98 μAcm^{-2} mM^{-1}) and long-term stability **(**Wang et al., 2006**)**.

Umar et al., (2009) have employed ZnO nanowires coated onto gold electrode for ultra-sensitive determination of hydrazine by amperometrically. The proposed biosensor has reproducible high sensitivity 12.76 μAcm^{-2} nM^{-1}, low detection limit, 84.7 nM, fast response time less than 5 s, good linear range from 500 - 1200nM with correlation coefficient of R = 0.9989.

Magnetic core–shell (Fe_3O_4–SiO_2) nanoparticles modified film onto the surface of carbon past electrode for covalently immobilization of Laccase enzyme, done by chemical cross-linking with glutaraldehyde and free aldehyde groups to determined the hydroquinone concentration. Magnetic core–shell nanoparticles provide microenvironment for retaining the bioactivity of laccase. The linear range for hydroquinone was determination as 1×10^{-7} to 1.375×10^{-4} M, with a detection limit of 1.5×10^{-8} M and 95% steady-state current was reached within 60 s (Zhang et al., 2007).

5. Conclusions

The unique electrocatalytic properties of the metal oxides and the ease of metal oxide nanostructured fabrication make them extremely interesting materials for electrochemical enzymatic biosensor applications. The application of nanostructured metal oxides in such sensing devices has taken off rapidly and will surely continue to expand. This article provides a review on current research status of electrochemical enzymatic biosensors based on various new types of nanostructured metal oxides such as nanotubes, nanorods, nanobelts, and nanowires. These nanostructured-based amperomatric biosensors represent a powerful detection platform for electrochemical enzymatic biosensors. It is a crucial requirement of consumers to design simple, affordable, reliable and portable electrochemical biosensors, which provides novel key features, including high sensitivity, selectivity, fast response, low detection limit, fast response with minimum intereferences from bulk species and could operate at low potential. Such electrochemical biosensors could be useful for diagnosing and monitoring infectious disease, monitoring the pharmokinetics of drugs, detecting cancer, and disease biomarkers, analyzing breath, urine and blood for drugs of abuse. It is only a matter of time before such protocols are used for routine diagnostic applications.

6. References

Ansari, A.A.; Kaushik, A.; Solanki, P.R.; Malhotra, B.D. (2008), Sol–gel derived nanoporous cerium oxide film for application to cholesterol biosensor. *Electrochem. Commun.* 10: 1246-1249.

Ansari, A.A.; Kaushik, A.; Solanki, P.R.; Malhotra, B.D. (2009), Electrochemical cholesterol sensor based on tin oxide-chitosan nanobiocomposite film. *Electroanalysis*, 21: 965-972.

Ansari, A.A.; Solanki, P.R.; Malhotra, B. D. (2009), Sol-gel derived nanostructured tin oxide film for glucose. *Sensor Letter* 7:64-71.

Ansari, A.A.; Solanki, P.R.; Malhotra, B.D. (2008), Sol-gel derived nanostructured cerium oxide film for glucose sensor.*Appl. Phys. Lett.* 92: 263901-3.

Ansari,A.A.; Sumana,G.; Khan R.; Malhotra, B.D.(2009), Polyaniline-cerium oxide nanocomposite for hydrogen peroxide sensor. *J.of Nanosc. and Nanotechnology* 9: 1–7.

Ansari,A.A.; Solanki,P.R.; Malhotra, B.D. (2009), Hydrogen peroxide sensor based on HRPimmobilized nanostructured cerium oxide film. *J. Biotechnology* 142:179–184

Ansari,A.A.; Sumana, G.; Pandey, M.K.; Malhotra, B.D. (2009), Sol-gel derived titanium oxide-cerium oxide biocompatible nanocomposite film for urea sensor; *J. Material Research* 24(5): 1667-1673.

Bai, Y.H.; Du, Y.; Xu, J.J.; Chen, H.Y. (2007), Choline biosensors based on a bi-electrocatalytic property of MnO_2 nanoparticles modified electrodes to H_2O_2. *Electrochem. Commun.*, 9: 2611–2616.

Bai, Y.H.; Zhang, H.; Xu, J.J.; Chen, H.Y. (2008), Relationship between nanostructure and electrochemical/biosensing properties of MnO_2 nanomaterials for H_2O_2/choline. *J. Phys. Chem. C* 112: 18984–18990.

Cao, D. F.; He, P.L.; Hu, N.F. (2003), Electrochemical biosensors utilising electron transfer in heme proteins immobilised on Fe_3O_4 nanoparticles.*Analyst* 128: 1268-1274.

Cao, D.; Hu, N. (2006), Direct electron transfer between hemoglobin and pyrolytic graphite electrodes enhanced by Fe_3O_4 nanoparticles in their layer-by-layer self-assembly films.*Biophys. Chem.*, 121: 209-217.

Chen, J.; Zhang, W.D.; Ye, J.S. (2008), Nonenzymatic electrochemical glucose sensor based on MnO_2/MWNTs nanocomposite. *Electrochem. Commun.* 10: 1268-1271.

Chopra, N.; Gavalas, V.G.; Bachas, L.G.; Hinds, B.J.; Bachas, L.G. (2007), Functional one-dimensional nanomaterials: applications in nanoscale biosensors. *Analytical Letters* 40(11): 2067-2096.

Chow, D.C.; Johannes, M.S.; Lee, W.K.; Clark, R.L.; Zauscher, S.; Chilkoti, A. (2005), Nanofabrication with biomolecules, *Materials Today*, 8: 30-39.

Cui, X.; Li, Z.; Yang, Y.; Zhang, W.; Wang, Q. (2008), Low-potential sensitive hydrogen peroxide detection based on nanotubular TiO_2 and platinum composite electrode. *Electroanalysis* 20: 970- 975.

Curulli, A.; Valentini, F.; Padeletti, G.; Viticoli, M.; Caschera, D.; Palleschi, G. (2005), Smart (Nano) materials: TiO_2 nanostructured films to modify electrodes for assembling of new electrochemical probes. *Sens. and Actuat. B* 111–112: 441–449.

Duan, G.; Li, Y.; Wen, Y.; Ma, X.; Wang, Y.; Ji, J.; Wu, P.; Zhang, Z.; Yang, H. (2008), Direct electrochemistry and electrocatalysis of hemoglobin/ZnO-chitosan/nano-Au modified glassy carbon electrode. *Electroanal.* 20(22):2454- 2459

Elyacoubia, A.; Zayeda, S.I.M.; Blankert, B.; Kauffmann, J.M. (2006), Development of an amperometric enzymatic biosensor based on gold modified magnetic nanoporous microparticles. *Electroanalysis* 18(4): 345-350

Hrbac, J.; Halouzka, V.; Zboril, R.; Papadopoulos, K.; Triantis, T. (2007), Carbon electrodes modified by nanoscopic iron(III) oxides to assemble chemical sensors for the hydrogen peroxide amperometric detection. *Electroanalysis* 19(17): 1850-1854.

Hubalek, J.; Hradecky, J.; Adam, V.; Krystofova, O.; Huska, D.; Masarik, M.; Trnkova, L.; Horna, A.; Klosova, K.; Adamek, M.; Zehnalek, J.; Kizek, R. (2007), Spectrometric and voltammetric analysis of urease–nickel nanoelectrode as an electrochemical sensor. *Sensors*, 7: 1238-1255.

Jia, N.; Zhou, Q.; Liu, L.; Yan, M.; Jiang, Z. (2005), Direct electrochemistry and electrocatalysis of HRPimmobilized in sol–gel-derived tin oxide/gelatin composite films. *J. Electroanal. Chem*. 580: 213–221

Jia, N.-Q.; Xu, J.; Sun, M.-H.; Jiang, Z.-Y.(2005), A mediatorless hydrogen peroxide biosensor based on HRPimmobilized in tin oxide sol-gel film. *Anal. Lett.* 38(8):1237-1248.

Kerman, K.; Saito, M.; Yamamura, S.; Takamura, Y.; Tamiya, E. (2008), Nanomaterial-based electrochemical biosensors for medical applications. *Trends in Anal. Chem.*,27: 585-592.

Khan, R.; Kaushik, A.; Solanki, P. R.; Ansari, A. A.; Pandey, M. K.; Malhotra, B.D. (2008), Zinc oxide nanoparticles-chitosan composite film for cholesterol biosensor. *Anal. Chim. Acta* 616: 207-213.

Kim, H. J.; Yoon, S. H.; Choi, H. N.; Lyu, Y. K.; Lee, W. Y. (2006), Amperometric glucose biosensor based on sol-gel-derived zirconia/Nafion composite film as encapsulation matrix. *Bull. Korean Chem. Soc.* 27(1): 65-70.

Kouassi, G.K.; Irudayaraj, J.; McCarty, G. (2005), Examination of cholesterol oxidase attachment to magnetic nanoparticles. *J. of Nanobiotechnology*, 3(1):1-9.

Kumar, S.A.; Chen, S.M. (2008), Nanostructured zinc oxide particles in chemically modified electrodes for biosensor applications. *Anal. Lett.*, 41(2): 141-158.

Kumar, S.A.; Lo P.H.; Chen, S.M. (2008), Electrochemical synthesis and characterization of TiO_2 nanoparticles and their use as a platform for flavin adenine dinucleotide immobilization and efficient electrocatalysis. *Nanotechnology* 19:255501-7

Li, Q.; Luo, G.; Feng, J.; Zhou, Q.; Zhang, L.; Zhu, Y. (2001), Amperometric detection of glucose with glucose oxidase absorbed on porous nanocrystalline TiO_2 film. *Electroanalysis*, 13(5): 413-416.

Li, Y.F.; Liu, Z.M.; Liu, Y.L.; Yang, Y.H.; Shen, G.L.; Yu, R.Q. (2006), A mediator-free phenol biosensor based on immobilizing tyrosinase to ZnO nanoparticles. *Anal. Biochem.*, 349: 33–40.

Lin, M.S.; Leu, H.J. (2005), A Fe_3O_4-based chemical sensor for cathodic determination of hydrogen peroxide.*Electroanalysis*, 17(22): 2068-2073.

Liu S.; Chen, A. (2005), Coadsorption of HRPwith thionine on TiO_2 nanotubes for biosensing. *Langmuir* 21: 8409-8413.

Liu, A.; Wei, M.; Honma, I.; Zhou, H. (2005), Direct electrochemistry of myoglobin in titanate nanotubes film. *Anal. Chem*., 77:, 8068-8074

Liu, A.; Wei, M.; Honma, I.; Zhou, H. (2006), Biosensing properties of titanate-nanotube films: selective detection of dopamine in the presence of ascorbate and uric acid. *Adv. Funct. Mater.*, 16: 371–376

Liu, B.; Cao, Y.; Chen, D.; Kong, J.; Deng, J. (2003), Amperometric biosensor based on a nanoporous ZrO_2 matrix. *Anal. Chim. Acta* 478: 59-66.

Liu, S.; Dai, Z.; Chen, H.; Ju, H.(2004), Immobilization of hemoglobin on zirconium dioxide nanoparticles for preparation of a novel hydrogen peroxide biosensor. *Biosens. Bioelectron.* 19: 963-969.

Liu, Y. L.; Yang, Y. H.; Yang, H. F.; Liu, Z. M.; Shen, G. L.; Yu, R. Q. (2005), Nanosized flower-like ZnO synthesized by a simple hydrothermal method and applied as matrix for horseradish peroxidase immobilization for electro-biosensing. *J. Inorg. Biochem.* 99: 2046-2053.

Liu, Z.; Liu, Y.; Yang, H.; Yang, Y.; Shen, G.; Yu, R. (2005), A mediator-free tyrosinase biosensor based on ZnO sol-gel matrix. *Electroanalysis*, 17(12):1065-1070

Liu, Z.; Wang, J.; Xie, D.; Chen, G. (2008), Polyaniline-coated Fe_3O_4 nanoparticle–carbon-nanotube composite and its application in electrochemical biosensing. *Small*, 4(4): 462–466.

Lo, P.H.; Kumar, S.A.; Chen, S.M. (2008), Amperometric determination of H_2O_2 at nano-TiO_2/DNA/thionin nanocomposite modified electrode. *Colloids and Surfaces B: Biointerf.* 66:266–273.

Lu, X.; Wen, Z.; Li, J.(2006), Hydroxyl-containing antimony oxide bromide nanorods combined with chitosan for biosensors. *Biomaterials* 27: 5740–5747

Lu, X.; Zhang, H.; Ni, Y.; Zhang, Q.; Chen, J. (2008),Porous nanosheet-based ZnO microspheres for the construction of direct electrochemical biosensors. *Biosensors and Bioelectronics* 24:93–98

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

Qu, S.; Wang, J.; Kong, J.; Yang, P.; Chen, G. (2007),Magnetic loading of carbon nanotube/nano-Fe_3O_4 composite for electrochemical sensing.*Talanta* 71: 1096–1102

Saha, S.; Arya, S.K.; Singh, S.P.; Sreenivas, K.; Malhotra, B.D.; Gupta, V. (2009), Nanoporous cerium oxide thin film for glucose biosensor. *Biosensors and Bioelectronics* 24:2040–2045

Salimi, A.; Sharifi, E.; Noorbakhsh, A.; Soltanian, S. (2007), Immobilization of glucose oxidase on electrodeposited nickel oxide nanoparticles: Direct electron transfer and electrocatalytic activity. *Biosens. Bioelectron.* 22: 3146-3153.

Singh, S.P.; Arya, S.K.; Pandey, P.; Malhotra, B.D.; Saha, S.; Sreenivas, K.; Gupta, V. (2007), Cholesterol biosensor based on rf sputtered zinc oxide nanoporous thin film. *Appl. Phys. Lett.*, 91: 063901-3.

Solanki, P. R.; Kaushik, A.; Ansari, A. A.; Malhotra, B. D. (2009), Nanostructured zinc oxide platform for cholesterol sensor.*Appl. Phys. Lett.* 94, 163903-3.

Solanki,P.R.; Kaushik,A.; Ansari,A.A.; Malhotra, B.D. (2008), Zinc oxide-chitosan nanobiocomposite for urea sensor, *Appl. Phys. Lett.*, 93:163903-3

Tong, Z.; Yuan, R. ; Chai, Y.; Xie, Y.; Chen, S. (2007), A novel and simple biomolecules immobilization method: electro-deposition ZrO_2 doped with HRP for fabrication of hydrogen peroxide biosensor. *J. Biotechnology* 128: 567-575.

Tong, Z.; Yuan, R.; Chai, Y.; Chen, S.; Xie, Y. (2007), Direct electrochemistry of horseradish peroxidase immobilized on DNA/electrodeposited zirconium dioxide modified, gold disk electrode. *Biotechn. Lett.*, 29: 791-795.

Topoglidis, E.; Astuti, Y.; Duriaux, F.; Gratzel, M.; Durrant, J. R. (2003), Direct electrochemistry and nitric oxide interaction of heme proteins adsorbed on nanocrystalline tin oxide electrodes.*Langmuir*, 19: 6894-6900.

Topoglidis, E.; Lutz, T.; Willis, R.L.; Barnett, C.J.; Cass, A.E.G.; Durrant, J.R. (2000), Protein adsorption on nanoporous TiO2 films: a novel approach to studying photoinduced protein/electrode transfer reactions. *Faraday Discuss*., 116, 35-46

Umar, A.; Rahman, M.M., Al-Hajry, A.; Hahn, Y.B.; (2009), Enzymatic glucose biosensor based on flower-shaped copper oxide nanostructures composed of thin nanosheets. *Electrochem. Commun*. 11:278–281

Umar, A.; Rahman, M.M.; Hahn, Y.B.(2009),Ultra-sensitive hydrazine chemical sensor based on high-aspect-ratio ZnO nanowires. *Talanta* 77: 1376–1380.

Umar, A.; Rahman, M.M.; Vaseem, M.; Hahn, Y.B. (2009), Ultra-sensitive cholesterol biosensor based on low-temperature grown ZnO nanoparticles. *Electrochem. Commun.,* 11:118–121

Valentini, F.; Palleschi, G.(2008), Nanomaterials and analytical chemistry, *Analytical Letters*, 41: 479–520.

Viticoli, M.; Curulli, A.; Cusma, A.; Kaciulis, S.; Nunziante, S.; Pandolfi, L.; Valentini, F.; Padeletti, G. (2006), Third-generation biosensors based on TiO_2 nanostructured films. *Mater. Sci. Engin. C*, 26: 947-951.

Wang, J. (2005), Nanomaterial-based electrochemical biosensors. *Analyst*, 130: 421–426.

Wang, J. X.; Sun, X. W.; Wei, A.; Lei, Y.; Cai, X. P.; Li, C. M.; Dong, Z. L. (2006), Zinc oxide nanocomb biosensor for glucose detection. *Appl. Phys. Lett.*,88, 233106-3.

Wang, J.; Snchez, A. A. (2006), Biocatalytically induced formation of cupric ferrocyanide nanoparticles and their application for electrochemical and optical biosensing of glucose. *Small*, 2(1):129–134

Wang, K.; Xu, J.J.; Chen, H.Y. (2006), Biocomposite of cobalt phthalocyanine and lactate oxidase for lactate biosensing with MnO_2 nanoparticles as an eliminator of ascorbic acid interference. *Sens. and Actuat. B* 114:1052–1058.

Wei, A.; Sun, X.W.; Wang, J.X.; Lei, Y.; Cai, X.P.; Li, C.M.; Dong, Z.L.; Huang, W. (2006), Enzymatic glucose biosensor based on ZnO nanorod array grown by hydrothermal decomposition. *Appl. Phys. Lett*, 89, 123902-3.

Xiang, C.; Zou, Y.; Sun, L.X.; Xu, F. (2009), Direct electrochemistry and enhanced electrocatalysis of horseradish peroxidase based on flowerlike ZnO–gold nanoparticle–Nafion nanocomposite. *Sens.and Actuat. B* 136: 158–162

Xiao, P.; Garcia, B.B.; Guo, Q.; Liu, D.; Cao, G. (2007), TiO_2 nanotube arrays fabricated by anodization in different electrolytes for biosensing. *Electrochem. Commun.* 9:2441–2447

Xu, J.J.; Luo, X.L.; Du, Y.; Chen, H.Y. (2004), Application of MnO_2 nanoparticles as an eliminator of ascorbate interference to amperometric glucose biosensors. *Electrochem. Commun.,* 6:1169–1173

Xu, X.; Zhao, J.; Jiang, D.; Kong, J.; Liu, B.; Deng, J. (2002), TiO_2 sol-gel derived amperometric biosensor for H_2O_2 on the electropolymerized phenazine methosulfate modified electrode, *Anal. Bioanal. Chem.* 374:1261–1266

Yang, M.; Yang, Y.; Yang, Y.; Shen, G.; Yu, R. (2005), Microbiosensor for acetylcholine and choline based on electropolymerization/sol–gel derived composite membrane.*Anal. Chim. Acta,* 530: 205-211.

Yang, Y.; Guo, M.; Yang, M.; Wang, Z.; Shen, G.; Yu, R. (2005), Determination of pesticides in vegetable samples using an acetylcholinesterase biosensor based on

nanoparticles ZrO_2/chitosan composite film. *Intern. J. Environ. and Anal.Chem.* 85(3) 163–175.

Yang, Y.; Yang, H.; Yang, M.; Liu, Y.; Shen, G.; Yu, R. (2004), Amperometric glucose biosensor based on a surface treated nanoporous ZrO_2/Chitosan composite film as immobilization matrix. *Anal. Chim. Acta*, 525: 213-220.

Yao, S.; Xu, J.; Wang, Y.; Chen, X.; Xu, Y.; Hu, S. (2006), A highly sensitive hydrogen peroxide amperometric sensor based on MnO_2 nanoparticles and dihexadecyl hydrogen phosphate composite film. *Anal. Chim. Acta*, 557: 78-84.

Yu, J.; Ju, H. (2002), Preparation of porous titania sol-gel matrix for immobilization of horseradish peroxidase by a vapor deposition method. *Anal. Chem.,* 74: 3579-3583.

Zang, J.; Li, C.M.; Cui, X.; Wang, J.; Sun, X.; Dong, H.; Sun, C.Q. (2007), Tailoring zinc oxide nanowires for high performance amperometric glucose sensor. *Electroanalysis* 19(9): 1008-1014.

Zhang, F.; Wang, X.; Ai, S.; Sun, Z.; Wan, Q.; Zhu, Z.; Xian, Y.; Yamamoto, K. (2004), Immobilization of uricase on ZnO nanorods for a reagentless uric acid biosensor. *Anal. Chim. Acta*, 519: 155-160.

Zhang, F.F.; Wang, X.L.; Li, C.X.; Li, X.H.; Wan, Q.; Xian, Y.Z.; Jin, L.T.; Yamamoto, K. (2005), Assay for uric acid level in rat striatum by a reagentless biosensor based on functionalized multi-wall carbon nanotubes with tin oxide. *Anal. Bioanal. Chem.*, 382: 1368-1373.

Zhang, H. L.; Zou, X. Z.; Lai, G. S.; Han, D. Y.; Wanga, F.; (2007), Direct electrochemistry of hemoglobin immobilized on carbon-coated iron nanoparticles for amperometric detection of hydrogen peroxide. *Electroanalysis* 19(18): 1869-1874.

Zhang, T.; Tian, B.; Kong, J.; Yang, P.; Liu, B. (2003) A sensitive mediator-free tyrosinase biosensor based on an inorganic–organic hybrid titania sol–gel matrix. *Analytica Chimica Acta* 489: 199–206

Zhang, Y.; He, P.; Hu, N. (2004), Horseradish peroxidase immobilized in TiO_2 nanoparticle films on pyrolytic graphite electrodes: direct electrochemistry and bioelectrocatalysis. *Electrochimica Acta* 49:1981–1988

Zhang, Y.; Zeng, G.M.; Tang, L.; Huang, D.L.; Jiang, X.Y.; Chen, Y.N. (2007), A hydroquinone biosensor using modified core–shell magneticnanoparticles supported on carbon paste electrode. *Biosen. and Bioelectron.* 22:2121–2126.

Zhao, G.; Lei, Y.; Zhang, Y.; Li, H.; Liu, M. (2008), Growth and favorable bioelectrocatalysis of multishaped nanocrystal Au in vertically aligned TiO_2 nanotubes for hemoprotein. *J. Phys. Chem. C*, 112:14786–14795

Zhao, G.; Xu, J. J.; Chen, H. Y. (2006), Fabrication, characterization of Fe_3O_4 multilayer film and its application in promoting direct electron transfer of hemoglobin.*Electrochem. Commun.* 8:148-154.

Zhao, G.; Xu, J.J.; Chen, H.Y. (2006), Interfacing myoglobin to graphite electrode with an electrodeposited nanoporous ZnO film. *Anal. Biochem.,* 350():145–150

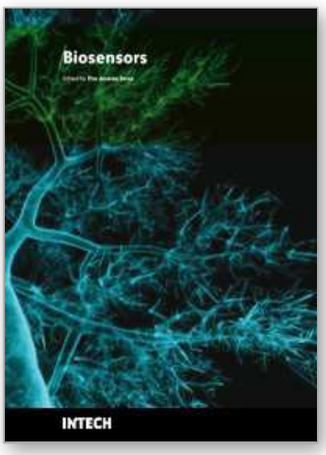
Zhao, Z.W.; Chen, X.J.; Tay, B.K.; Chen, J.S.; Han, Z.J.; Khor, K.A. (2007), A novel amperometric biosensor based on ZnO:Co nanoclusters for biosensing glucose. *Biosens. Bioelectron.*, 23: 135-139.

Zhao, G.; Feng, J.J.; Zhang, Q.L.; Li, S.P.; Chen, H.Y. (2005), Synthesis and characterization of prussian blue modified magnetite nanoparticles and its application to the electrocatalytic reduction of H_2O_2. *Chem. Mater.*, 17: 3154–3159

Zhu, X.; Yuri, I.; Gan, X.; Suzuki, I.; Li, G. (2007), Electrochemical study of the effect of nano-zinc oxide on microperoxidase and its application to more sensitive hydrogen peroxide biosensor preparation. *Biosens. and Bioelectron.* 22: 1600-1604.

Zong, S.; Cao, Y.; Zhou, Y.; Ju, H. (2007), Hydrogen peroxide biosensor based on hemoglobin modified zirconia nanoparticles-grafted collagen matrix. *Anal. Chim. Acta*, 582: 361-366.

Zong, S.; Cao, Y.; Zhou, Y.; Ju, H. (2007), Reagentless biosensor for hydrogen peroxide based on immobilization of protein in zirconia nanoparticles enhanced grafted collagen matrix. *Biosens. Bioelectron.*, 22: 1776-1782.



Biosensors

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A biosensor is defined as a detecting device that combines a transducer with a biologically sensitive and selective component. When a specific target molecule interacts with the biological component, a signal is produced, at transducer level, proportional to the concentration of the substance. Therefore biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. Bringing together researchers from 11 different countries, this book covers a wide range of aspects and issues related to biosensor technology, such as biosensor applications in the fields of drug discovery, diagnostics and bacteria detection, optical biosensors, biotelemetry and algorithms applied to biosensing.

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