Zinc Oxide: A Novel Material for Biosensors (A review)

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Abstract: Biosensors provide great potential application in the area like health care, food industry, environmental monitoring etc. The biosensors' performance relies upon their matrix material which plays an important role in determining the sensitivity, stability and shelf-life of a biosensor. Nowadays Zinc Oxide (ZnO) is a key promising materials for biosensor application with improved analytical performance due to its various properties like high isoelectric point, high electron communication, non toxicity etc. It also imparts a steady immobilization for biomolecules maintaining their biological activity. In this review we mainly focused on ZnO based enzyme biosensors (glucose, cholesterol, etc.) and immunosensors. We also reviewed some papers on DNA biosensors based on ZnO.

1. Introduction:
There is necessity of having analytical devices which are able to perform quick and accurate analysis for monitoring and regulating various parameters in the areas like food industry, drug development, forensic, clinical diagnose etc [1]. A biosensor (figure 1) is nothing but an analytical device consisting of bioreceptors and transducer which converts biological response in to a measureable signal for monitoring a biological analyte, whose excess concentration can harm humans lives [2,3,4]. International Union of Pure and Applied Chemistry (IUPAC) defines biosensor as “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical components usually by electrical, thermal or optical signals” [6]. A bioreceptor is the most essential element of a biosensor which is responsible for the selective recognition of the analytes (glucose, cholesterol, urea etc.) and generating the physicochemical signal observed on the transducer i.e. it determines the sensitivity of the device [7]. A transducer is a device which can change a broad range of physical, chemical or biological effect into a digital signal (electrochemical, optical, calorimetric etc) with high sensitivity and minimum disturbance and play an important role in biosensor [8]. There are different kind of biosensors classified on the basis of bioreceptors types and transducer (Table I and II). Researchers have great interest towards

![Fig 1. Schematic diagram of biosensor [5]](image-url)
biosensors due to its portability, high specificity, simple to use and compatibility with data processing technologies [9]. In today’s world semiconductor materials in nano (10^{-9}) size earned much interest in biosensor’s application due to their unique properties. Among all the metal oxides Zinc Oxide (ZnO) is a key technological material due to its wide range of potential applications [10-15]. ZnO belongs to the richest family of nanostructures [15, 16].

ZnO nanostructures not only give high surface area [14, 17], good chemical and photochemical stability [14, 17], optical transparency [17], non-toxicity [14, 16] and biocompatibility [14, 17, 18] but also possesses high electron communication [12, 14], increased sensitivity, enhanced analytical performance and easy preparation [19]. ZnO is also comparatively stable around biological pH values which make it companionable with biological fluids and species. In addition to this ZnO is a suitable material for immobilization of biomolecules with low isoelectric point (IEP) because of its high IEP (9.5) [24, 20, 21]. These properties attracted ZnO for the biosensor application. It occurs frequently in nature, it is an inorganic compound also known as zincite. It is generally found as a white crystalline powder, which is nearly insoluble in water [22]. ZnO is one of the hardest II-VI semiconductor materials whose covalence lies at the boundary between ionic and covalent semiconductors with direct wide band gap of energy 3.37 eV at room temperature near UV spectral region and possesses large exciton binding energy of 60 meV [16, 18, 23, 24]. Usually ZnO exhibit hexagonal wurtzite structure [18]. It possesses optical, semiconducting, magnetic, piezoelectric and pyroelectric properties, good thermal properties like high thermal conductivity, high thermal capacity, low coefficient of thermal expansion and high melting point [12, 14, 25]. ZnO also exhibit different properties like strong adsorption ability, high catalytic efficiency, high breakdown strength, exciton stability etc [12, 26]. This article provides a comprehensive review on the application of ZnO in various biosensors.

2. Enzyme Biosensors:

An enzyme biosensor is an analytical device that combines an enzyme with a transducer to produce a signal proportional to target analyte concentration. Enzymes have found extensive used as sensing elements in biosensor due to their simplicity and catalytic properties [27].

Table I. Classification of biosensor based on the types of the bioreceptor molecules.

<table>
<thead>
<tr>
<th>Bioreceptors</th>
<th>Types of Biosensor</th>
</tr>
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<tbody>
<tr>
<td>Enzyme</td>
<td>Catalytic or enzyme biosensor</td>
</tr>
<tr>
<td>Affinity-complex forming biomolecules (membrane receptor, protein, antibody etc.)</td>
<td>Affinity based biosensor</td>
</tr>
<tr>
<td>• Antibody</td>
<td>• Immunosensors</td>
</tr>
<tr>
<td>• DNA</td>
<td>• DNA-based biosensor</td>
</tr>
<tr>
<td>Cell</td>
<td>Whole-cell biosensor</td>
</tr>
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</table>

Table II. Classification of biosensor based on transduction principle.

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Types of biosensor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemistry</td>
<td>Electrochemical biosensor</td>
</tr>
<tr>
<td>• Current</td>
<td>• Amperometric</td>
</tr>
<tr>
<td>• Charge</td>
<td>• Coulometric</td>
</tr>
<tr>
<td>• Voltage</td>
<td>• Potentiometric</td>
</tr>
<tr>
<td>• Conductivity</td>
<td>• Conductometric</td>
</tr>
<tr>
<td>• Impedance</td>
<td>• Impedimetric</td>
</tr>
<tr>
<td>• Field-effect</td>
<td>• Field-effect transistor based</td>
</tr>
<tr>
<td>Optics</td>
<td>Optical biosensor</td>
</tr>
<tr>
<td>• Absorbtion</td>
<td>• Colorometric</td>
</tr>
<tr>
<td>• Chemiluminescence</td>
<td>• Chemiluminescent</td>
</tr>
<tr>
<td>• Fluorescence</td>
<td>• Fluorescent</td>
</tr>
<tr>
<td>• Refractive index</td>
<td>• Surface Plasmon Resonance</td>
</tr>
<tr>
<td>Mass</td>
<td>Piezo-electric biosensor</td>
</tr>
</tbody>
</table>
2.1. Glucose Biosensor:

Glucose which is one of the major products of photosynthesis acts as the major source of energy in human body. Cellular adenosine triphosphate is generated only by converting glucose into glycosis in hard working muscles and hypoxic conditions. Brain is critically dependent on the constant supply of glucose. It also works as the metabolic intermediate for the synthesis of complex molecules such as fats. If the amount of sugar is not regulated properly in the blood, disease such as diabetes can be developed [28]. Around 200 million people suffered from diabetes mellitus all over the world which is expected to increase over 300 million by 2030. So, regular examination of sugar level in blood to prevent emergencies is a must [29]. A. Wei et al. [30] developed a glucose biosensor based on immobilization of glucose oxidase onto the ZnO nanorods developed on standard gold electrode using hydrothermal decomposition. They observed the cyclic voltammetry result of GOx/ZnO nanorods/Au electrode with and without glucose and found that the cyclic voltogram changed significantly when 5mM of glucose is added to it. The developed biosensor showed great sensitivity of 23.1μA/mMcm² and fast response time below 5s. It also linear range between 0.01 and 3.45mM (correction coefficient, R=0.9995), detection limit of 0.01mM and apparent Michaelis–Menten constant of 2.9mM (2006, enzymatic) [30]. Mashkoor Ahmad et al. [31] synthesize Zinc Oxide nanofibres in the diameter range of 350-195nm by electrosprinning technique through high temperature calcination of Poly (Vinyl Pyrrolidine)/Zinc acetate composite nanofibres for the fabrication of amperometric glucose biosensor. A single Zinc Oxide nanofibre was transferred to gold electrode and functionalized through physical absorption with glucose oxidase. The prepared biosensor displayed high sensitivity of 70μA/mMcm² and a response time of less than 4s [31]. Y. Wei et al. [32] synthesized ZnO nanorods/Au hybrid nanocomposite by developing Au nanocrystals on the surface of ZnO nanorods through hydrothermal route. Fabrication of glucose biosensor was done by trapping glucose oxidase onto the ZnO nanorods/Au hybrid nanocomposite matrix by cross linking method using gluteraldehyde and nafion solutions. Cyclic voltammetry study of GEC/ZnO/Au/GOx/Nafion electrode revealed that the biosensor showed high sensitivity of 1492μA/mMcm², fast response inside 5s, limit of detection of 10mM and apparent Michaelis–Menten constant of 0.41mM [32]. P. Norouzi et al. [33] fabricated a new glucose electrochemical biosensor with acceptable reproduceibility, good stability and low interferences based on the immobilization of glucose oxidase (GOx) on Zinc Oxide nanoparticles (ZnO NPs) doped in nanographene (NGs) sheets. They had used fast fourier
Transformation continuous cyclic voltammetry for monitoring of glucose as a detection method. The fabricated biosensor showed the linear response range between 0.1 to 20 μM and the detection limit was 0.02 μM at a signal to noise ratio of 3 [33]. S. Saha et al. [34] had successfully developed a novel hybrid matrix of Zinc Oxide methylene blue (ZnO-MB) for glucose biosensor application. The prepared matrix was deposited on to the Indium Tin Oxide (ITO) coated glass by spin coating. Glucose oxidase (GOx) was immobilized on to the prepared electrode. The electron transfer of the composite increased due to the introduction of methylene blue. The value of Michaelis- Menten constant was 2.6mM. The proposed biosensor exhibited enhance sensing response (2μA/mM-cm^-2) with long shelf life (>10 weeks) [34]. An electrochemical biosensor based on highly porous biomorphic ZnO nanostructured prepared by aqueous sol-gel method was fabricated by Hengameh Fatemi et al. [35] for the detection of glucose. The glassy carbon electrode (GCE) was modified by dropping Nafion on the pretreated surface of the electrode and then porous ZnO and glucose oxidase (GOx) were cast on the Nafion modified GCE surface. To prevent leakage of GOx, Nafion was then drop on the surface of Nafion/ZnO-GOx. The Nafion porous S-ZnO nanostructure Nafion/ZnO-GOD/Nafion/GC electrode shows response time of 7s, high sensitivity 23.4μA/nMcm^-2. The electron transfer rate constant, k_s of glucose oxidase is around 3.9s^-1 [35]. S. Palanisamy et al. [36] demonstrated an amperometric glucose biosensor based on zinc oxide (ZnO), electrochemically grown on multiwalled carbon nanotube modified glassy carbon electrode (GCE) which showed linear range of 0.2-27.2 mM with a detection limit of 20μM and sensitivity of 4.18. The fabricated biosensor exhibited good selectivity, good storage ability, acceptable reproducibility, repeatability. Glucose oxidase (GOx) was immobilized on MWCNT/ZnO composite and negatively charged GOx provide good stability to the immobilized GOx [36]. Gozde Aydogdu et al. [37] developed ZnO nanoparticles (ZnO NPs) modified carbon paste enzyme electrodes (ZnO NPs MCPE) for the determination of glucose in human serum. They developed highly sensitive electrochemical glucose oxidase (GOx) biosensor for the determination of glucose performed by immobilization of GOx within ZnO NPs MCPE. GOx, ZnO NPs modified carbon paste provided a micro-environment. It also provided a necessary pathway of electron transfer between the electrode and GOx. They characterized the bare and ZnO NPs modified electrode electrochemically. The developed biosensor exhibited high analytical performance with wide linear range (9.1x10-3-14.5 mM), high selectivity, sensitivity reproducibility, reusability, and storage stability [37]. M. Marie et al. [38] investigate a glucose electrochemical sensor based on Zinc Oxide nanorod grown on indium tin oxide coated glass substrate by hydrothermal sol-gel growth method. The high quality grown ZnO nanorods were characterized using XRD, SEM, absorption spectroscopy, Raman spectroscopy. Glucose oxidase was immobilized ZnO/ITO was characterized by cyclic voltammetry. The amperometric response of the developed biosensor was 3S. The sensitivity and selectivity of the proposed biosensor.

![Image](A) (A) (b) Cyclic voltammograms of ZnO-MB/ITO film at different scan rate (inset shows the variation of anodic and cathodic peak current with potential scan rate) (b) CV of GOx/ZnO-MB/ITO bio-electrode with different glucose concentration (Inset shows CV of ZnO MB/ITO and GOx/ZnO-MB/ITO electrodes). (B) Cyclic voltammograms of bare and ZnONPs modified carbon paste electrodes in 0.1 M pH 7.0 PBS in absence (a, c) and presence (b, d) of 1.0 mM H_2O at scan rate of 50 mV s^-1 [34], [37].
2.2. Cholesterol biosensor:

Cholesterol is an important lipid which is found in the cell membranes of all animal and human cells. It is the precursor for other biological material like steroids. Letha disease like arteriosclerosis, cerebral thrombosis, myocardial infarction, coronary disease and lipid metabolism dysfunction can be caused due to high cholesterol accumulation [39].

A Radio frequency sputtered Zinc Oxide nanoporous thin film based cholesterol biosensor was fabricated by S.P Singh et al. [40]. Physisorption technique was used to immobilize cholesterol oxidase on to modified gold electrode (ZnO/Au). The bio-electrode was studied by UV response and cyclic Voltammetry. The prepared Zinc Oxide thin film exhibited a desirable matrix for biomolecule immobilization and also fictionalization with various biomolecules enhanced the electron communication characteristic of ZnO. The developed biosensor provided a linear response and high sensitivity in the cholesterol concentration ranging from 25-400 mg/dl and stability about 10 week. Michaelis-Menten constant of 2.1 mM pointed the increased affinity of ChOx [40]. Raju Khan et al. [41] fabricated Zinc Oxide nanoparticles (ZnONPs)-Chitosan (CHIT) hybrid composite film based cholesterol biosensor. They immobilized Cholesterol Oxidase onto the prepared film by physical adsorption to detect the cholesterol in the sample. Electrochemical response of the prepared bio-electrode investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The bioelectrode exhibited linearity from 5 to 300 mg dl-1 of cholesterol with detection limit 5 mg dl-1 [42]. An ultra sensitive biosensor based on ZnO nanoparticles grown at low temperature on Au electrode for the detection of cholesterol was fabricated by Ahmad Umar et al. [42]. Cholesterol Oxidase was immobilized on prepared electrode by physisorption technique. The fabricated biosensor exhibit high sensitivity (23.7 µA mM⁻¹ cm⁻²), response time <5s, limits of detection 0.37±0.02 mM (based on S/N ratio). Pratima R. Solanki et al. [43] reported a cholesterol sensor based on Nanostructured Zinc Oxide (Nano-ZnO) platform. They synthesized Nano-ZnO film onto indium tin oxide coated electrode by sol-gel method. Cholesterol Oxidase (ChOx) was immobilized by physical adsorption on to Nano-ZnO/ITO film for the detection of cholesterol. The Nano-ZnO platform increased the electron transfer and gave suitable environment between ChOx and ITO electrode. The electrochemical response of the prepared bioelectrode was investigated by Cyclic Voltammetry (CV). The biosensor exhibit fast response time (10s), sensitivity of 0059µA/mgdll⁻¹cm², low detection limit of 0.5mg/dl, low Michaelis-Menten constant K_m=0.98mg/dl and detection range from 5 to 400mg/dl [43]. A high sensitive cholesterol biosensor based on ChOx immobilized well crystallized flower-shaped ZnO structures made of perfectly hexagonal shaped ZnO nanorods grown on gold electrode by simple low temperature solution method had been reported by Ahmad Umar et al. [44] which provided an extremely high and reproducible sensitivity (61.7 µAµM⁻¹cm⁻²) with a response time <5s and detection limit of 0.012 µM. The low value of Michaelis-Menten constant K_m=2.57 mM indicates the high affinity of the biosensor towards cholesterol [44]. Mashkoor Ahmad et al. [45] reported a high sensitive amperometric cholesterol biosensor, successfully made up with Pt-incorporated fullerene like ZnO nanosphere (Pt-ZONS) modified carbon electrode by electrophoistion. Cholesterol Oxidase was immobilized onto the modified electrode by physical adsorption. To avoid probable enzyme leakage and remove foreign interferences Nafion solution was dropped onto the prepared electrode. The fabricated biosensor exhibited a reproducible sensitivity of 1866.4 mMAM⁻¹cm⁻², linearity ranging from 0.5-15µm, response time 5s [45]. M. Q. Israr et al. [46] developed an electrochemical biosensor based on hexagonal ZnO nanorods (125-250 nm) directly grown on silver (Ag) wire using aqueous chemical approach for cholesterol sensing. They immobilized cholesterol oxidase enzyme by physical adsorption technique onto the grown ZnO nanorods. The electrochemical response of ChOx/ZnO/Ag biosensor was studied as a logarithmic function of cholesterol concentration. The fabricated biosensor showed high sensitivity of 35.2 mV per decade with good linearity, good response time at 10s and half life of 3 months [46]. A cholesterol biosensor based on ZnO nanostructured thin film was fabricated by Neha Batra et al. [47]. A vapor phase transport technique was used to synthesize ZnO nanostructured films onto platinum coated silicon (Pt/Si) substrate. Physical adsorption technique was used to immobilized cholesterol oxidase (ChOx) onto the ZnO based Pt/Si electrode. The electrochemical response of the prepared electrode was studied by cyclic voltammetry. The fabricated biosensor provided high stability, good selectivity high sensitivity of 153 mA mM⁻¹ cm⁻², a linear response in the range of 0.12 to 12.93 mM cholesterol concentration and low Michaelis-Menten constant of 1.08 mM [47]. Neha Batra et al. [48] fabricated a cholesterol biosensor based on Al doped ZnO thin film prepared by radio frequency sputtering. Cholesterol oxidase of low IEP (~4.7) was
immobilized on the surface of the prepared film matrix (Al:ZnO/Pt/glass) via physical adsorption for the detection of cholesterol. Cyclic voltammetry technique was used to study the electrochemical response of the fabricated bioelectrode. The prepared Al:ZnO/Pt/glass bioelectrode is highly stable, shows sensitivity of 173 μA/mMcm$^2$ and linear response time ranging from 0.6-12.9mM of cholesterol concentration [48]. Rafiq Ahmad et al. [49] reported a cholesterol biosensor based on aspect ratio controlled ZnO nanorods grown on silver electrodes in solution at 90°. Cholesterol oxidase enzyme was immobilized onto prepared electrode by physical adsorption. With larger ARZnO NRs, more immobilization of enzymes and faster electron conduction were obtained. Using cyclic voltammetry they investigated the performance of the biosensor and it provided a highly reproducible sensitivity (74.10 μA mM$^{-1}$ cm$^{-2}$), a linear response up to 16.0 mM, a fast response time of <2s and a detection limit of 0.0015μM (S/N=3). It also exhibit first-rate anti-interference ability against electroactive species like glucose, ascorbic acid, L-cysteine and uric acid [49].

A biosensor based on solution-gated field effect transistor fabricated with ZnO nanorods for the detection of cholesterol was reported by Rafiq Ahmad et al. [50]. Solution process was used for the fabrication of ZnO nanorods arrays on Si/SiO$_2$ via site selective growth of nanorods. Immobilization of cholesterol oxidase was done by physical adsorption process. The reported sensor exhibited a real time response for cholesterol concentration of 0.001-45 nM with high selectivity and sensitivity of 10μA cm$^{-2}$ mM$^{-1}$. It also showed limit of detection of about 0.05μM [50]. Vinod Kumar et al. [51] fabricated a FET admittance voltammetric biosensor for the detection of cholesterol based on MWCNT-ZnO nanoparticles. The prepared biosensor was fabricated by immobilization of cholesterol oxidase on to Zinc Oxide nanoparticles and Multiwall carbon nanotubes (MWCNTs) modified gold electrode. The proposed biosensor provided response time <8s, limit of detection 0.050±0.002 nM and stability for four weeks [51]. Rafiq Ahmad et al. [52] fabricated a cholesterol biosensor based on ZnO nanotube (ZNT) arrays grown by one step chemical process on Silicon/Silver (Si/Ag) substrate, which provided suitable platform for regular examination of cholesterol in human blood serum samples. For the detection of cholesterol they immobilized cholesterol oxidase using physical adsorption on to the Si/Ag/ZNT electrode. The developed cholesterol biosensor was studied by cyclic voltammetry and it provided high sensitivity (79.40μA/mMcm$^2$), wide linear range (10μM-130mM), fast response time -2s, low detection limit (0.5nM (S/N=3)) [52]. Qiong Wu et al. [53] recently reported a highly selective and sensitive amperometric cholesterol biosensor. The fabricated biosensor was based on ZnO films on Silver nanowire-graphene oxide and Chiston modified indium tin oxide (Ag NWs/GO-CS/ITO) electrode. ZnO nanocrystals were deposited onto the Ag NWs/GO-CS/ITO electrode by spin coating and Cholesterol Oxidase (ChOx) was immobilized on it for the detection of cholesterol. The Ag NWs - ZnO composite increased the electron communication. The electrochemical response of the prepared bio-electrode was studied by cyclic voltammetry and impedance measurement. The biosensor provided good reproducibility, stability, sensitivity of 9.2 μA μM$^{-1}$ cm$^{-2}$, linearity in the range of 0.25-400 mg dL$^{-1}$ and good selectivity towards cholesterol. The detection limit and the Michaelis-Menten constant ($K_M^{app}$) were 0.287 μM (S/N=3) and 0.295 μM respectively. The low value of $K_M^{app}$ implies the high affinity of the biosensor to cholesterol. For this sensor long term stability and exceptional anti-interference capability against electroactive species were observed [53].

![FESEM images of ZnO nanotubes before ChOx immobilization](image_url)
3. Immunosensors:

Affinity ligand-based biosensors solid-state devices in which immunochemical reaction is coupled to a transducer termed as immunosensors. Its fundamental basis is the specificity of the molecular recognition of antigens by antibodies to form a stable complex [54].

Xuxiao Lu et al. [55] fabricated a reagentless immunosensor based on gold nanowires and ZnO nanorods composites films onto the glassy carbon electrode for the detection of α-1-fetoprotein (AFP) in human serum. They synthesized gold nanowires and ZnO nanorods using potentiostatic electrodeposition. The α-1-fetoprotein antibody was immobilized onto the modified glassy carbon electrode. The developed immunosensor showed an outstanding amperometric response for APF ranging from 0.5 to 160.0ngmL\(^{-1}\) with a detection limit of 0.1ngmL\(^{-1}\). The proposed immunosensor showed high sensitivity, quick response, good reliability and long term stability [55]. For the immobilization of fluorescently labeled IgG antibodies on planar ZnO surfaces, Christopher D. Corso et al. [56] investigated the use of two different silane molecules (3glycidyloxypropyl) trimethoxysilane (GPS), and (3-mercaptopropyl) trimethoxysilane (MTS). The surface characteristics and the antibody surface coverage were analyzed by using atomic microscopy, water contact angle measurements and fluorescence microscopy. In immobilizing antibodies to the ZnO surface both GPS and MTS employed procedures were effective, but on average, greater surface coverage was offered by
MTS. We achieved uniform and repeatable surface coverage using both methods which is a necessary requirement for biosensor functionalization the necessary requirement is uniform and repeatable surface coverage, which was achieved by both methods. The antibody functionality was studied by ZnO-based acoustic resonators. The results showed that the sensitivity and specificity to the ZnO-based devices was imparted by immobilization procedure [56]. Anees A. Ansari et al. [57] co-immobilized rabbit immunoglogulin antibodies (r-IgGs) and brovine serum albumin (BSA) for the detection of ochratoxin-A (OTA) onto the nanostructured ZnO (Nano-ZnO) film deposited on the indium-tin-oxide (ITO) glass plate. Sol-gel technique was used to prepare Nano-ZnO film. Nano-ZnO/ITO electrode and BSA/r-IgGs/Nano immunoelectrode were characterized by Fourier Transform infrared spectroscopy (FTIR) spectroscopy, scanning electron microscope (SEM), electrochemical impedance spectroscopy (EIS) techniques. The prepared immunoelectrode provided detection limit of 0.006nM/dm$^3$, response time of 25s, linearity as 0.006-0.01nM/dm$^3$, sensitivity of 189$\Omega$/nM/dm$^2$ cm$^2$, regression coefficient of 0.997 [57]. For the detection of carbohydrate antigen 19-9 Baoxiang Gu et al. [58] fabricated an immunosensor based on ZnO quantum dots. By using colloidal procedure ZnO quantum dots were prepared. Through electrochemical and optical technique ZnO quantum dots and CA19-9 antibody (Ab)-Ag sandwich immunosensor assembled on the functioized Si substrate. The sensor exhibited higher sensitivity of 0.0047μA/U/ml, broad linear range 0.1-180U/ml, lower detection limit 0.04U/ml, good stability, reliability, reproducibility and the linear response of 1-180U/ml [58].

Fig 7. The Photoluminescence spectra of the immunosensors with a series concentration of CA 19-9 inserted with the spectral intensity vs. concentration of CA 19-9 in logarithmic style plot [58].
Silver nanoparticles decorated ZnO nanotubes based potentiometric immunosensor was fabricated by Z.H Ibupoto et al. [59] for the detection of d-dimer. ZnO nanotubes were synthesized using hydrothermal methods by chemical etching of nanorods. Electrodeposition technique was used to deposit silver nanoparticles on to the prepared nanotubes. The prepared immunosensor exhibited detection limit of \(1.00 \times 10^5\) µg/ml, fast response time (less than 5s) with high sensitivity, selectivity, storage ability and good reproducibility [59]. A interdigitated capacitive immunosensor based on ZnO nanorod deposited onto Au electrode was fabricated by P. Sanguino et al. [60]. By cross linking process anti horseradish peroxidase (anti-HRP) was immobilized onto the surface of ZnO nanorods. The prepared biosensor gained maximum response in the frequency range of 5-7 KHz, giving rise to the prospect of simplified single frequency detection system to guide antigen detection in complex biological sample [60]. Md. Azahar Ali et al. [61] fabricated an efficient, highly sensitive level-free, highly reproducible, selective immunosensor based on mesoporous Zinc Oxide nanofibers (ZnOnFs) with fiber diameter ranging from 50-150 nm for the detection of breast cancer biomarker. These mesoporous Zinc Oxide nanofibers were synthesized by electrospinning. Electrophoretically ZnOnFs were deposited on indium tin coated (ITO) glass substrates and conjugated with antibody of human epidermal growth factor receptor 2 (ErbB2) by covalent or physical electrostatic interactions. Surface oxygen plasma treatment on to the ZnOnFs surface created functional groups such as -COOH, -OH etc. because of the presence of residual carbon in ZnOnFs. Oxygen plasma treated ZnOnFs were effective for conjugation of anti ErbB2. Both fabricated untreated and oxy-plasma treated immunoelectrode were tested for comparison. For level free detection of ErbB2 they used electrochemical impedance spectroscopy (EIS). The charge transfer resistance \(R_{CT}\) in EIS spectra increased with the increasing concentration of ErbB2 due to the interaction of specific binding sites of the antigen and antibody on the transducer surface form an immunocomplex layer that obstructs the electron transfer from the bulk solution. The \(R_{CT}\) value of anti ErbB2-ZnOnFs electrode is higher than that of anti ErbB2-ZnOnFs may be because of the available functional group created after the treatment of oxygen plasma on to the ZnOnFs surface. Anti ErbB2-ZnOnFs electrode \((K_a=404.8 \text{ kM}^{-1} \text{s}^{-1})\) had high affinity towards the biomarker (ErbB2 antigen) compared to that of Anti ErbB2-ZnOnFs electrode \((K_a=165.6 \text{ kM}^{-1} \text{s}^{-1})\). The fabricated immunosensor provided high sensitivity \((7.76\text{K} \Omega \mu \text{M}^{-1})\), fast response time \((128\text{s})\) in a wide detection test range \((1.0f-0.5\mu\text{M})\) [61].
An immunosensor based on a nanohybrid of platinum nanoparticles-porous ZnO sphere-hemin (Pt-pZnO-hemin) was fabricated by Zhen-Hua Yang et al. [62] to detect influenza. Hydrothermal method and ester like bridging between p-ZnO and hemin was used to synthesize nanohybrid of Pt-pZnO-hemin. The developed sensor provided high specificity, acceptable reproducibility and good stability for influenza detection [62].

4. Other biosensors:

ZnO provided good chemical stability, wonderful biocompatibility, easy surface modification, and various types of nanostructures which make it promising material for DNA biosensor. A simple method for the immobilization of oligonucleotides onto ZnO surface by electrochemical covalent grafting of diazonium salts was reported by Chun Wang et al. [63]. The, X-ray photoelectron spectroscopy, surface contact angle system, and electrochemical workstation were employed to characterize the functionalization process was characterized using X-ray photoelectron spectroscopy, atomic force microscope, surface contact angle system and electrochemical workstation. The fabricated biosensor distinguished four-base, one-base mismatched and complementary DNA sequences. A linear relationship between the fluorescence intensity and the concentration of the complementary DNA solution was observed within the range from $10^{-6}$ to $10^{-9}$ M [63]. Maumita Das et al. [64] fabricated nanostructured Zinc Oxide (nsZnO) films onto conducting indium tin oxide (ITO) coated glass plate using cathodic electro-deposition for electrochemical DNA biosensor. Using electrostatic interactions between the positively charged ZnO (IEP=9.5) and the negatively charged DNA (IEP~4) probe DNA specific to M. tuberculosis was immobilized onto the prepared film. The DNA-nsZnO/ITO bioelectrode showed different characteristics like range of detection $1\times10^{-6}$ to $1\times10^{-12}$ M, detection limit of $1\times10^{-12}$ M and $1\times10$–$13$ M for complementary target and genomic DNA respectively, response time of 60s , reusability of about 10 times and stability up to 4 months (kept at 4°C) [64].

In addition to glucose and cholesterol enzyme biosensors other enzyme based on ZnO are reported by many researchers. Most of phenolic
compounds can harm human health and the environment, so determination of these phenolic compounds is very important. For determination of phenolic compounds Jianwen Zhao et al. [65] developed biofunctional ZnO nanorod microarrays on the boron doped nanocrystalline diamond (BDND) based tyrosinase biosensor. Using a low-temperature solution method firstly ZnO nanorod microarrays were deposited on BDND thin film, and then using co-condensation approach ZnO nanorods were functionalized with 3-aminopropyltriethoxysilane (APTES) and tetraethoxysilane (TEOS) mixture. Tyrosinase was immobilized by the covalent binding onto functionalized ZnO nanorod surfaces. The biosensor exhibited a linear response range and sensitivity of 1-175 μM and 576.2 μA mmol⁻¹ cm⁻² for p-cresol, 1-150 μM and 339.3 μA mmol⁻¹ cm⁻² for 4-chlorophenol, 1-150 μM and 287.1 μA mmol⁻¹ cm⁻² for phenol respectively. The estimated low detection limit was 0.1, 0.25 and 0.2 μM (sb/m=3) for p-cresol, 4-chlorophenol and phenol respectively [65]. Z. H Ibupto et. al. [66] successfully developed a L-Ascorbic acid biosensor base on immobilized ascorbic oxidase enzyme with cross linking molecule 3-glycidoxypropyl 1-trimethoxysilane (GPTS) on to the well aligned, perpendicularly grown ZnO nanorod on to the gold substrate by hydrothermal growth method. The proposed biosensor showed sensitivity of 32MV/decade, fast response time less than 10 sec, better selectivity, and good stability. These qualities made the biosensor favorable for determination and monitoring of vitamin C in food, drugs and real sample [66].

A Co-Doped ZnO Nanomaterial based Hydrogen Peroxide Biosensor was fabricated by Wen Zhang et. al. [67]. Co-doped ZnO nanofilms had good conductivity and fast electron transfer in electrocatalytic reduction. The sensitivity of the proposed biosensor were 22.7 μA/mM and 2.93 mM, respectively with a broad linear response in the range of 0.05 to 1.15 mM and the detection limit was 13 μM (S/N=3) within 10 s at an applied working potential of -0.3 V [67]. A nitrogen doped Zinc oxide thin films based biosensor for the detection of uric acid was fabricated by K. Jindal et.al. [68]. N-doping improving the charge transfer characteristic. The bio-electrode based on the nitrogen-doped ZnO thin film matrix exhibits better sensitivity (1.1 mA mM⁻¹ cm⁻2) with linearity over a wide range (0.05 mM to 1.0 mM) of uric acid concentration. The Michaeli-Menten constant (Km) value was 0.10mM [68].

5. Conclusion:
In this review we have discussed the application of ZnO in biosensors. We have discussed the use of ZnO in glucose, cholesterol, immnosensors and others biosensors. The biocompatibility, bio-safety and other biological properties of ZnO have been pointed out. We have discussed the use of ZnO in glucose, cholesterol, immnosensors and others biosensors.

4. References


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