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REVIEW

Bilirubin enzyme biosensor: potentiality and recent advances towards clinical bioanalysis

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Abstract Bilirubin detection plays a major role in healthcare. Its high concentration in human serum is lethal and must be determined accurately. Clinically, it is vital for assessing patients with deleterious health conditions such as jaundice or icterus, hepatitis, mental disorders, cerebral palsy and brain damage especially in the case of neonates. In evaluating the drawbacks regarding the conventional methodology of bilirubin detection, there is need for a superior analytical tool. Bilirubin oxidase (BOx)-based sensors have been designed for the ultrasensitive analysis of bilirubin and quality deliverance of treatment and this review highlights the different mechanisms of bilirubin detection using different modified electrodes. Further, it also addresses the exploitation of highly attractive electrocatalytic properties of elite nanoparticles such as gold and zirconia- coated silica nanoparticles in enhancing the reproducibility and specificity of bilirubin biosensors.

Keywords Bilirubin · Bilirubin oxidase · Biosensor · Conducting polymers · Nanoparticles

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Introduction

Bilirubin ($C_{33}H_{36}N_4O_6$) is important in the physiological system. It is the focus of intense clinical research. It is a yellow tetrapyrrole formed as the breakdown product of heme catabolism in red blood cells and exists in human serum as an unconjugated form (Avramescu et al. 2004). Free bilirubin, also known as bilirubin IX, has a lipophilic nature. In the blood circulatory system, most of the unconjugated bilirubin is bound to the albumin to form a water-soluble complex and excreted as bilirubin glucuronides from the hepatic cells of the liver into the bile (Wang et al. 2006; Andreu et al. 2002). Bilirubin possesses significant antioxidant properties (Baranano et al. 2002; Lui et al. 2008) as it prevents the oxidation of vitamin A and polyunsaturated fatty acids. Bilirubin is a potent scavenger of peroxyl radicals (Bulmer et al. 2008) and therefore fulfills a useful physiological function. It is normal to have some bilirubin in the blood; an average level is 0.3–1.9 mg/100 ml. A low concentration of bilirubin in the blood is associated with coronary artery disease and iron deficiency. When the level of serum bilirubin surpasses to 2.5 mg/100 ml, the condition is known as hyperbilirubinemia. Free bilirubin is unexcretable and hence it is better to determine the concentration of this free bilirubin in blood serum to assess the risk caused by hyperbilirubinemia in jaundiced patients. In hyperbilirubinemia, bilirubin binds to and deposits on various tissues causing disorders in the metabolism of bilirubin and

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leads to deleterious effects such as jaundice or icterus, hepatitis, mental disorders, cerebral palsy, brain damage and even death (especially in the case of neonates) (Khan and Tayyab 2001; Kaplan and Hammerman 2005). In both adults and infants, the yellowing of skin and eyes, dark urine, itchy skin, fatigue and low appetite are some of the symptoms related to jaundice. Neonatal jaundice is common as more or less every newborn has an elevated level of bilirubin which can be serious if left untreated. Several disorders linked to high level of bilirubin in serum are shown in Fig. 1. Therefore, the clinical determination of serum bilirubin levels is carried out in the newborns at about 24 h age in most of the hospitals on a routine basis. Transcutaneous bilirubinometry is used as a screening tool for the diagnosis of jaundice in hospitals but it is not reliable for the accurate measurement of serum bilirubin in neonates (Samanta et al. 2004). Thus, it is essential to find new methods that can accurately measure the bilirubin levels in biological fluid.

Various approaches have been developed for the estimation of bilirubin concentration in the serum samples. The most prevalent detection methods are: a direct spectroscopic measurement and a diazo reaction. However, these methods have limitations too. The interference of other heme proteins in the blood hinders the direct spectroscopic measurement of bilirubin while the pH dependence of the diazo reaction makes it even less reliable. Fluorimetry and polarography are other methods used to analyse bilirubin content in serum samples. These standard/conventional techniques have limitations regarding tedious sample preparation, time-consumption, less specificity, high cost and complexity in achieving on-site, real-time monitoring of sample. To overcome the shortcomings mentioned above, researchers have developed biosensors that are innovative, robust, sensitive and automated analytical devices to meet the growing demand for the screening of bilirubin in jaundiced patients.

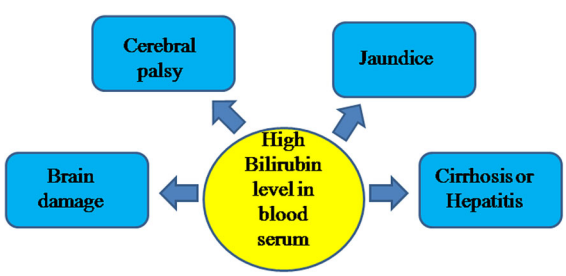

Fig. 1 Several disorders related to high level of bilirubin

Biosensors: basic concept and classification

A biosensor is basically a device that transforms certain biological process into signal which can be read and analyzed. It is made up of a biological sensing element or bioreceptor that constitutes of enzymes, antibodies, nucleic acids etc. and a physical (e.g., optical, mass, or electrochemical) transducer. The transducer part collects information from physico-chemical change (i.e. electron transfer, heat transfer, pH change, mass change, uptake or release of specific ions or gases) generated by the interaction between the bioelement and the analyte being tested. Then the transducer converts this change into electric signals. The electric signal is amplified, interpreted and finally displayed accounting to the concentration of analyte present in the sample. The basic components of a biosensor are depicted in Fig. 2. Biosensors have an interdisciplinary design which combines computational abilities of microprocessors with the sensitivity and specificity of biological systems. Biosensors are classified into following categories depending on the type of transducer used in their fabrication as shown in Fig. 3.

Electrochemical biosensor

The basic principle of a electrochemical biosensor is that the production or consumption of ions or electrons during a chemical reaction between the immobilized biomolecule and target analyte influence measurable electrical properties (e.g. electric current or potential) of the solution. Depending on their operating principle, the electrochemical biosensors can employ amperometric, conductometric and potentiometric transducers for the conversion of the chemical information into a measurable amperometric signal.

Amperometric biosensor

An amperometric biosensor is a self-contained integrated device which provides quantitative analytical information by measuring the electronic current produced from the oxidation or reduction of an

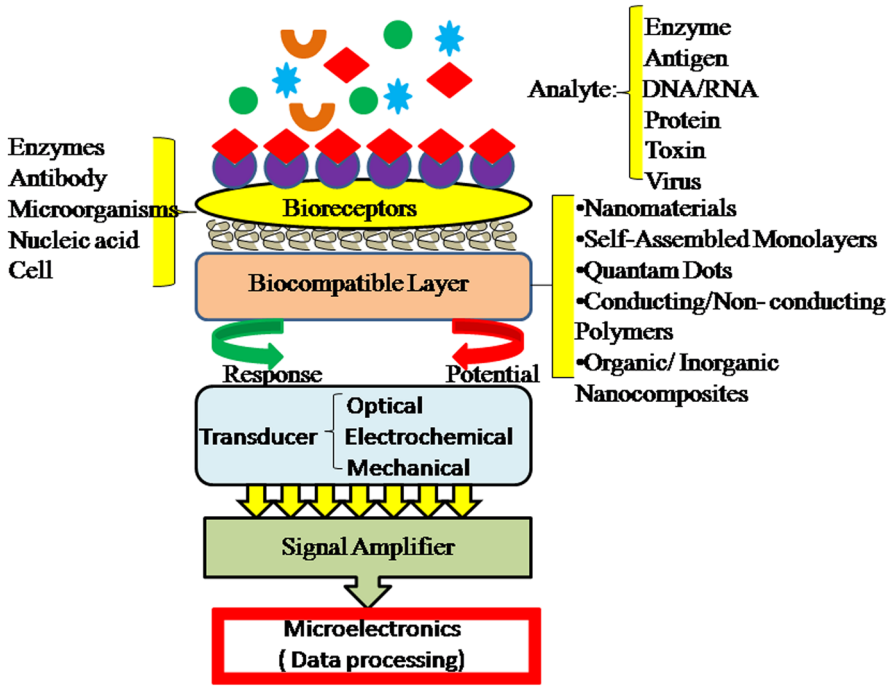

Fig. 2 Basic principle of a biosensor

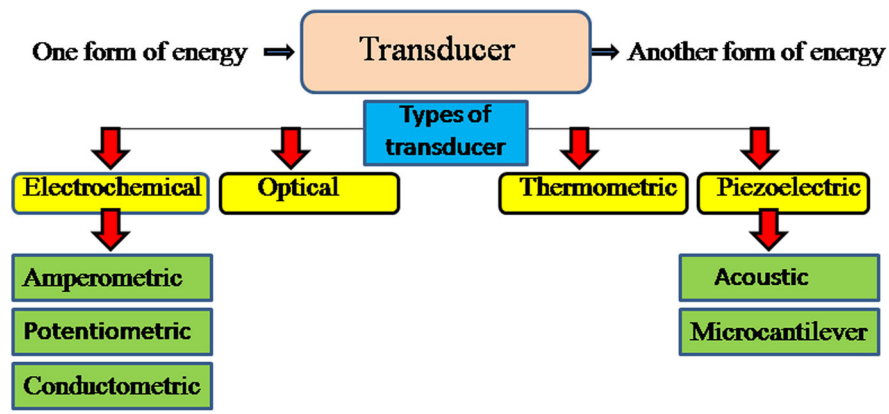

Fig. 3 Different types of transducers used in biosensors

electroactive biological element. The magnitude of the electric current produced at the surface of electrode as a result of the electrocatalytic reaction of the electroactive species is directly proportional to the concentration of the analyte present in the sample.

Potentiometric biosensor

A potentiometric biosensor employs ion-selective electrodes to transduce the biological reaction into the electric signal. In this type of biosensor, a biorecognition element is combined with a transducer that senses the concentration variation of an ionic species and then the analytical signal being logarithmically correlated with the concentration of analyte is recorded.

Conductometric biosensor

A conductometric biosensor can detect the changes in the electrical conductance of sample solution due to the presence of the analyte. The ionic species concentration changes as a result of the reaction between the biomolecule and analyte which in turn leads to change in the electrical conductivity or current flow of the solution.

Optical biosensor

The basic principle involved in optical biosensors relies on the optical measurements like fluorescence, absorbance and chemiluminescence. The optical transducers make use of photons to detect the analyte concentration. These biosensors utilize an enzyme system that converts the target analyte into product which is either oxidized or reduced at the surface of working electrode.

Thermometric biosensor

Thermometric biosensors or calorimetric biosensors depend upon the basic property of some biological reactions which are endothermic or exothermic in nature. The difference in the temperature between the substrate and product within the reaction medium as a result of heat absorbed or released is measured by thermistors. Thermal biosensors can detect even a small change in the temperature.

Piezoelectric biosensor

Piezoelectric biosensors work on the principle of acoustics (sound vibrations), thus they are also known as acoustic biosensors. Piezoelectric crystals and the vibration of these crystals with positive and negative charges showing characteristic frequencies form the basics of these biosensors. Electronic devices can be used to measure the alterations in the resonance frequencies due to adsorption of certain molecules on the crystal surface.

Enzymes involved in the fabrication of biosensors for bilirubin detection

Among the various multicopper oxidases (MCOs), bilirubin oxidase (BOx) has been used for the preparation of biosensors. Electrochemical amperometric bilirubin biosensors have an advantage of the specificity and selectivity of bilirubin oxidase to substitute the classical analytical methods of bilirubin estimation. These biosensors are comparatively more rapid and require no sample pre-treatment. Bilirubin oxidase (BOx, EC 1.3.3.5) is a 'blue' multicopper oxidase (MCO) and consists of three different copper redox sites (Mano 2012). BOx has four coppers in the active site which are classified on the basis of their spectroscopic and magnetic properties into three types- type 1, type 2, and type 3. The type 1 copper is connected to the type 2 and type 3 coppers by a His-Cys-His tripeptide to form a trinuclear copper cluster. There is intramolecular electron transfer from the type 1 copper to the trinuclear copper center. The reduction of O_2 to water takes place at the type2/type3 copper center while the substrate oxidation occurs at the type 1 copper ion (Shleev et al. 2004) as presented in Fig. 4.

Bilirubin oxidase (BOx) from *Myrothecium verrucaria* has a monomeric structure and molecular mass of 66 kDa. Other bilirubin oxidases like Tt-BOx obtained from *Trachyderma tsunodae* and Mv-Box obtained from *Myrothecium verrucaria* has also been reported for the electrochemical detection of bilirubin. Durand et al. (2012) identified and isolated a novel bilirubin oxidase (BOx) from the rice blast fungus *Magnaporthe oryzae*. The yield of BOx isolated from *Magnaporthe oryzae* is almost twice than that of *Myrothecium verrucaria.* It has the lowest K_m value and high k_{cat}/K_m ratio for conjugated bilirubin (1513 mM^{-1} s^{-1}) which makes it desirable for the detection of bilirubin in medical samples. BOx shows high activity even under alkaline or neutral pH and hence it is an enzyme of great importance. The isoelectric point of BOx is 4.2 (Tanaka and Murao 1982) and unlike other multicopper oxidases, it is catalytically active in high chloride concentrations. Murao and Tanaka reported that BOx catalyze the oxidation of both conjugated and unconjugated bilirubin to biliverdin (Eq. 1) which is then further oxidized to an unknown compound(s), (Eq. 2)

$$\text{Bilirubin} + \frac{1}{2}O_2 \overset{\text{BOx}}{\rightarrow} \text{Biliverdin} + H_2O \quad (1)$$

$$\text{Biliverdin} + O_2 \rightarrow \text{Purple compound(s)} + H_2O \quad (2)$$

The property of BOx O_2 as the other substrate and having a wide range of substrate specificity suggest its role in various industrial applications like development of biofuels cells and biosensors. Dehghani et al. (2016) fabricated an amperometric biosensor using catalase (EC 1.11.1.6) from human erythrocytes and a carbon paste electrode modified with ZnS nanoparticles for the detection of bilirubin. The ZnS nanoparticles act as a bioelectrochemical facilitator for the direct transfer of electrons. This bilirubin biosensor

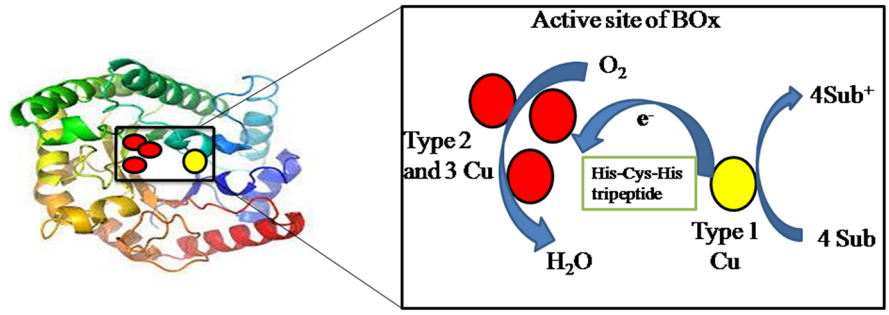

Fig. 4 Schematic showing active site of bilirubin oxide

exhibited a high sensitivity, good reproducibility, stability and low interference by other agents. These results demonstrated that ZnS nanoparticles are an attractive material for the construction of efficient nano based biosensors. Also the fast electron transfer frequency and excellent catalytic capability to the oxidation of bilirubin to biliverdin showed that the catalase retains its bioactivity fine.

Bienzyme system

Wang and Ozsoz (1990) developed a polishable amperometric electrode incorporating horseradish peroxidase and bilirubin oxidase embedded within a graphite-epoxy matrix. This bienzyme electrode was renewable (by polishing) and offered low-potential detection of bilirubin without any interference from other electroactive species. Thus it monitored H_2O_2 liberated at oxidase-based amperometric electodes. BOx catalyzed the oxidation of bilirubin to biliverdin. During this chemical reaction, liberated H_2O_2 was measured in the presence of co-immobilized peroxidase

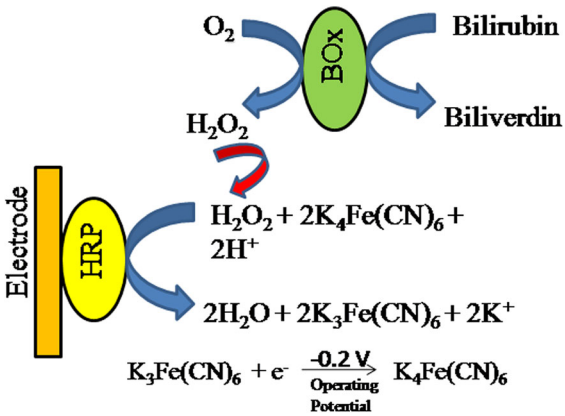

Fig. 5 Schematic representation showing coupling of bilirubin oxidase and HRP for bilirubin detection

and dissolved ferrocyanide as shown in Fig. 5. The liberated potassium ferricyanide was then detected amperometrically by reduction. The reduction current was directly proportional to the concentration of bilirubin. The linear response was up to 10^{-4} M and limit of detection was 4×10^{-6} M. The fabrication scheme was easy and the response time was short.

Methods to connect BOx on the surface of electrodes

There are two groups of BOx electrodes on the basis of the electrical connection between the redox enzyme and the electrode. The first group employs a direct connection of enzyme on the surface of electrode (DET) (Liu et al. 2013) and the second group—MET (mediated electron transfer) was a redox mediator to shuttle electrons from the electrode to the enzyme. Redox mediators are chemical species like Os and Ru complexes, ferrocenes etc. DET is generally preferred over the MET because of its simplicity and decreased overpotentials. The proper orientation of the enzyme and close proximity of the redox center of the enzyme to the surface of electrode enhances the influence of DET. But the number of enzymes that are capable of DET are substantially fewer (Salaj-Kosla et al. 2013). Due to the multiple orientation of the enzyme, the rate of electron transfer may be slow in DET (Léqer and Bertrand 2008). On the other hand, a redox mediator increases the rate of electron transfer by decreasing the distance between the redox center of the enzyme and the electrode surface. A mediator is actually a low molecular weight artificial electroactive species which acts as shuttle or bridge between the redox center of the enzyme and the surface of electrode for the transfer of electrons. The role of a mediator in the transfer of electrons has been depicted in Fig. 6.

Different biosensors designed for the detection of bilirubin

Various methods for the determination of bilirubin have been developed (Table 1). It is worth comparing the analytical performances of the available biosensors based on enzymes, chemically-modified electrodes and other polymer composites. Shoham et al. (1995) reported a bilirubin biosensor using gold electrodes with the self-assembled multilayer of BOx. When stored at 4 °C, the shelf-life of this biosensor was more than three months and the limit of detection was 17 μM. Later an enzymatic biosensor with the detection limit of 0.7 μM was reported by Fortuney et al. (1996). A crosslinked adduct of bilirubin oxidase, BSA and glutaraldehyde was immobilized on a platinum electrode and the level of bilirubin was estimated by monitoring the increase in H_2O_2 during the reaction. A fiber optic fluorescence biosensor was developed by Li and Rosenzweig (1997). A miniaturized form of this biosensor was designed for the rapid analysis of bilirubin. In a fiber optic biosensor, there is indirect method for detecting bilirubin which involves the fluorescence quenching of tris(4,7-diphenyl-1,10-phenanthroline) ruthenium chloride, $[Ru(dpp)_3]$, by dissolved O_2. O_2 is depleted in the oxidation of bilirubin to biliverdin, catalyzed by the enzyme bilirubin oxidase (BOx). The storage stability of the fiber optic biosensor was about 20 days in 8% (v/v) isobutanol in a phosphate buffer at pH 6. The linear response was between 10^{-7} and 3×10^{-4} M and the limit of detection of the sensor was 50 μM. The sensor has the advantage of short response time of about 10 s.

Klemm et al. (2000) described the method of indirect monitoring of bilirubin through its reaction with oxygen to biliverdin by chemically bonding the bilirubin oxidase to different types of pre-activated membranes and measuring the amperometric signals by depletion of O_2 by using an oxygen electrode. A detection limit of 8 μM was achieved by this biosensor and it was fast (<3 min) and interference free. The linearity range of the biosensor was up to 2×10^{-4} M which corresponds to the elevated concentrations of bilirubin in newborn infants and adults. Rahman et al. (2008) fabricated a bilirubin biosensor by complexing Mn(II) ion with a conducting polymer poly-5,2′-5′,2″-terthiophene-3-carboxylic acid $(PolyTTCA)^+$ and then a thin film of polyethyleneimine (PEI) with embedded ascorbate oxidase (AsOx) enzyme was coated on the surface of biosensor. The formation of Mn–O bond between the Mn(II) and the conducting polymer was confirmed by XPS and cyclic voltammetry was used to characterize the PolyTTCA–Mn(II) complex. The PolyTTCA–Mn(II)/PEI–AsOx biosensor specifically detect bilirubin by the mediated electron transfer mechanism of the Mn(II) ion. Ascorbate oxidase (AsOx) was used to avoid interferences from other biologically active compounds like ascorbic acid. Various experimental parameters such as temperature, pH and applied potential which affect the detection of bilirubin were optimized. The bilirubin sensor exhibited response time of less than 5 s.

Nanoparticle-based bilirubin biosensors

Wang et al. (2009) developed a ferrocenecarboxamide-modified electrode with gold nanoparticles and multiwall carbon nanotubes. The modified electrode had an enhanced electrocatalytic effect on bilirubin. This biosensor exhibited short response time of less than 5 s, an excellent broad linear range of 1 to 100 μM as well as a good detection limit of 0.12 μM.

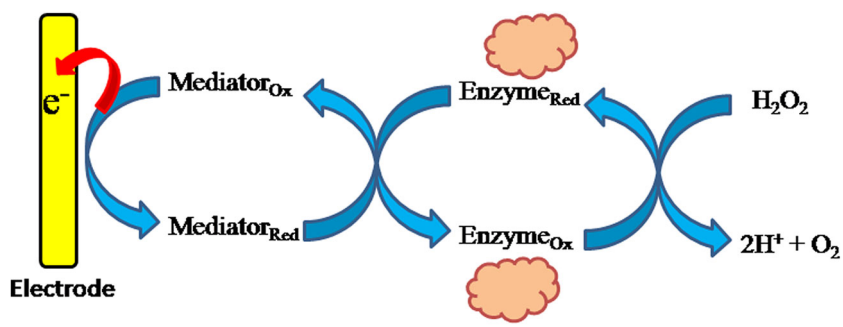

Fig. 6 Role of a mediator in election transfer

Table 1 Various Bilirubin biosensors fabricated for the determination of bilirubin in serum

Mode of detection	Transducer	Method of monitoring	Response time	Minimum detection limit	Linear range	Storage stability	References
Amperometric	Nanostructured gold electrode	Decrease in O_2	–	6 ± 1 μM	6×10^{-6}–3×10^{4} M	–	Pita et al. (2013)
Amperometric	Au/MPTS/AuNPs/BOx electrode	Oxidation of bilirubin	–	1.4 μM	1×10^{-6}–5×10^{3} M	80 days	Kannan et al. (2011)
Amperometric	BOx immobilized on a oxygen electrode	Decrease in O_2	<3 min	8×10^{-6} M	1–2.0×10^{-4} M	30 days	Klemm et al. (2000)
Amperometric	Poly-TTCA-Mn(II)/PEI-AsOx on GC electrode	Mediated electron transfer by Mn(II)	<5 s	40 ± 3.8 nM	1×10^{-7}–5×10^{-5} M	60 days	Rahman et al. (2008)
Amperometric	BOx/SiO_2@ZrONPs/CHIT/Au electrode	Increase in H_2O_2	2 s	0.1 μM	2×10^{-8}–25×10^{-5} M	120 days	Batra et al. (2013)
Fiber optic	[$Ru(dpp)_3$] as fluorescent probe	Fluorescence quenching	10 s	50 μM	1×10^{-7}–3×10^{-4} M	20 days	Li et al. (1997)
Amperometric	BOx immobilized on a platinum electrode	Increase in H_2O_2	–	700 μM	1×10^{-6}–3×10^{-4} M	30 days	Fortuney et al. (1996)
Amperometric	Incorporation of BOx and HRP within a graphite-epoxy matrix	Increase in H_2O_2	–	4000 μM	4×10^{-6}–1×10^{-4} M	–	Wang et al. (1990)
Amperometric	Ferrocenecarboxamide modified MWCNT/AuNPs electrode	Oxidation of bilirubin	<5 s	120 μM	1×10^{-6}–1×10^{-4} M	30 days	Wang et al. (2009)
Amperometric	BOx/PPyNPs/PANI/Au electrode	Increase in H_2O_2	2 s	0.1 μmol/L	0.01–320 μmol/L	60 days	Batra et al. (2015)
Amperometric	BOx/nano Au/CHIT/ micro AuE	Increase in H_2O_2	2 s	0.005 μM	1×10^{-8}–5×10^{-4} M	30 days	Narang et al. (2015)
Amperometric	BOx/GONP@Ppy/FTO	Increase in H_2O_2	2 s	0.1 nM	1×10^{-8}–5×10^{-4} M	150 days	Chauhan et al. (2016)
Colorimetric/ Fluorimetric techniques	(2,2′-((1E,1′E)-((6-bromopyridine-2,3-diyl) bis(azanylylidene)) bis(methanylylidene diphenol) (BAMD)	FRET mechanism	–	2.8 pM	1×10^{-12}–5×10^{-4} M	870 ps	Ellairaja et al. (2017)

Kannan et al. (2011) developed a highly specific and efficient electrochemical bilirubin biosensor by incorporating BOx onto the gold nanoparticles (AuNPs). Bilirubin was detected by using gold nanoparticles and enzyme on sol–gel film modified electrode. The use of sol-gel technology involves the formation of three dimensional silicate networks by the condensation of precursors of silicon alkoxide. In the development of sensing devices, this sol-gel derived 3-D network offer amazing application because of its chemical inertness and high thermal stability (Collinson 2007; Kannan and John 2010). The properties of gold nanoparticles (AuNPs) such as high surface energy, high specific surface area and high conductivity make them a promising nanomaterial for the biosensors (Kannan and John 2011; Rosi and Mirkin 2005; Shipway et al. 2000; Daniel and Astruc 2003; Zhong and Maye 2001; Guo et al. 2010; Si et al. 2011; Guo et al. 2011). AuNPs have been extensively used for the immobilization of various oxidases in the construction of biofuel cell (Murata et al. 2009) and development of biosensors (Zhang et al. 2005; Yang et al. 2006; Jena and Raj 2006; Hoshi et al. 2007; Rakhi et al. 2009; Saxena et al. 2011). Biosensors based on the enzyme/nanoparticles complex showed better performance in terms of sensitivity and stability than other existing enzyme and polymer based electrodes. The low detection limit for the bilirubin at the Au/MPTS/AuNPs/BOx electrode was 1.4 nM. The modified electrode had a stable response for long time. The analytical performance of this biosensor was superior to that of previously reported biosensors. Batra et al. (2013) reported a highly sensitive electrochemical bilirubin biosensor using zirconia coated silica nanoparticles with the detection limit of 0.1 nM and response time of 2 s. BOx was covalently immobilized onto SiO_2@-ZrONPs/CHIT-modified Au electrode through glutaraldehyde coupling. This enzyme electrode acted as the working electrode and zirconia coated silica nanoparticles and CHIT contributed to the electrocatalytic behaviour for the H_2O_2 electron transfer to the electrode. The biosensor exhibited a wider linear range from 2×10^{-8} to 25×10^{-5} M with excellent sensitivity.

An oxygen biosensor based on the nanostructured bielectrodes with the linear range of 6–300 μM and a detection limit of 6 ± 1 μM was reported by Pita et al. (2013). Bilirubin oxidase was covalently immobilized on the scaffold of gold disk electrodes modified with the gold nanoparticles. Pita et al. (2013) aimed to design a sensitive biosensor which could be operated even in buffer solutions and complex physiological fluids. This is the first ever reported biosensor that involves the direct electron transfer of bilirubin oxidase for the monitoring of depletion of O_2 by chronoamperometric measurement. Batra et al. (2015) fabricated a bilirubin biosensor by covalently immobilizing the *Myrothecium verrucaria* bilirubin oxidase onto the polypyrrole nanoparticles (PPyNPs) and polyaniline (PANI) nanocomposite film, electrodeposited onto gold electrode. The biosensor had excellent sensitivity with the minimum detection limit of 0.1 μM and wide linear range. Narang et al. (2015) simultaneously developed a third generation biosensor by employing gold nanorods and gold microelectrodes for immobilizing the bilirubin oxidase enzyme and amplifying the signal for bilirubin detection. The biosensor exhibited good working range of 0.01 to 500 μM. An electrochemical biosensor was developed by Chauhan et al. (2016) by fabricating a working electrode (BOx/GONP@Ppy/FTO) for the detection of bilirubin. A linear response with the range of 0.01–500 μM was exhibited and the biosensor could be used many times after storing it at 4 °C.

Fluorimetry and colorimetry based bilirubin biosensors

In the field of designing bilirubin biosensors, Santhosh et al. (2014) for the first time demonstrated a highly reliable and sensitive method based on the fluorometry and colorimetry for the determination of free bilirubin in human serum. The biosensor used HAS (natural carrier of bilirubin) stabilized gold nanoclusters as detection probe. The principle behind this newer method is the static interaction between bilirubin and HSA-AuNCs followed by the fluorescence quenching phenomena to obtain a interference free determination of bilirubin. The minimum limit of detection through this fluorometric method was 248 ± 12 nM. The biosensor is applicable at wide range of pH and temperature. Further, the similar nature of HSA-AuNCs as that of a peroxidase was exploited to oxidize the free bilirubin to a colorless compound in the colorimetric method which can be monitored visually. In the colorimetric method, a detection limit

of 200 ± 19 nM was achieved followed by the decrease in absorbance at 440 nm.

Colorimetric and fluorometric methods have been successfully applied for detection of free bilirubin in blood serum sample. A new approach was reported by Ellairaja et al. (2017) for synthesizing an imine to develop an optical biosensor for the determination of bilirubin in human urine and blood samples. 2,2′-((1E,1′E)-(6-Bromopyridine-2,3-diyl)bis(azanylylidene)) bis(methanylylidene diphenol (BAMD) was used to design a fluorescent platform for the detection of bilirubin using both fluorimetric and colorimetric techniques. The biosensor showed minimum detection limit of 2.8 pM and linear range of 1 pM to 500 μM for highly selective detection of bilirubin even in the presence of interfering metal ions.

Conclusion and future perspectives

In the development of a biosensor, two aspects are significant—the biological component that determines the selectivity and the transducer that determines the sensitivity. To guarantee the maximum selectivity, the active site of a biological molecule must be accessible physically or/and chemically and it should be free from steric effects. The tendency is to produce more sophisticated and specific surface transducers using nanotechnological tools and surface engineering to get the best biosensor device. If this happens, health workers will believe more in this enzyme-based analytical methodology and they may derive benefits from it in the instant of giving to the patient an unequivocal diagnostic of disease. Enzymatic biosensors have indispensable applications in multitudinous fields. Yet such vital devices have not found a commercial niche except glucose biosensors. There is exasperation along with an unwavering belief that such smart devices must find a valuable place in the near future and must satisfy unmet needs. The round-the-clock monitoring of biological samples would be possible with the development of smartphone-based biosensors for point-of-care testing (POCT) applications. Such continuous monitoring biosensors would definitely support medical researchers in revealing the factors that designate the imminent onset of disease, thus leading to improved diagnosis and cure. Finally the field of smart domestic sensors with large potential distribution among the general population is on the horizon.

Conflict of interest The authors report no conflicts of interest.

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