

A Brief Review of How to Construct an Enzyme-Based H₂O₂ Sensor Involved in Nanomaterials

Uwitonze Nestor*, Hitimana Frodouard, Muhizi Theoneste

Department of Chemistry, School of Science, College of Science and Technology, University of Rwanda, Kigali, Rwanda

Email: *Uwitonze.nestor@yahoo.com

How to cite this paper: Nestor, U., Frodouard, H. and Theoneste, M. (2021) A Brief Review of How to Construct an Enzyme-Based H₂O₂ Sensor Involved in Nanomaterials. *Advances in Nanoparticles*, 10, 1-25. <https://doi.org/10.4236/anp.2021.101001>

Received: May 8, 2020

Accepted: December 22, 2020

Published: December 25, 2020

Copyright © 2021 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

This article briefly reviews how to construct an enzyme based hydrogen peroxide sensor involving nanomaterials, which has the advantages of high efficiency, good sensitivity and selectivity, fast response time and an extended range of linearity with lower detection limit. Glucose biosensor is constructed by immobilizing glucose oxidase enzyme on the polycarbonate membrane and the protective cover is then filled with a physiological phosphate buffer, pH 7.4. The novel blocking hydrophobic membrane which is only permeable to hydrogen peroxide is used to eliminate electrochemical interferences. This constructed enzyme based H₂O₂ biosensor is miniaturized by the involvement of nanomaterials like carbon nanotubes, platinum nanoparticles and silver nanoparticles and it can achieve the effective microscopic detection of glucose. The introduction of nanomaterials including some pure metals (Ag, Au, Pd, Ni, Pt, and Cu), metal oxide (ZnO and TiO₂), bimetallic (Au/Ag and Au/Pt) and carbon (nanotubes and graphene) nanomaterials in the construction of the enzyme based H₂O₂ biosensor improves its sensitivity and performance by enhancing the enzymatic activity, and allows the introduction of many new signal transduction technologies in biosensors. This review article summarizes the working principles of glucose oxidase based hydrogen peroxide sensor, importance of involving nanomaterials in biosensor manufacturing, basic characteristics and components of a biosensor, generations glucose biosensors, procedure of making hydrogen peroxide based biosensor, synthesis of nanomaterials involved in hydrogen peroxide biosensor, and finally some examples of nanomaterials which intervene in hydrogen peroxide biosensor.

Keywords

Biosensor, Glucose Oxidase, Hydrogen Peroxide, Carbon Nanotubes, Nanoparticles

1. Introduction

In past few years, all over the world faced a public health problem of diabetes mellitus which is a metabolic disorder resulted from the deficiency of insulin and the unusually high concentration of sugar in blood. The normal range of concentration of glucose in blood is between 80 - 120 mg/dl or 4.4 - 6.6 mmol/l. This over concentration disease can be one of the causes of death and disability in the world. To diagnose and manage the diabetes mellitus, a closed surveillance of levels of blood glucose is required and the higher number of diabetics tests performed every day has made glucose to be most frequently analyte. This giant work has stimulating scientists to develop new biosensing concepts and a glucose biosensor based on enzyme were designed to make ease-to-use blood sugar testing and expecting their similar importance in continuous glucose monitoring [1] [2]. It is in this regard that enzyme based hydrogen peroxide electrodes have been widely developed and used glucose oxidase in the presence of oxygen for glucose measurements. The glucose probes based on hydrogen peroxide are highly generated today and being also used in clinical, industrial, environmental, and food analyses [2]. The enzyme based H_2O_2 sensors have been commonly used to detect the by-product of oxidases for glucose, alcohol, lactate, lysine, cholesterol, D-amino acid, urate, oxalate and glutamate respectively and the hydrogen peroxide is also a substrate for other enzyme like the horse-radish peroxidase [3] [4]. Before the development of enzyme based glucose/ H_2O_2 sensor, the concentration of glucose/ H_2O_2 is determined by using different analytical methods such as chromatography, titrimetry, chemiluminescence, spectrophotometry, electrochemistry, phosphorescence and fluorescence but, most of them show their own technical disadvantages including low sensitivity and selectivity, time losing, vulnerability to electroactive substances and expensive and /or complicated instrumentation. These techniques have been substituted by an enzyme based hydrogen peroxide sensors because they are cheapest and have high selectivity and sensitivity, quick operation, simplicity, rapid response, feasibility, and with effective commercial applications [5]. Recently, Nanomaterials such as nanoparticles made from pure metal (Au, Ag, Ni, Pt, Pd and Cu), metal oxide (ZnO and TiO_2); carbon nanotubes and carbon graphene are also involved in construction of enzyme based hydrogen peroxide biosensor. Particularly, the nanomaterials have been involved to initiate the development of highest performance miniaturized glucose oxidase based hydrogen peroxide biosensors which have faster response, good stability, higher sensitivity and selectivity with longer storage time. These resulted biosensors devices are smart, portable and implantable [3] [5] [6].

The use of Nanomaterials in electrochemical sensors has shown a potential positive effect especially in enhancement of sensitivity, optimization of selectivity, decreasing of detection limit, and extension of storage period [4]. Based on recent papers published in the field of nanomaterials development, the following keywords have been used in Google Scholar: NPs, QDs, NTs, NCs, NWs, NRs,

and NFs. Because of the vast progress and revolution in nanotechnology, most papers were published since 2010 as illustrated in **Figure 1(a)**.

The pie chart in **Figure 1(b)** indicates the impact factors of nanomaterials (Top chart) and the variation of citations and articles for nanomaterials (Bottom chart) since 2014 to 2018. It is obviously clear that the number of citations is continuously increasing.

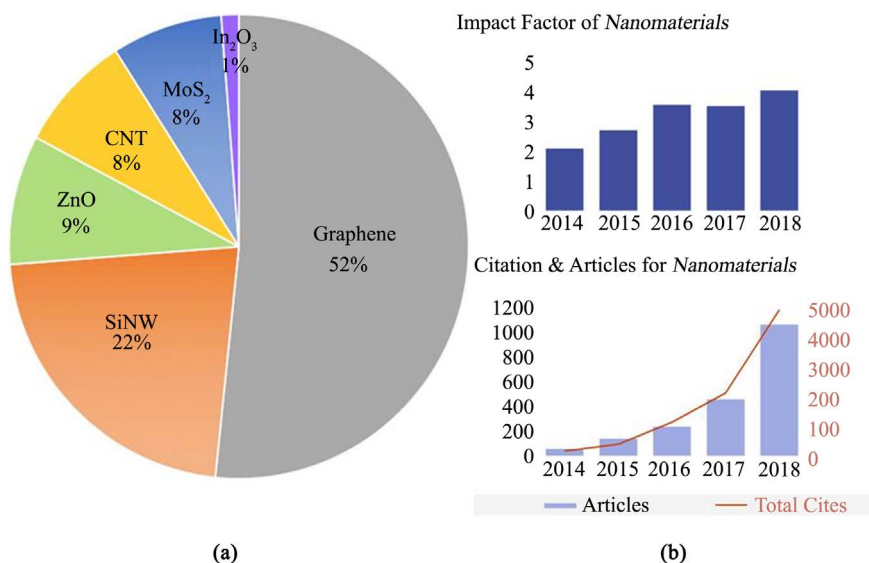
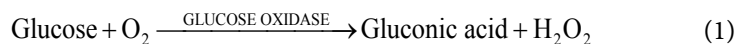


Figure 1. (a) Distribution of publications on one and two-dimensional (1D and 2D) nanomaterials in the FET based biosensor literature according to the ISI Web of Science from 2010 to July 2019; (b) Evolution of Impact Factor, Citations and Publications for Nanomaterials [7].

2. Working Principle of Enzyme Based Hydrogen Peroxide Biosensor

A glucose probe operates based to the reaction below which is catalyzed by the enzyme glucose oxidase and it is oxygen dependent [8].



The oxygen consumed during the above oxidative reaction was detected by applying a negative potential to the cathode electrode made in platinum according to the reduction reaction below [8]:



The hydrogen peroxide (H₂O₂) being produced as a by-product of biological processes is highly sensitive determined by using an enzyme based hydrogen peroxide biosensor through the anodic monitoring based on the anodic reaction below that involves the oxidation of H₂O₂ product [9]



Therefore, the above resulting type of biosensor offered good accuracy and precision in connection with 100 μL blood samples [9].

Even though these probes have sufficient stability and precision but in the presence of electroactive interferences at the platinum electrode, they lack accuracy. The interference-free hydrogen peroxide sensors were developed by introducing an electrochemical interference blocking membrane but which cannot block the interference from acetaminophen and slightly ascorbic acid drugs when the probe was left in the measuring solution for a long time. The presence or the variation of these drugs inhibits the continuous monitoring of glucose by using this probe.

Another problem of glucose sensors is its limited linearity of (30 mg/dl) and the required dilution procedures in many practical applications. To overcome this problem, an interference-free hydrogen peroxide based glucose electrode with a fast reaction on platinum electrode, good response range or linear range of 1 - 1200 mg/dl and a detection limit of 0.5 mg/dl glucose is developed [5].

3. Importance of Nanomaterials Involved in Biosensor Manufacturing

The use of nanomaterials is required today in miniaturization of glucose biosensors that can be synthesized in various shapes and dimension depending on the desired application. The synthesis method used to make required nanoparticles is widely selected by depending on the desired size, appropriate properties of surface and type of concerned material for example semiconductors, metals, ceramics, polymers, etc. The synthesis methods of preparing diverse types of nanoparticles (NPs) are grouped into three that are physical, chemical and bio-assisted methods [10]. The involvement of nanomaterials in enzymatic hydrogen peroxide biosensors has enhanced their working performance like improving the sensitivity and selectivity, lowering the limit of detection, increasing the response time and lengthening the storage period compared to an unmodified probe which used the direct oxidation systems [11].

The introduction of nanotechnology has had a deep influence in the area of biosensors in which the highly understood effect is the reduction of devices dimensions and the extent increase of sensitivity and selectivity of nanobiosensors. The novel nanomaterials are used to improve the biological activity of biological recognition element and the miniaturization of biosensors materials at the nanoscale level as a result of the nanotechnology development [12]. The introduction of nanomaterials will increase the loading surface of the enzyme, contact with more detectors, and improve the speed of electron transfer. The nanomaterials, such as Pt nanoparticles, Ag nanoparticles, carbon nanotubes, graphene, Au nanoparticles, polyaniline and their hybrid nanostructure may enhance effective performance of the enzyme based biosensors [13].

4. Basic Characteristics and Components of a Biosensor

4.1. Basic Characteristics of a Biosensor

A good enzyme based hydrogen peroxide biosensor should have the following

discussed characteristics [14]:

- ❖ **Linearity** is the maximum linear value of the sensor calibration curve. Linearity of the sensor must be high for the detection of high substrate concentration;
- ❖ **Sensitivity** is the value of the electrode response per concentration of substrate;
- ❖ **Selectivity** is the way of reducing the interference of chemicals for obtaining the correct result;
- ❖ **Response time** is the required time for having 95% of the response;
- ❖ **Stability and Reusability** is the ability of the electrode to measure the concentration of analyte for at least 4 weeks using the same electrode and same membrane to produce the good expected results;
- ❖ **Reproducibility of the biosensor** is the ability of the biosensor to generate the same responses for a duplicated experimental set-up and it is characterized by the precision and accuracy of the transducer and electronics in a biosensor.

Therefore, a novel enzyme based hydrogen peroxide as well as the glucose electrodes are useful as analytical device for practical analysis when the examination of their calibration curves, pH and temperature is done and showing that all the above discussed characteristics are excellent [14] [15].

4.2. Components of Biosensors

Based on the IUPAC definition, an enzyme based hydrogen peroxide biosensor is a self-contained analytical device made by a biological recognition element combined with a chemical or a physical transducer which normally exhibits specific and powerful interaction to an analyte or to a target molecule including glucose, alcohol, glutamate, urea, cholesterol, D-amino acid, oxalate etc and tends to provide the quantitative or semi-quantitative analytical information as mentioned in **Figure 2**. It is an electrode that yields a readable signal through its electronic component that is integrated with a biological component [5] [6].

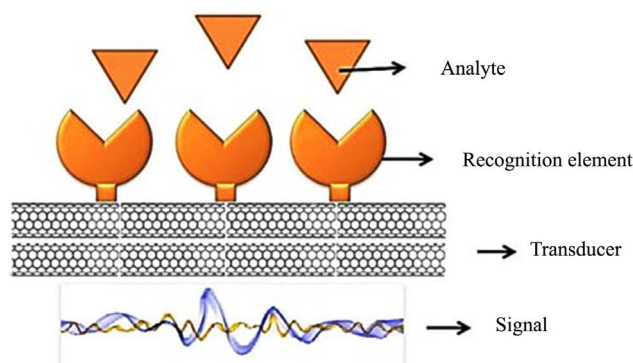


Figure 2. Schematic presentation of a biosensor [8].

As illustrated from **Figure 3**, a good working biosensor consists of three major components discussed below [8]:

- ❖ **Bioreceptors** that bind the specific form to the sample and they are connected to an electrochemical interface where specific biological processes occur by giving rise to a signal;
- ❖ **A transducer** that converts the specific biochemical reaction in an electrical signal;
- ❖ **A signal processor** that converts the electronic signal into a meaningful physical parameter and finally a proper interface displays the results to the operator.

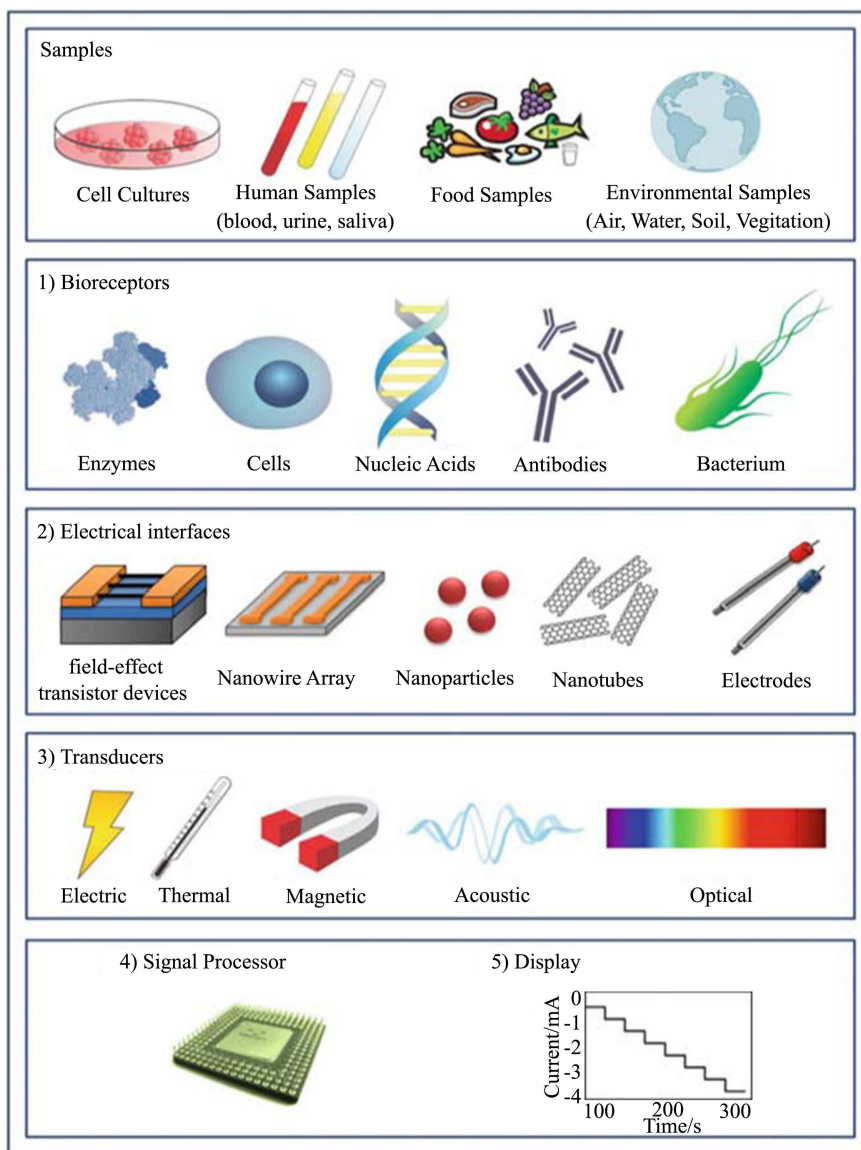


Figure 3. Components of a typical Biosensors [8].

5. Generations Glucose Biosensors

5.1. First-Generation Glucose Biosensors

First-generation glucose biosensors employ the molecular oxygen as natural

coenzyme to produce and detect hydrogen peroxide (Equations (1) and (3) above). The hydrogen peroxide can also be generated by the biocatalytic reaction involving the reduction of the enzymatic flavin group (FAD) by reacting with glucose into a reduced form of the enzyme (FADH₂) and gluconolactone [14].



The obtained reduced enzymatic Flavin group is reoxidized by natural molecular oxygen to reproduce its oxidized form and hydrogen peroxide.



The detections of hydrogen peroxide formation have the advantage of being simpler, especially when miniaturized devices are used. Such detections are commonly carried out on a platinum electrode at a moderate anodic potential of around +0.6 V (vs Ag/AgCl). The selective detection of the GOx-generated hydrogen peroxide at low-potential is possible also by coupling with another enzyme such as horseradish peroxidase (HRP) that catalyzes the oxidation of peroxide (Refer to **Figure 4**). The coupling of carbon nanotubes (CNTs) with platinum nanoparticles has been shown to be extremely useful for improving the sensitivity and speed of GOx-based glucose biosensors (down to 0.5 μM within 3 s) [14].

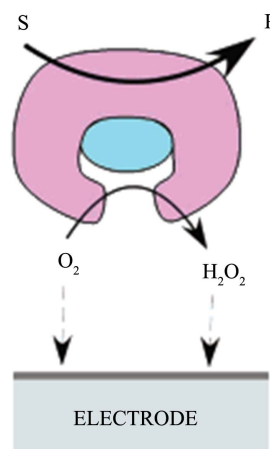
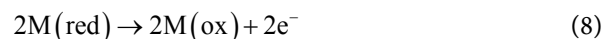
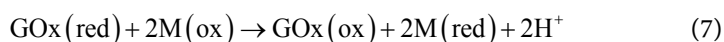
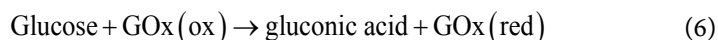


Figure 4. Amperometric enzyme electrodes for glucose based on the use of natural oxygen cofactor [14].

5.2. Second-Generation Glucose Biosensors

The second generation of glucose biosensor did not use oxygen as an electron acceptor but it is replaced by a nonphysiological electron acceptor known as a mediator. Electrons are not directly transferred by Glucose oxidase to conventional electrodes due to the presence of a thick protein layer that covers its flavin adenine dinucleotide (FAD) redox center and introducing an intrinsic barrier to direct electron transfer. The reaction scheme below demonstrates how electrons are carried by synthetic mediator between the FAD redox center and the surface of the electrode [14] [16].



where M(ox) and M(red) are the oxidized and reduced forms of the mediator. The reoxidation of the reduced form of the mediator at the electrode surface produces a current signal which is proportional to the glucose concentration. Such mediation cycle is displayed in **Figure 5** [16].

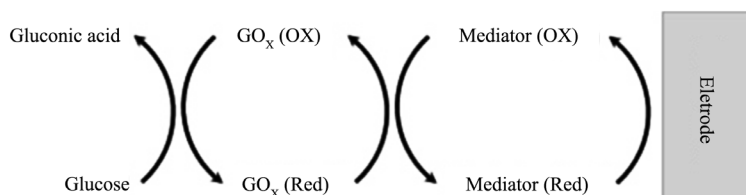


Figure 5. Sequence of events that occur in “second-generation” (mediator-based) glucose biosensors-mediated system [16].

The mediator should be insoluble, nontoxic and chemically stable in its both reduced and oxidized forms. If the rate of electron transfer through the mediator is higher than the rate of the reaction of enzyme with oxygen, the competition of oxygen will be reduced (Refer to **Figure 6**) [17].

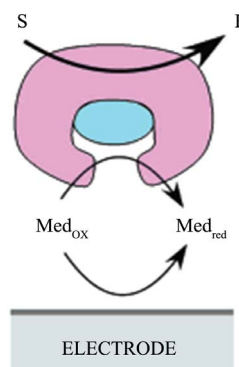


Figure 6. Amperometric enzyme electrodes for glucose based on the use of artificial redox mediators [17].

5.3. Third-Generation Glucose Biosensor

The third generation of glucose biosensor would like to remove the mediator and develop a non-reagent glucose biosensor that operates at low potential closed to the redox potential of the enzyme (Refer to **Figure 7**). The main advantage of the third generation glucose biosensor is the absence of mediators that leads to their very high selectivity at very low operating potential. This will also allow a direct transfer of electron from glucose to the electrode through the active enzyme site. Some critical challenges should be overcome to make the realization of this direct electron transfer successful. One route for constructing third-generation

amperometric glucose biosensors is to employ conducting organic salt electrodes based on charge-transfer complexes such as tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCNQ). Some authors claimed that this type of biosensor cannot allow a direct electron transfer because of the direct oxidation of the enzyme at the surface of crystal and the selectivity of glucose measurements at 0.1 V against Ag/AgCl electrode. Later they proposed that the electron transfer of GOx at TTF-TCNQ electrodes is mediated and involves corrosion of the TTF-TCNQ to produce dissolved components of these organic salts that mediate the electron transfer of the enzyme. Thereafter, an oxidized boron-doped diamond mediatorless electrodes based on direct electron transfer was developed for glucose detection [14] [18].

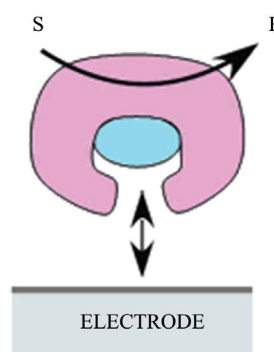


Figure 7. Amperometric enzyme electrodes based on direct electron transfer between GOx and the electrode [18].

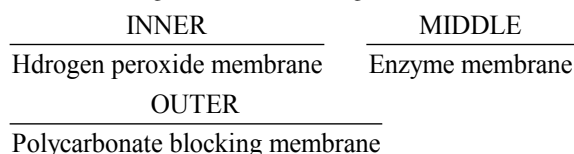
6. Procedure of Making Hydrogen Peroxide Based Sensor

An electrochemical HPLC detector of a Tacussel PRG-GLUC is used to perform the studies of constant anodic potential at +0.65 V. Gould medium gain preamplifier, model 13-4615-10 and Gould differentiator amplifier model 13-4615-71 are both used for measuring the rate. In an alternative way, the Universal Sensors Amperometric Biosensor *Detector* (ABD, cat. no. 3001, Universal Sensors Inc. Metairie, LA) is used for both constant potential studies and rate measurements. A dual pen Houston Microscribe strip chart recorder is used to record the steady-state current and rate measurements. The combined working electrode (Pt) and reference *electrode* (silver/silver chloride) in a Universal Sensors Hydrogen Peroxide Electrode (Cat. no. 4106) contained in the same electrode jacket made the base sensor. A protective cover glass wall beaker is thermostated by an MS-20 LAUDA Heating Circulator from Brinkmann Instruments to study temperatures [14] [19].

The enzyme based hydrogen peroxide sensor is constructed by protecting a 1cm² piece of the blocking membrane made in Polycarbonate on the extreme end of a reversed electrode protective cover with an O-ring. Glucose oxidase enzyme is immobilized on the polycarbonate membrane and the protective cover is then filled with a physiological phosphate buffer, pH 7.4. Inside the protecting cover, the combined working/reference electrode is introduced and screwed

down until the end of the platinum electrode is pressed against the blocking membrane in polycarbonate. Then, a thin layer of electrolytic solution is formed between the working electrode and the inner membrane while the reference electrode is exposed to the body of the filling solution. The very thin enzyme membranes with a thickness of 5 - 10 μm approximately is prepared by mixing 20 U of glucose oxidase with 10 μL of phosphate buffer of pH 7.5 that contains 2.5% glutaraldehyde on the blocking polycarbonate membrane. There is a good conduction between the working and the reference electrodes because they are on the same side of the hydrophobic membrane. The sensor is then dunked in 10 mL of the physiological buffer for balancing and a recording of the current and rate of current change is done after injection of aliquots of hydrogen peroxide [14].

The construction procedure of the enzyme based hydrogen peroxide sensor is summarized in the following membrane configuration [2]



Hence, the enzyme based hydrogen peroxide sensor is assembled as follow:

Pt/Hydrogen Peroxide membrane/Glucose Oxidase enzyme/Polycarbonate membrane [15].

The glucose oxidase enzyme is highly used in the construction of the enzyme based glucose sensor since 950 s. During the construction of this enzyme based sensing electrode, the electroactive species such as uric and ascorbic acid from samples of human blood or body fluids are excluded. The sensing performance of enzyme based glucose biosensor is determined by enzyme immobilization and dependent to nanomaterials morphology [20].

7. Synthesis of Nanomaterials Involved in Hydrogen Peroxide Biosensor

The construction and measurement of enzyme based hydrogen peroxide biosensor use platinum as working electrode and silver/silver chloride as standard reference electrode [4] [5]. Therefore, the Pt NPs, Ag NPs and carbon nanotubes (CNTs) nanomaterials can be synthesized to miniaturize the hydrogen peroxide electrode. The chemical methods involving microemulsion technique is used to synthesize platinum and silver nanoparticles. The reduction strategy is used in microemulsion technique for easy preparation of metal NPs. The effective synthesis of metal NPs is controlled by some key parameters such as water/surfactant molar ratio (u), type of continuous phase, metal ion concentration, type and concentration of the reducing agent, structure and amount of the surfactant employed. The production of Pt cubic NPs is performed by reducing H_2PtCl_6 precursor with sodium borohydride and various amount of hydrochloric acid were introduced in the $\text{H}_2\text{PtCl}_6/\text{NaBH}_4$ microemulsion system to control the Pt NPs shape/surface. The results of the studies of the Ag NPs synthesis and investiga-

tion of the effect of water/surfactants ratio on the particle size and distribution revealed that the size of particles decreases continuously from 5 to 1.5 nm while the water/surfactants ratio reduced from 15 to 2.5. Therefore, during the synthesis of metal NPs, the particle size is reduced by increasing the concentration of surfactants such as sodium bis(2-ethylhexyl) sulfosuccinate (AOT) concentration on the Ag NPs synthesis and an appropriate concentration of a reducing agent is used because both less and excess amount of reducing agent inhibits the synthesis process by affecting the colloids stability [1] [21]. Carbon nanotubes (CNTs) have therefore been employed as electrical connectors between the electrode surface and the redox center of the enzyme. For instance, glucose oxidase can be reconstituted on a 1.4 nm gold nanocrystal functionalized with the FAD cofactor. The dithiol connector allows gold nanoparticle to be immobilized onto the gold electrode surface and acts as an “electrical nanoplug” for the electrical wiring of its redox-active center [14]. An example of the whole process of nanoparticles synthesis involved in hydrogen peroxide biosensor is presented in **Figure 8** and **Figure 9**.

The other metals nanomaterials such as Pd, Rd, Au, Ni, Cu and Ir NPs can be synthesized by using the reverse micelle microemulsion strategy and employing $\text{Pd}(\text{NH}_2)_4\text{Cl}_2$, RhCl_2 , NiCl_2 and IrCl_3 , as the precursors with the reducing agents which are the active hydrogen and hydrazine. These nanomaterials are synthesized and used as matrix to immobilize the heme enzymes for improving the sensing performances of hydrogen peroxide (Refer to **Figure 10**). The synthesis of the above metal nanomaterials requires different reagent such as hydrazine in synthesis of Cu NPs which displayed the growing of water and oxide content during the control of NPs size and structure respectively; cationic water to oil ratio of water/CTAB (cetyl trimethylammonium bromide)/n-hexanol system to reduce nickel chloride by using hydrazine to synthesize Ni NPs; the alkaline 2, 7-dihydroxynaphthalene (DNP) in CTAB micellar media under 30 min continuous UV-irradiation is used to reduce the Au(III) ions for synthesizing the size and shape-selective Au NPs. The size and shape of Au NPs is dependent to the Au(III): CTAB ratio. The Rh NPs are synthesized in the similar way with Au NPs by using DNP as reducing agent and CTAB as surfactant with 6hours of continuous UV-irradiation [4] [5].

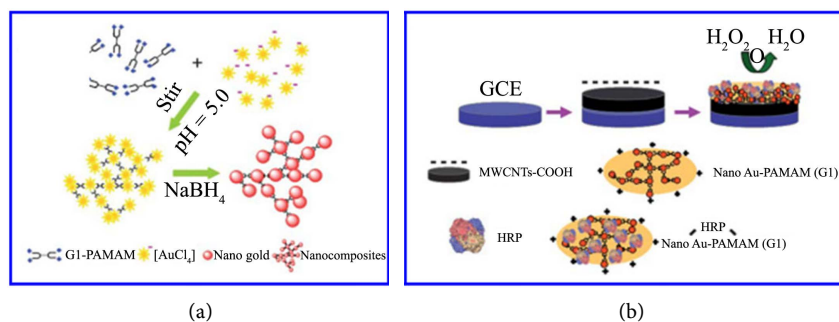


Figure 8. (a) Synthesis process of PAMAM-Au nanocomposite, (b) Preparation of the enzyme modified electrode [5].

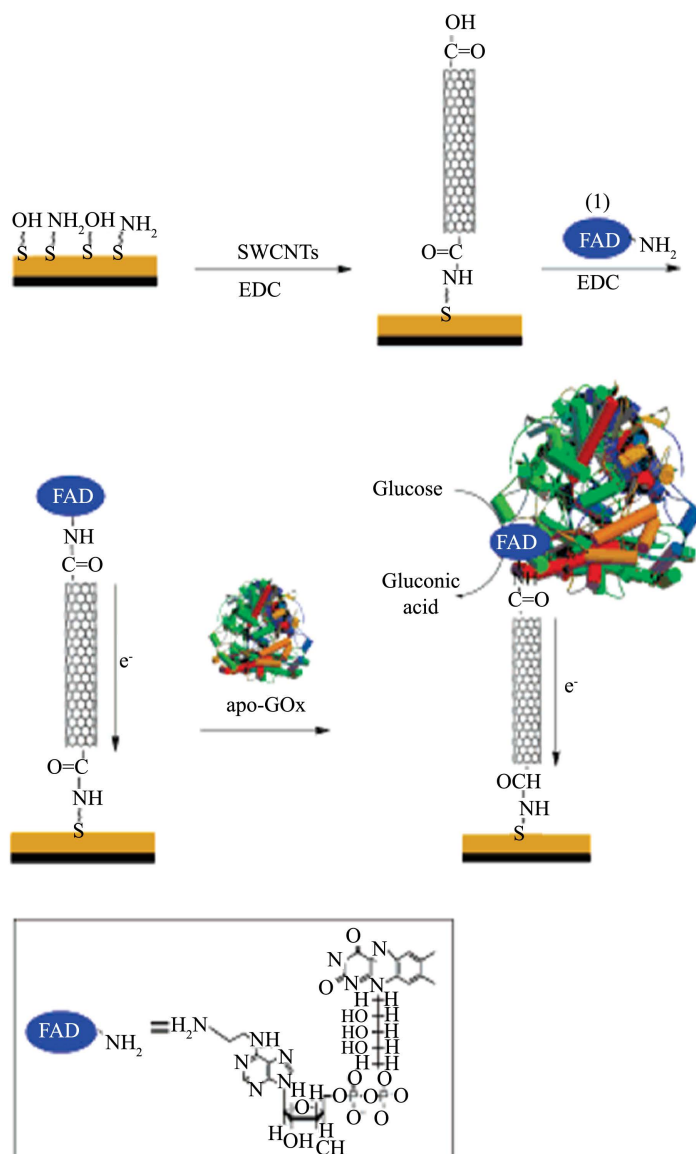


Figure 9. Carbon nanotube (CNT) connectors with long-range electrically contacted to glucose oxidase electrode [22].

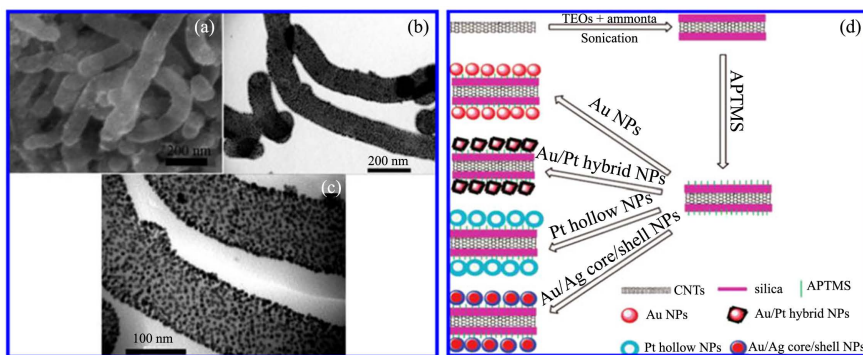


Figure 10. SEM (a) and TEM ((b), (c)) images of Au/Pt bimetallic nanoparticles supported on coaxial nanocables. (d) Procedure to design diverse metal nanoparticles/coaxial nanocable composite nanostructures [24].

Bimetallic nanoparticles can be also synthesized and used in hydrogen peroxide sensing since they show a highest catalytic selectivity and activity and a best resistance to deactivation compare to their corresponding monometal nanoparticles. These properties are due to the addition of the second metal which brings indispensable variations in particle shape, size, and surface morphology. They are usually in forms of alloys resulted to the mixture of monometallic nanoparticles. Recently, the most synthesized bimetallic nanoparticles are Au/Ag and Au/Pt NPs that are used to catalyze the detection of hydrogen peroxide. Meanwhile, the construction of third generation H_2O_2 biosensor will be easy when using the Au/Pt bimetallic nanoparticles [20] [23]. The synthesis of Gold-silver bimetallic NPs with variable mole fractions is performed under water-in-oil microemulsions of water/aerosol OT/isooctane system by using hydrazine to reduce HAuCl_4 and AgNO_3 [20]. The Gold-Platinum bimetallic nanoparticles were synthesized by preparing a series of room-temperature ionic liquids (RTILs) containing various functional groups that were used as substrate for electrodeposition to deposit the Au/Pt nanostructures. In addition, the other bimetallic nanoparticles including Pt/Pd, Pt/Ir, Pt/Cu, Pd/Cu, Rh/Pd and Ru/Rh were utilized to construct an enzyme based H_2O_2 biosensor but also used for construction the non-enzymatic electrochemical biosensor for sensing and determining glucose and H_2O_2 [20] [23].

Another novel biosensor used to detect H_2O_2 was reported by Zhao *et al.* [25] as an electrode with the surface of 0.3 cm^2 which is constructed by directly increasing mesoporous ZnO NFs based on chitosan enzyme. The SEM images of ZnO NFs and 3D porous ZnO framework for high and low magnification respectively are shown by **Figure 11(a)** and **Figure 11(b)**. All the above discussed nanomaterials contributed to the higher performance of enzyme based H_2O_2 biosensors [4].

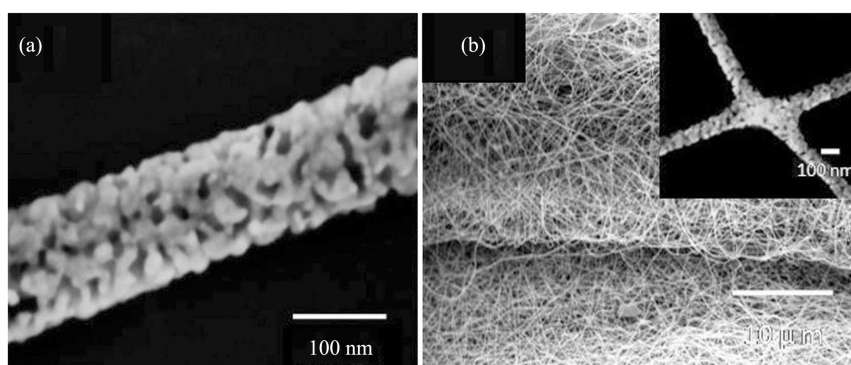


Figure 11. (a) SEM images of ZnO NF and (b) 3D porous ZnO framework and Inset SEM image of nature connection between ZnO NFs [4].

8. Examples of Nanomaterials Involved in Enzyme Based Hydrogen Peroxide Biosensor

The carbon nanotubes have been shown to be useful for development of glucose

nanobiosensors based on conductivity due to the unique electrical properties of unidimensional nanomaterials. In this case, a glucose sensing devices were made by connecting two carbon band microelectrodes with polyaniline(PANI) film covered with a glucose oxidase enzyme/poly(1, 2-diaminobenzene) layer. A typically small pocket sized, lighter and battery operated is manufactured based on its precision and it is used for home blood glucose testing (**Figure 12**). Such devices offer significant affirmation for obtaining the desired clinical information in a simpler, faster, and cheaper manner compared to traditional attempts. These blood glucose personal testing electrochemical biosensors are fully suited and have played a crucial role in the move to simple one-step blood sugar testing. Since blood glucose home testing devices are used daily to diagnose potentially life-threatening events they must be of extremely high quality. The majority of home blood glucose monitors rely on disposable screen-printed enzyme electrode test strips. Such single-use electrode strips are mass produced by the rapid and simple thick-film microfabrication or vapor deposition process. In these devices each strip contains both printed working and reference electrodes in which the working one is coated with the needed reagents such as enzyme, mediator, stabilizer, surfactant, linking, and binding agents and membranes as shown in figure below [1].

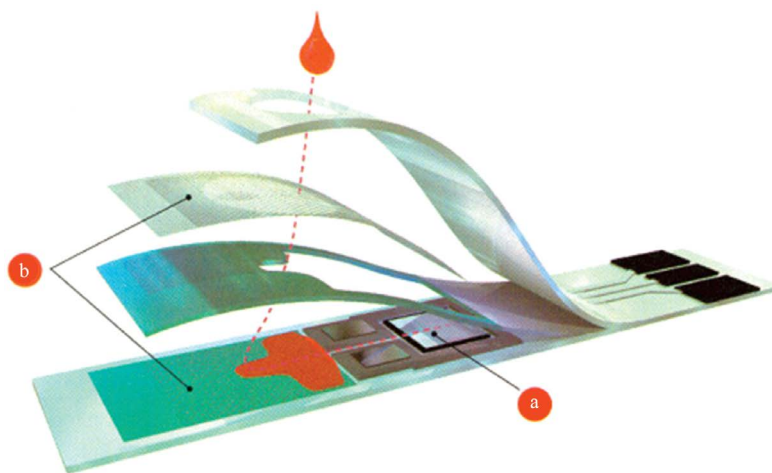


Figure 12. Cross section of a commercial strip for self-testing of blood glucose: (a) Electrode system; (b) Hydrophobic layer (drawing the blood) [1] [14].

Most of these devices count on a ferricyanide mediator, except for the Abbott devices that employ a ferrocene derivative or an osmium-based redox polymer. In all cases, the diabetic patient pricks the finger, places the small blood droplet on the sensor strip, and obtains the blood glucose concentration within 5 - 30 s. Some of the modern meters allow a collection of submicroliter amplitudes of blood from the forearm and this minimizes the pain and discomfort associated with puncturing the skin. For instance, the FreeStyle monitor of Abbott counts on coulometric strips with a 50 μm gap thin-layer cell for attempts of 300 nL blood samples.

Gold nanoparticles have been widely employed for biomaterials immobilization for the efficiency retaining of their activity and their electron transfer improvement for the application in the arrangement of biosensors. Au nanoparticles are biocompatible materials that can interact strongly with a certain number of proteins by promoting greatly the direct electron transfer in the redox reactions of proteins. The enzyme based hydrogen peroxide sensors involving Au nanomaterials can work with or without mediators. **Table 1** and **Table 2** summarize the sensing performance parameters of some examples for enzyme based H₂O₂ sensors involving Au nanomaterials with or without mediators [26]. The percentage was obtained by comparing the response current after a period of storage with the original response current of the modified electrode.

Table 1. Examples of enzymatic nano-Au based mediator H₂O₂ biosensor [4].

Sensing materials	Immobilization mode of mediators	Linear range (μM)	Detection limit (μM)	Stability	References
HRP-labeled Au colloids	In test solution	0.39 - 330	0.15	90% (2 weeks)	[27]
Au/SPAN-HRP-CS	In test solution	10 - 2000	1.6	85% (1 week)	[28]
HRP-nano-Au	In test solution	12 - 1100	6.1	75% (5 weeks)	[29]
HRP/nano-Au/Thi/p-ABSA	Adsorption	2.6 - 8800	0.64	70% (1 month)	[30]
HRP-[1]n	Adsorption	0.15 - 8600	0.07	85% (30 days)	[31]
CSHMs/HRP-GNPs-Fe(CN) ₆ ^{3-/4-} -CSHMs	Entrapment	3.5 - 1400	0.8	78% (1 month)	[32]
HRP-GNPs/Thi/GNPs/MWCNTs-Chits	Electropolymerization	0.5 - 1500	0.0375	97% (2 weeks)	[33]
HRP/GNPs-Thi/Chit	Covalent linking	0.1 - 100	0.05	92% (20 days)	[34]
Hb/CMCS-GNPs/TiO ₂ -PTATB	Covalent linking	1.4 - 1600	0.37	94% (20 days)	[35]

MWCNTs, multi-walled carbon nanotubes, SPAN, self-doped polyaniline nanofibers, CS or Chit chitosan, HRP, horse-radish peroxidase, ABSA, amino-benzene sulfonic acid, CSHMs, chitosan/silica sol-gel hybrid membranes, GNPs or AuNPs gold nanoparticles.

Table 1 shows that the mediator based hydrogen peroxide biosensors could hold a larger linear range and the very low detection limits of about 0.1 - 0.01 μM.

Table 2. Examples of enzymatic nano-Au based mediatorless H₂O₂ biosensor [4].

Sensing materials	Linear range (μM)	Detection limit (μM)	Stability
Hb-GNACs	1 - 140	0.93	93.6% (1 month)
HRP/AuNPs/poly(St-co-AA) nanospheres	8 - 7000	4	97.8% (60 days)
HRP/AuNPs	8 - 3000	2	83% (12 weeks)
HRP/{Au/Cys} ₄ /Nf	1.6 - 2400	0.5	86% (2 weeks)
HRP/AuNPs/sol-gel	5 - 10000	2	No change 120 days
cyt. c/nanoporous Au/ITO	10 - 12000	6.3	Constant for at least 1 month
HRP/3D-PAMAM-Au NC	18 - 20800	6.72	82% (4 weeks)
Hb/Au nanoflowers/CNTs/GCE	1 - 600	7.3	-

Continued

HRP/GNPs-TNTs	5 - 1000	2.1	90% (3 days)
HRP/CaCO ₃ -AuNPs/ATP/Au	0.5 - 5200	0.1	96.4% (30 days)
HRP/AuNPs/CdTe-CdS/G-AuNP	0.0001 - 0.012	0.000032	97.2% (2 weeks)
Au/TiO ₂	0.1 - 12000	0.45	No changes half a month
Cat/AuNPs/graphene-NH ₂	0.3 - 600	0.05	95% (15 days)
Hb/GNPs/Hb/MWNT	0.21 - 3000	0.08	85% (1 month)
Hb/Au@Fe ₃ O ₄	3.4 - 4000	0.67	92% (1 week)
Hb/C@Au	50 - 13500	1.67	93.6% (1 month)

GNACs, gold nanoparticle-assembled capsules, St-co-AA, styrene-co-acrylic acid, AuNPs or GNPs: Au nanoparticles, 3D-PAMAM-Au NC, three-dimensional polyamidoamine Au nanocomposite, Hb, hemoglobin, MWNTs, multi-wall nanotubes, G, graphene.

Table 2 shows that the mediatorless based hydrogen peroxide biosensors could hold a greater extent linear range and the very high detection limits than the ones with mediators.

The sensing parameters of this electrode were improved by the introduction of metal oxides nanomaterials and they are listed and compared in **Table 3**. The functionalized glucose oxidase, GOx, enzyme based biosensor involving Au NPs, chitosan, conducting polymers, CNTs, graphene and other metal nanomaterials shown a high sensing performance. The noble metals with a good resistance to oxidation like Au and Pt are better to be functionalized on electrode while for other metal it is better to use the metal oxide nanomaterials because the sensing performance parameters of nonoxide metal nanomaterials based electrodes were destroyed and the respective layer of insulating native oxide is obtained at its sensing interface. The detection range GOx operated enzyme glucose sensors based on Au NPs was extended from 10 μ M to 52 mM with a very low detection limit of less than 0.0986 μ M after incorporation of some other nanomaterials like for example molybdenum sulphide, carbon nanotubes and conducted polymers. Nevertheless, the results from the recent experimental studies show that the commercial applications are obstructed by expensiveness of enzymes, critical conditions of operation, complicated immobilization procedure and lower stability. The stability of an enzyme based electrode sensor depends on environmental storage conditions such as pH, humidity, temperature, ionic detergents and other toxic chemicals that affect significantly the activity of immobilized glucose oxidase enzyme [14].

Table 3. Performance parameters of glucose oxidase functioned enzyme glucose sensor based on nanomaterials [3].

Materials	Linear range (μ M)	Detection limit (μ M)	Sensitivity (μ A/mM)	Response time (s)	Retention period (days)	References
Au NPs-Pb NWs	5 - 2200	2	135.5	<5	70 (80.7%)	[36]

Continued

Au NPs/graphene	43.6 - 261.6	8.9	64	-	120 (80%)	[37]
Au NPs/MoS ₂	10 - 300	2.8	-	-	27 (89%)	[38]
Au NPs/CNTs	500 - 8000	-	16.6	<10	30 (94.6%)	[39]
Au NPs/GR-MWNTs	10 - 52,000	4.1 - 950	0.695 - 0.238	-	60 (94.37%)	[40]
Au NPs/DTP-Ph-NH ₂	100 - 25,000	50	-	-		[41]
Au NPs/DTP-Ph-Ph-NH ₂	50 - 1000	0.0986	-	-		
Au nanodendrite	28 - 8400	7	47.76	- 3	10 (84%)	
ZnO NF	250 - 19,000	1	70.2	<4	120	[43]
ZnO/chitosan NFs	200 - 12,000	-	- 0.016	3	40 (85%)	[25]
Porous ZnO film	200 - 5600	10	50.58	<3	21 (94%)	[43]
ZnO NRs/prickly ZnO-Cu	1000 - 15,000	40	0.097	<6	15 (95%)	[44]
ZnO NRs	10 - 23,000	0.1	110.76	<1	45 (94%)	[45]
CP NFs	125 - 11,250	-	1900	30 - 35	10 (85%)	[46]

GOx, glucose oxidase, NPs, nanoparticles, NWs, nanowires, NFs, nanofibers, NRs, nanorods, CP, conducting polymers, CNT, carbon nanotubes, MWNTs, multi-wall carbon nanotubes, NBs, nanobelts, G, graphene, GO, graphene oxide, IL, ionic liquid, MFs, microfibers, NTs, nanotubes, MWs, microwires, NCs, nanocubes.

Hydrogen peroxide is the very important byproduct of glucose and its determined concentration is helpful in determining the glucose content in the blood. Various nanomaterials from metals and metal oxides are involved to improve sensing parameters of the biosensors used in the detection of hydrogen peroxide/glucose based on the glucose oxidase enzyme or on the horse-radish peroxidase enzyme. These improved parameters are mainly sensitivity, linear range, detection limit, and response time and retention period. **Table 4** enumerated and compared the distinctive sensing performance parameters of the enzyme based H₂O₂ sensors involving nanomaterials are shown in **Table 3** [4].

Table 4. Performance parameters of H₂O₂ sensor based on nanomaterials.

Materials	Linear range (μM)	Detection limit (μM)	Sensitivity (μA/mM)	Response time (s)	Retention period (days)	References
Au/TiO ₂ NTs	5 - 40,000	2	≈170	<5	21 (95%)	[47]
Au NPs/BC NFs	1 - 500	<1	≈60	<10	-	[48]

Continued

G/Cu ₂ O NCs	300 - 7800	20.8	-	<7	-	[49]
ZnO NFs/Chitosan	10 - 1560	1	573	<3	28 (90%)	[25]
G/peptide NFs-Ag NWs	50 - 5000	10.4	≈10	-	15 (87%)	[50]
Pt NPs/ OMCs	0.1 - 3200	0.08	24.43	4 - 5	14 (92.6)	[51]
Pt/Ni nanofilm	10 - 60	2.2	200	<5	60 (>90%)	[52]
Pt NPs/CNTs	100 - 75,000	0.61	≈0.07	-	15 (95.5%)	[53]
WO ₃ /TiC/carbon NFs	10 - 500	0.003	386	<15	30 (90%)	[54]
Co ₃ O ₄ NPs	0.4 - 200	0.24	389.7	<4	60 (-100%)	[55]

NTs, nanotubes, BC, bacteria cellulose, NFs, nanofibers, G, graphene, NCs, nanocubes, NWs, nanowires, NPs, nanoparticles, OMCs, ordered mesoporous carbons, CNTs, carbon nanotubes.

The oxidation of H₂O₂ is also catalyzed by the heme enzyme horse-radish peroxidase (HRP) during the recycling of hydroquinone and benzoquinone in which the reduction current is increased significantly as shown in **Figure 13** of the mechanism of phosphate buffer solution enzyme-based hydrogen peroxide biosensor [4].

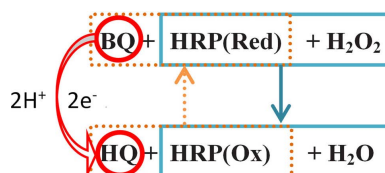


Figure 13. Mechanism of PBS enzyme-based H₂O₂ sensor [4].

As proposed by Kafi and his co-workers [47], a novel biosensor used to determine the concentration of hydrogen peroxide as well as of glucose is the one based on Gold-modified TiO₂ NTs which resulted to their modification with HRP and chitosan. It is highly advantageous due to its high quality detection performance and unique properties of TiO₂ NTs including the effective biocompatibility, cheap, environmental friendly, and chemical/thermal stability. Its sensing performance parameters are shown in first row of **Table 3** and it must be kept in 0.1M PBS with a pH = 7 at 4 °C to maintain its bioactivity. Even if the enzyme based biosensors show the high performance activity in detecting and determining the concentration of H₂O₂ and glucose but the high cost, temperature susceptibility, pH and electrochemical interfering species as well as their natural instability will hinder their commercial applications. Therefore, the synthetic enzyme based biosensors are explored and carried out in the future works as the best feasible solution [4].

Table 5 indicates the analytical performance of Ag nanoparticles based non-enzymic H₂O₂ sensors. As observed from **Table 5**, they also exhibited favourable catalysis effect with wide linear range and achieved the detection of H₂O₂ at low potential. Furthermore, the non-enzymic H₂O₂ sensors obtained relatively higher

stability than enzyme biosensors (as shown in **Table 5**). However, the non-enzymic H_2O_2 sensors generally hold the detection limits of 10^{-5} - 10^{-7} M, which is not as low as 10^{-7} - 10^{-8} M for the enzyme H_2O_2 sensors [5].

Table 5. Examples of Ag nanoparticles based non-enzymic H_2O_2 sensors [5].

Sensing material	Detection potential (V)	L. R. (M)	D. L. (M)	Stability
Ag-DNA/GCE	-0.45	2.0×10^{-6} - 2.5×10^{-3}	6.0×10^{-7}	
AgPs-SWCNT electrode	-0.3	1.6×10^{-5} - 1.8×10^{-2}	2.76×10^{-6}	89.92% (30 days)
Ag NPs/ZnO/GCE	-0.25	2.0×10^{-6} - 5.5×10^{-3}	4.2×10^{-7}	
Ag NPs/CHIT-GO/cysteamine/Au	-0.4	6.0×10^{-6} - 1.8×10^{-2}	7.0×10^{-7}	98.8% (30 days)
AgNPs/PoPD/GCE	-0.5	6.0×10^{-6} - 6.73×10^{-2}	1.5×10^{-6}	98.2% (15 days)
Ag NPs/ATP/GCE	-0.3	1.0×10^{-5} - 2.15×10^{-2}	2.4×10^{-6}	no change (2 weeks)
Ag/GC electrode	-0.44	4.0×10^{-5} - 9.0×10^{-1}	1.0×10^{-5}	95% (100 days)
PQ11-AgNPs/GCE	-0.30	1.0×10^{-4} - 1.8×10^{-1}	3.39×10^{-5}	
Ag/GN-R/GCE	-0.40	1.0×10^{-4} - 4.0×10^{-2}	2.8×10^{-5}	
Ag@PMPD-Ag/GCE	-0.30	1.0×10^{-4} - 1.7×10^{-1}	2.5×10^{-6}	
SWCNT/AgNPs/CCE	-0.20	1.0×10^{-5} - 8.0×10^{-3}	2×10^{-7}	
Ag-3D/graphite electrode	0.6	5.0×10^{-5} - 2.5×10^{-3}	1.0×10^{-6}	92% (30 days)
Polydopamine-Ag hollow/GCE	-0.30	9.2×10^{-5} - 2.0×10^{-2}	1.97×10^{-6}	90% (10 days)
Ag/graphene/GCE	-0.3	1.0×10^{-4} - 1.0×10^{-1}	5.0×10^{-7}	

AgPs or Ag NPs silver particles, SWCNT, single-walled carbon nanotube, CHIT, chitosan, GO, Graphene oxide, PoPD, poly(o-phenylenediamine), PEDOT, poly[3,4-ethylenedioxythiophene], ATP, attapulgit, GN, graphene nanosheet, PMPD, poly(m-phenylenediamine), MWCNT or MWNTs: multiwalled carbon nanotubes, CCE, carbon-ceramic electrode, DA, dopamine, AA, ascorbic acid, UA, uric acid, AC, acetaminophen.

In the recent study conducted by Tavakkoli and his team [56], they have prepared a nanobiocomposite from multiwalled carbon nanotubes and zein nanoparticles. It was dispersed in water/ethanol and drop cast onto a glassy carbon electrode. The modified electrode can be used for electroreduction of H_2O_2 (typically at a working potential of -0.71 V vs. Ag/AgCl). The electrochemical properties of the electrode were performed by cyclic voltammetry **Figure 14(a)**, linear sweep voltammetry **Figure 14(b)**, chronoamperometry **Figure 14(c)**, and electrochemical impedance spectroscopy **Figure 14(d)** Response to H_2O_2 was linear in the 0.049 to $22 \mu\text{M}$ concentration range, and the detection limit was 35 nM at pH 7.0 . The sensor was successfully utilized for the measurement of H_2O_2 in a synthetic urine sample, and for monitoring the release of H_2O_2 from human dermal fibroblasts and human hepatocellular carcinoma cells.

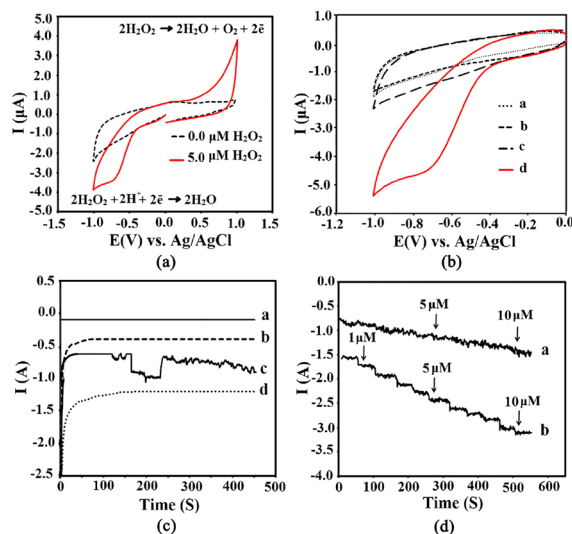


Figure 14. (a) The CVs of GC/ZNBs/fMWCNTs in the absence and presence of 5.0 μM H_2O_2 in the range of 1.0 to -1.0 V vs. Ag/AgCl, at $100 \text{ mV}\cdot\text{s}^{-1}$; (b) The CVs of bare GCE and GC/ZNBs/ fMWCNTs in the absence ((a) and (b)) and presence ((c) and (d)) of 5.0 μM H_2O_2 , respectively, at $100 \text{ mV}\cdot\text{s}^{-1}$; (d) Chronoamperometry of (a) GC/ZNBs, (b) bare GCE, (c) GC/ MWCNTs and GC/ZNBs/fMWCNTs in 5.0 μM H_2O_2 at -0.75 V. (d) Amperometry of 1.0 - 10.0 μM H_2O_2 at (a) bare GCE and (b) GC/ZNBs/fMWCNTs. All experiments were performed in Ar-saturated phosphate buffer (pH 7.0) [56].

9. Conclusions

The combination of the platinum, hydrogen peroxide membrane, glucose oxidase enzyme and 0.015 μm polycarbonate membrane makes the electrode which is very useful for glucose sensing in many practical applications such clinical analysis, industrial and environmental processes.

A novel enzyme based hydrogen peroxide probe was developed and widely studied. This constructed electrode has showed a great performance in terms of its basic characteristics such stability reproducibility, sensitivity, response time, reusability, large linear range, and high selectivity without electrochemical interferences resulting from the protection of the platinum electrode surface by new blocking membrane.

The use of carbon nanotubes as nanomaterials has led to the fabrication of home blood glucose testing sensor used by many diabetic patients because it is easy-to-use device.

Even if the enzyme based biosensors show the high performance activity in detecting and determining the concentration of H_2O_2 and glucose but the high cost, temperature susceptibility, pH and electrochemical interfering species as well as their natural instability will hinder their commercial applications. Therefore, the synthetic enzyme based biosensors are explored and carried out in the future works as the best feasible solution. Metals and Metal oxides nanoparticles have been widely employed for biomaterials immobilization for the efficiency retaining of their activity and their electron transfer improvement for the application in the arrangement of biosensors and they can work with or without me-

diators. Besides, the use of metals and metal oxides nanomaterials, the bimetallic nanoparticles including Au/Ag, Au/Pt, Pt/Pd, Pt/Ir, Pt/Cu, Pd/Cu, Rh/Pd and Ru/Rh were also utilized to construct an enzyme based H₂O₂ biosensor for sensing and determining glucose and H₂O₂. Therefore, the combination of nanomaterials and the miniaturized sensing electrodes may offer a high performance and adequate platform for H₂O₂ detection.

Acknowledgements

Authors are grateful to the University of Rwanda, School of Science and Department of Chemistry for providing the facilities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Meier, J., *et al.* (2019) Hydrogen Peroxide Sensors for Biomedical Applications. *Chemosensors*, **7**, 64. <https://doi.org/10.3390/chemosensors7040064>
- [2] Elias, H. and Vayssié, S. (2000) Reactive Peroxo Compounds Generated *in Situ* from Hydrogen Peroxide: Kinetics and Catalytic Application in Oxidation Processes. In: *Peroxide Chemistry: Mechanistic and Preparative Aspects of Oxygen Transfer*, Wiley-VCH, Weinheim, 128-138. <https://doi.org/10.1002/3527600396.ch6>
- [3] Imlay, J.A. and Linn, S. (1987) Mutagenesis and Stress Responses Induced in *Escherichia coli* by Hydrogen Peroxide. *Journal of Bacteriology*, **169**, 2967-2976. <https://doi.org/10.1128/JB.169.7.2967-2976.1987>
- [4] Li, J., *et al.* (2017) Recent Developments in Electrochemical Sensors Based on Nanomaterials for Determining Glucose and Its Byproduct H₂O₂. *Journal of Materials Science*, **52**, 10455-10469. <https://doi.org/10.1007/s10853-017-1221-4>
- [5] Chen, S., *et al.* (2013) Electrochemical Sensing of Hydrogen Peroxide Using Metal Nanoparticles: A Review. *Microchimica Acta*, **180**, 15-32. <https://doi.org/10.1007/s00604-012-0904-4>
- [6] Sakr, M.A., *et al.* (2020) Performance-Enhanced Non-Enzymatic Glucose Sensor Based on Graphene-Heterostructure. *Sensors*, **20**, 145. <https://doi.org/10.3390/s20010145>
- [7] Syedmoradi, L., *et al.* (2019) A Review on Nanomaterial-Based Field Effect Transistor Technology for Biomarker Detection. *Microchimica Acta*, **186**, 739. <https://doi.org/10.1007/s00604-019-3850-6>
- [8] Mehta, A., *et al.* (2007) A Novel Multivalent Nanomaterial Based Hydrogen Peroxide Sensor. *Sensors and Actuators A: Physical*, **134**, 146-151. <https://doi.org/10.1016/j.sna.2006.05.028>
- [9] Posch, H.E. and Wolfbeis, O.S. (1989) Optical Sensor for Hydrogen Peroxide. *Microchimica Acta*, **97**, 41-50. <https://doi.org/10.1007/BF01197282>
- [10] Merkoçi, A. (2009) *Biosensing Using Nanomaterials*. John Wiley & Sons, Hoboken. <https://doi.org/10.1002/9780470447734>
- [11] Khanna, V.K. (2008) New-Generation Nano-Engineered Biosensors, Enabling Nanotechnologies and Nanomaterials. *Sensor Review*, **28**, 39-45.

- <https://doi.org/10.1108/02602280810850017>
- [12] Chen, Y., *et al.* (2017) Nanomaterials-Based Sensitive Electrochemiluminescence Biosensing. *Nano Today*, **12**, 98-115. <https://doi.org/10.1016/j.nantod.2016.12.013>
- [13] Huang, L., *et al.* (2011) Characterising a Technology Development at the Stage of Early Emerging Applications: Nanomaterial-Enhanced Biosensors. *Technology Analysis & Strategic Management*, **23**, 527-544. <https://doi.org/10.1080/09537325.2011.565666>
- [14] Wang, J. (2008) Electrochemical Glucose Biosensors. *Chemical Reviews*, **108**, 814-825. <https://doi.org/10.1021/cr068123a>
- [15] Yoon, J., *et al.* (2020) Highly Sensitive Biosensors Based on Biomolecules and Functional Nanomaterials Depending on the Types of Nanomaterials: A Perspective Review. *Materials*, **13**, 299. <https://doi.org/10.3390/ma13020299>
- [16] Scheller, F.W., *et al.* (1991) Second Generation Biosensors. *Biosensors and Bioelectronics*, **6**, 245-253. [https://doi.org/10.1016/0956-5663\(91\)80010-U](https://doi.org/10.1016/0956-5663(91)80010-U)
- [17] Wang, J. (2002) Glucose Biosensors: 40 Years of Advances and Challenges. *Sensors Update*, **10**, 107-119. [https://doi.org/10.1002/1616-8984\(200201\)10:1<107::AID-SEUP107>3.0.CO;2-Q](https://doi.org/10.1002/1616-8984(200201)10:1<107::AID-SEUP107>3.0.CO;2-Q)
- [18] Koopal, C., Bos, A. and Nolte, R. (1994) Third-Generation Glucose Biosensor Incorporated in a Conducting Printing Ink. *Sensors and Actuators B: Chemical*, **18**, 166-170. [https://doi.org/10.1016/0925-4005\(94\)87077-2](https://doi.org/10.1016/0925-4005(94)87077-2)
- [19] Tang, F., *et al.* (2000) Glucose Biosensor Enhanced by Nanoparticles. *Science in China Series B: Chemistry*, **43**, 268-274. <https://doi.org/10.1007/BF02969521>
- [20] Rad, A.S., *et al.* (2011) A Review on Glucose and Hydrogen Peroxide Biosensor Based on Modified Electrode Included Silver Nanoparticles. *Science in China Series B: Chemistry*, **6**, 3671-3683.
- [21] Gautret, P., *et al.* (2020) Hydroxychloroquine and Azithromycin as a Treatment of COVID-19: Results of an Open-Label Non-Randomized Clinical Trial. *International Journal of Antimicrobial Agents*, **2020**, Article ID: 105949.
- [22] Patolsky, F., Weizmann, Y. and Willner, I. (2004) Long-Range Electrical Contacting of Redox Enzymes by SWCNT Connectors. *Angewandte Chemie International Edition*, **43**, 2113-2117. <https://doi.org/10.1002/anie.200353275>
- [23] Zhang, J., Feng, M. and Tachikawa, H. (2007) Layer-by-Layer Fabrication and Direct Electrochemistry of Glucose Oxidase on Single Wall Carbon Nanotubes. *Biosensors and Bioelectronics*, **22**, 3036-3041. <https://doi.org/10.1016/j.bios.2007.01.009>
- [24] Guo, S., *et al.* (2009) Carbon Nanotube/Silica Coaxial Nanocable as a Three-Dimensional Support for Loading Diverse Ultra-High-Density Metal Nanostructures: Facile Preparation and Use as Enhanced Materials for Electrochemical Devices and SERS. *Chemistry of Materials*, **21**, 2247-2257. <https://doi.org/10.1021/cm900300v>
- [25] Zhao, M., *et al.* (2013) A Single Mesoporous ZnO/Chitosan Hybrid Nanostructure for a Novel Free Nanoprobe Type Biosensor. *Biosensors and Bioelectronics*, **43**, 226-230. <https://doi.org/10.1016/j.bios.2012.11.041>
- [26] Wang, F., *et al.* (2013) Cysteine-Mediated Aggregation of Au Nanoparticles: The Development of a H₂O₂ Sensor and Oxidase-Based Biosensors. *ACS Nano*, **7**, 7278-7286. <https://doi.org/10.1021/nn402810x>
- [27] Xiao, Y., Ju, H.-X. and Chen, H.-Y. (1999) Hydrogen Peroxide Sensor Based on Horseradish Peroxidase-Labeled Au Colloids Immobilized on Gold Electrode Surface by Cysteamine Monolayer. *Analytica Chimica Acta*, **391**, 73-82.

- [https://doi.org/10.1016/S0003-2670\(99\)00196-8](https://doi.org/10.1016/S0003-2670(99)00196-8)
- [28] Chen, X., *et al.* (2011) A Novel H₂O₂ Amperometric Biosensor Based on Gold Nanoparticles/Self-Doped Polyaniline Nanofibers. *Bioelectrochemistry*, **82**, 87-94. <https://doi.org/10.1016/j.bioelechem.2011.05.004>
- [29] Lei, C.-X., *et al.* (2004) An Amperometric Hydrogen Peroxide Biosensor Based on Immobilizing Horseradish Peroxidase to a Nano-Au Monolayer Supported by Sol-Gel Derived Carbon Ceramic Electrode. *Bioelectrochemistry*, **65**, 33-39. <https://doi.org/10.1016/j.bioelechem.2004.06.002>
- [30] Yang, G., Yuan, R. and Chai, Y.-Q. (2008) A High-Sensitive Amperometric Hydrogen Peroxide Biosensor Based on the Immobilization of Hemoglobin on Gold Colloid/l-Cysteine/Gold Colloid/Nanoparticles Pt-Chitosan Composite Film-Modified Platinum Disk Electrode. *Colloids and Surfaces B: Biointerfaces*, **61**, 93-100. <https://doi.org/10.1016/j.colsurfb.2007.07.014>
- [31] Chen, S., *et al.* (2006) Amperometric Hydrogen Peroxide Biosensor Based on the Immobilization of Horseradish Peroxidase (HRP) on the Layer-by-Layer Assembly Films of Gold Colloidal Nanoparticles and Toluidine Blue. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, **18**, 471-477. <https://doi.org/10.1002/elan.200503424>
- [32] Li, W., *et al.* (2008) Immobilization of Horseradish Peroxidase on Chitosan/Silica Sol-Gel Hybrid Membranes for the Preparation of Hydrogen Peroxide Biosensor. *Journal of Biochemical and Biophysical Methods*, **70**, 830-837. <https://doi.org/10.1016/j.jprot.2007.11.010>
- [33] Li, S., *et al.* (2012) Hydrogen Peroxide Biosensor Based on Gold Nanoparticles/Thionine/Gold Nanoparticles/Multi-Walled Carbon Nanotubes-Chitosans Composite Film-Modified Electrode. *Applied Surface Science*, **258**, 2802-2807. <https://doi.org/10.1016/j.apsusc.2011.10.138>
- [34] Kang, X.B., *et al.* (2012) Study on a Hydrogen Peroxide Biosensor Based on Horseradish Peroxidase/GNPs-Thionine/Chitosan. *Electrochimica Acta*, **62**, 327-334. <https://doi.org/10.1016/j.electacta.2011.12.034>
- [35] Chen, S., *et al.* (2008) A New Enzyme Immobilization Technique Based on Thionine-Bovine Serum Albumin Conjugate and Gold Colloidal Nanoparticles for Reagentless Amperometric Biosensor Applications. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, **20**, 418-425. <https://doi.org/10.1002/elan.200704072>
- [36] Wang, H., *et al.* (2009) A Novel Glucose Biosensor Based on the Immobilization of Glucose Oxidase onto Gold Nanoparticles-Modified Pb Nanowires. *Biosensors and Bioelectronics*, **25**, 142-146. <https://doi.org/10.1016/j.bios.2009.06.022>
- [37] Chen, Y., *et al.* (2011) Fabrication of Gold Nanoparticles on Bilayer Graphene for Glucose Electrochemical Biosensing. *Journal of Materials Chemistry*, **21**, 7604-7611. <https://doi.org/10.1039/c1jm10293a>
- [38] Su, S., *et al.* (2014) Direct Electrochemistry of Glucose Oxidase and a Biosensor for Glucose Based on a Glass Carbon Electrode Modified with MoS₂ Nanosheets Decorated with Gold Nanoparticles. *Microchimica Acta*, **181**, 1497-1503. <https://doi.org/10.1007/s00604-014-1178-9>
- [39] Zhang, H., *et al.* (2011) A Novel Glucose Biosensor Based on Direct Electrochemistry of Glucose Oxidase Incorporated in Biomediated Gold Nanoparticles-Carbon Nanotubes Composite Film. *Sensors and Actuators B: Chemical*, **158**, 23-27. <https://doi.org/10.1016/j.snb.2011.04.057>
- [40] Devasenathipathy, R., *et al.* (2015) Glucose Biosensor Based on Glucose Oxidase Im-

- mobilized at Gold Nanoparticles Decorated Graphene-Carbon Nanotubes. *Enzyme and Microbial Technology*, **78**, 40-45.
<https://doi.org/10.1016/j.enzmictec.2015.06.006>
- [41] Azak, H., *et al.* (2016) Electrochemical Glucose Biosensing via New Generation DTP Type Conducting Polymers/Gold Nanoparticles/Glucose Oxidase Modified Electrodes. *Journal of Electroanalytical Chemistry*, **770**, 90-97.
<https://doi.org/10.1016/j.jelechem.2016.03.034>
- [42] Ahmad, M., *et al.* (2010) A Single ZnO Nanofiber-Based Highly Sensitive Amperometric Glucose Biosensor. *The Journal of Physical Chemistry C*, **114**, 9308-9313.
<https://doi.org/10.1021/jp102505g>
- [43] Zhou, S., *et al.* (2013) Direct Growth of Vertically Aligned Arrays of Cu(OH)₂ Nanotubes for the Electrochemical Sensing of Glucose. *Sensors and Actuators B: Chemical*, **177**, 445-452. <https://doi.org/10.1016/j.snb.2012.11.035>
- [44] Yang, C., Xu, C. and Wang, X. (2012) ZnO/Cu Nanocomposite: A Platform for Direct Electrochemistry of Enzymes and Biosensing Applications. *Langmuir*, **28**, 4580-4585. <https://doi.org/10.1021/la2044202>
- [45] Ahmad, R., *et al.* (2012) Highly Selective Wide Linear-Range Detecting Glucose Biosensors Based on Aspect-Ratio Controlled ZnO Nanorods Directly Grown on Electrodes. *Sensors and Actuators B: Chemical*, **174**, 195-201.
<https://doi.org/10.1016/j.snb.2012.08.011>
- [46] Palod, P.A. and Singh, V. (2015) Facile Synthesis of High Density Polypyrrole Nanofiber Network with Controllable Diameters by One Step Template Free Electropolymerization for Biosensing Applications. *Sensors and Actuators B: Chemical*, **209**, 85-93. <https://doi.org/10.1016/j.snb.2014.11.100>
- [47] Kafi, A., Wu, G. and Chen, A. (2008) A Novel Hydrogen Peroxide Biosensor Based on the Immobilization of Horseradish Peroxidase onto Au-Modified Titanium Dioxide Nanotube Arrays. *Biosensors and Bioelectronics*, **24**, 566-571.
<https://doi.org/10.1016/j.bios.2008.06.004>
- [48] Zhang, T., *et al.* (2010) Biotemplated Synthesis of Gold Nanoparticle-Bacteria Cellulose Nanofiber Nanocomposites and Their Application in Biosensing. *Advanced Functional Materials*, **20**, 1152-1160. <https://doi.org/10.1002/adfm.200902104>
- [49] Liu, M., Liu, R. and Chen, W. (2013) Graphene Wrapped Cu₂O Nanocubes: Non-Enzymatic Electrochemical Sensors for the Detection of Glucose and Hydrogen Peroxide with Enhanced Stability. *Biosensors and Bioelectronics*, **45**, 206-212.
<https://doi.org/10.1016/j.bios.2013.02.010>
- [50] Wang, Q., *et al.* (2015) *In Situ* Preparation of Porous Pd Nanotubes on a GCE for Non-Enzymatic Electrochemical Glucose Sensors. *Analytical Methods*, **7**, 8605-8610.
<https://doi.org/10.1039/C5AY01172H>
- [51] Bo, X., *et al.* (2011) Ultra-Fine Pt Nanoparticles Supported on Ionic Liquid Polymer-Functionalized Ordered Mesoporous Carbons for Nonenzymatic Hydrogen Peroxide Detection. *Biosensors and Bioelectronics*, **28**, 77-83.
<https://doi.org/10.1016/j.bios.2011.07.001>
- [52] Li, H., *et al.* (2013) A Photoelectrochemical Sensor Based on Nickel Hydroxyl-Oxide Modified n-Silicon Electrode for Hydrogen Peroxide Detection in an Alkaline Solution. *Biosensors and Bioelectronics*, **47**, 225-230.
<https://doi.org/10.1016/j.bios.2013.03.028>
- [53] Zhang, P., *et al.* (2014) Electrospun Doping of Carbon Nanotubes and Platinum Nanoparticles into the β -Phase Polyvinylidene Difluoride Nanofibrous Membrane for Biosensor and Catalysis Applications. *ACS Applied Materials & Interfaces*, **6**,

- 7563-7571. <https://doi.org/10.1021/am500908v>
- [54] Zhang, X., *et al.* (2013) Non-Enzymatic Hydrogen Peroxide Photoelectrochemical Sensor Based on WO₃ Decorated Core-Shell TiC/C Nanofibers Electrode. *Electrochimica Acta*, **108**, 491-496. <https://doi.org/10.1016/j.electacta.2013.07.064>
- [55] Han, L., Yang, D.-P. and Liu, A. (2015) Leaf-Templated Synthesis of 3D Hierarchical Porous Cobalt Oxide Nanostructure as Direct Electrochemical Biosensing Interface with Enhanced Electrocatalysis. *Biosensors and Bioelectronics*, **63**, 145-152. <https://doi.org/10.1016/j.bios.2014.07.031>
- [56] Tavakkoli, H., *et al.* (2020) Electrochemical Sensing of Hydrogen Peroxide Using a Glassy Carbon Electrode Modified with Multiwalled Carbon Nanotubes and Zein Nanoparticle Composites: Application to HepG2 Cancer Cell Detection. *Microchimica Acta*, **187**, 1-12. <https://doi.org/10.1007/s00604-019-4064-7>