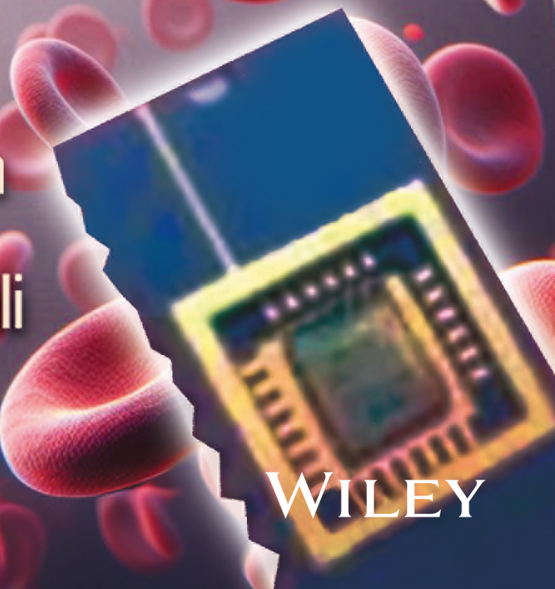
NANO-BIOSENSOR TECHNOLOGIES FOR DIAGNOSIS OF INFECTIOUS DISEASES

Edited By
Suvardhan Kanchi
Ayyappa Bathinapatla
Anitha Varghese
Phumlane Selby Mdluli



Scrivener Publishing

WILEY

Nano-Biosensor Technologies for Diagnosis of Infectious Diseases

Scrivener Publishing
100 Cummings Center, Suite 541J
Beverly, MA 01915-6106

Publishers at Scrivener
Martin Scrivener (martin@scrivenerpublishing.com)
Phillip Carmical (pcarmical@scrivenerpublishing.com)

Nano-Biosensor Technologies for Diagnosis of Infectious Diseases

Edited by

Suvardhan Kanchi

Dept. of Chemistry, CHRIST (Deemed to be University), Bengaluru, Karnataka, India

Ayyappa Bathinapatla

Dept. of Chemistry, CMRIT, Bangalore, India

Anitha Varghese

Dept. of Chemistry, CHRIST (Deemed to be University), Bangalore, Karnataka, India

and

Phumlane Selby Mdluli

Dept. of Chemistry, Durban University of Technology, Durban, South Africa

WILEY

This edition first published 2025 by John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA and Scrivener Publishing LLC, 100 Cummings Center, Suite 541J, Beverly, MA 01915, USA

For more information about Scrivener publications please visit www.scrivenerpublishing.com.

Wiley Global Headquarters
111 River Street, Hoboken, NJ 07030, USA

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Library of Congress Cataloging-in-Publication Data

ISBN 978-1-394-28766-6

Front cover art courtesy of Adobe Firefly and Wikimedia Commons
Cover design by Russell Richardson

Set in size of 11pt and Minion Pro by Manila Typesetting Company, Makati, Philippines

Printed in the USA

10 9 8 7 6 5 4 3 2 1

Contents

Preface

Welcome to *Nano-Biosensor Technologies for Diagnosis of Infectious Diseases*. This comprehensive volume explores the cutting edge of scientific innovation, presenting a curated collection of 16 meticulously crafted chapters that highlight the latest advancements in nano-biosensor technologies—a revolutionary approach to the diagnosis and management of infectious diseases.

Infectious diseases continue to pose significant global health challenges, underlining the critical need for rapid, accurate, and sensitive diagnostic tools. Situated at the intersection of nanotechnology and biomedicine, nano-biosensors offer unparalleled opportunities to meet these challenges by delivering highly sensitive and specific detection capabilities across various clinical settings.

Within these pages, readers will discover a treasure trove of cutting-edge research, ranging from foundational principles to practical applications. Authored by leading experts in the field, each chapter provides insights into current trends, emerging methodologies, and the innovative technologies that are driving the advancement of nano-biosensors for infectious disease diagnosis.

From innovative sensing mechanisms and fabrication techniques to their real-world implementation and clinical translation, this book offers a comprehensive overview of the current state of the art in nano-biosensor technologies. We hope this volume will serve as an invaluable resource for researchers, clinicians, policymakers, and anyone interested in leveraging the power of nanotechnology to combat infectious diseases and enhance global health outcomes.

We extend our sincere thanks to the contributors for sharing their expertise and insights, and to our readers, whose curiosity and commitment to advancing scientific knowledge propel progress in this vital field.

Finally, our gratitude goes to Martin Scrivener and the team at Scrivener Publishing for their support in bringing this volume to light.

The Editors
January 2025

1

Biosensor Technology: Basic Principles, Fundamentals, and History

Mariyam Thomas[1], Mathew George[2], Derry Holaday M. G.[3] and P. J. Jandas[4]*

[1]Department of Physics, St. Teresa's College, Ernakulam, Kerala, India
[2]Department of Physics, Sacred Heart College, Kochi, Kerala, India
[3]Department of Chemistry, University of Calicut, Malappuram, Kerala, India
[4]iNest Bioincubation Centre, Dr. Moopen's Medical College, Wayanad, Kerala, India

Abstract

Fabrication and modification of biosensors is one of the extensively studied areas today due to viable manufacture methods with commercialization potential and excellent performance characteristics in terms of rapid detection, cost effectiveness, high selectivity, and sensitivity. Biosensors have already explored many applications including protein sensing-based disease identification, understanding the stages and medication. Advents in this area show biosensors have the potential to find application in next generation medicine like personalized drug delivery and error free biomarker detection with extreme selectivity and sensitivity. The book chapter summarizes the concept of biosensors, conventional classifications, application areas and potential as a dependable biomedical tool. A special emphasis is given into the recent advancements in biosensors used for glucose sensing. The important role of the nonmaterial-based transducing bioreceptors in a biosensor performance is also discussed in detail.

***Keywords*:** Biosensors, biomarkers, bioreceptor, nanomaterials, SAW, QCM

**Corresponding author*: drjandasjanan@gmail.com

Suvardhan Kanchi, Ayyappa Bathinapatla, Anitha Varghese and Phumlane Selby Mdluli (eds.) Nano-Biosensor Technologies for Diagnosis of Infectious Diseases, (1–22) © 2025 Scrivener Publishing LLC

1.1 Introduction

Biological sensors, also called biosensors, are defined as analytical devices comprising a biological or biologically derived component [1] that decides their selectivity [2] capable of detecting an analyte [3] by the physiochemical component [2]. The real-time acoustic detection of carcinoembryonic antigen (CEA) was done using a bioreceptor of polyimide thin film doped with nanoparticles of Ti3C2Tx MXene-Au [13]. This work utilized thioglycolic acid arm linker mechanism. According to the immunoassay, biosensor response is linear to the concentration of CEA samples. Figure 1.1 represents the basic working principle of the CEA biosensor. The progress in the field of biosensors from the 1980s is immense with the development of life-essential devices such as pregnancy test kits which utilize biochemical or biological reactions.

The basic component of a biosensor includes a biological recognition element which detects specific analytes, then the transducer which converts the corresponding biological signal to an electrical signal (Figure 1.2). This enables rapid, accurate detection and monitoring of samples from medical, food, agriculture, environmental, and industry-based resources. According to International Union of Pure and Applied Chemistry (IUPAC), a biosensor

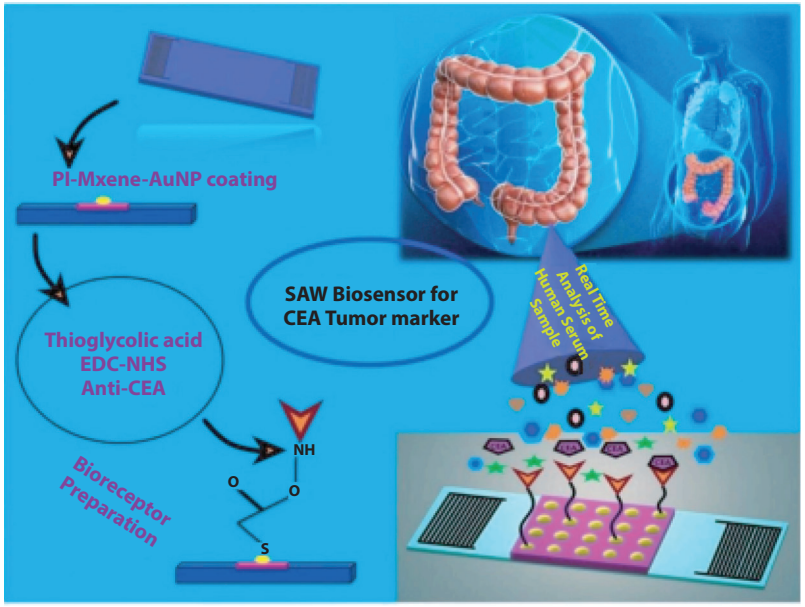


Figure 1.1 The real-time acoustic detection of carcinoembryonic antigen with polyimide thin film doped with nanoparticles of $Ti_3C_2T_x$ MXene-Au bioreceptor [13].

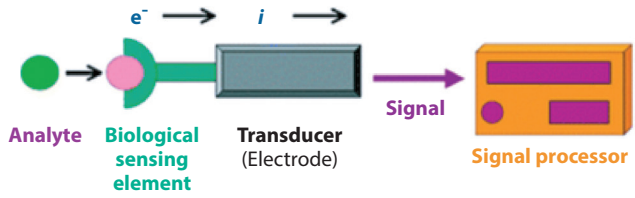


Figure 1.2 Scheme of a biosensor with an electrochemical transducer [14].

is "a device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" [4].

The three major parts of every biosensor include [1]:

i) Biological recognition element that facilitates the device to recognize the target molecule from the other chemicals present and the binding is followed [1].
ii) Signal conversion unit which is a transducer [4] that converts the specific binding process to a measurable signal [1].
iii) A signal processing system that includes a detector [5] and the detected signal is converted into readable form [1].

Glucose biosensors dominate in the industry by covering about 85% [6] and the work on the principle is based on the detection of disease indicator analytes such as glucose and insulin [7]. The first biosensors were developed in the early 1960s by Dr. Leland C Clarkin where an enzyme electrode with glucose oxidase (GOD) was employed for the measurement of the concentration of glucose [4]. The GOD catalyzes the formation of gluconolactone. The proportional increase in hydrogen peroxide concentration to the glucose concentration during the oxidation of β-D-glucose and the decline in oxygen concentration is detected electrochemically [8]. Effective glycemic monitoring has been achieved ever since the introduction of glucose biosensors. These devices also have a place in various applications such as food analysis and bioprocess monitoring [9]. Though techniques like radio labelling prevail for the measurement, the complications in the procedure, the bulk size of the equipment, sample destruction, and low spatial resolution problems prompt us to look for an effective technique, and biosensors pave its importance in this scenario [7]. Continuous, real-time glucose concentration monitoring in liquid samples ranging in

nanoliters is achieved through modern glucose biosensors which have miniaturized micro/nanoscale range sensors. These sensors achieve the measurement in single cells or isolated organelles because of their high spatial resolution [9].

Working Principle of Glucose Biosensor

The biosensor consists of a biological component that on specific binding produces a response which is transmuted into a quantifiable signal with the help of a transducer [2]. The basic classification of biosensors is given in the Figure 1.4. Most prevailing glucose biosensors have better sensitivity, reproducibility, and easy maintenance, are economical in nature, and are electrochemical in type. Generally, glucose measurements are based on either of the enzyme interactions viz hexokinase, glucose oxidase (GOx), or glucose-1-dehydrogenase, of which GOx is considered superior due to its immense selective nature. Moreover, it is easily available and can withstand extreme temperatures, pH conditions, and ionic strength compared to other enzymes. Figure 1.3 represents the catalysis process by immobilized glucose oxidase. In the process, flavin adenine dinucleotide (FAD) acts as a redox cofactor for the oxidation of β-D-glucose employing molecular oxygen, thus converting it into gluconic acid and peroxide (Figure 1.3). The mechanism of the process is shown in Scheme 1.1.

The FAD on reduction converts to $FADH_2$. Upon oxidation, H_2O_2 is formed which is oxidized at platinum electrode. It detects the electron transfer number thus detecting the concentration of glucose in the sample.

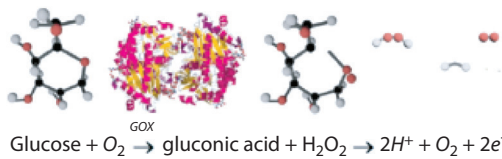


Figure 1.3 Schematic representation of glucose biosensing [10].

$$\text{Glucose} + \text{GOX} - \text{FAD}^+ \longrightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\text{GOx - FADH}_2 + \text{O}_2 \longrightarrow \text{GOx} - \text{FAD} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 \longrightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}$$

Scheme 1.1 Mechanism of the glucose detection.

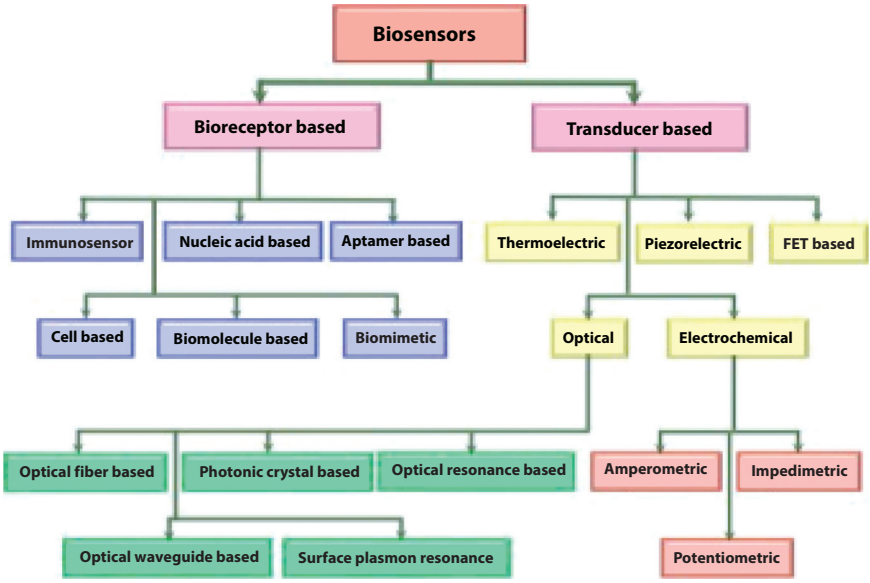


Figure 1.4 Different types of biosensors.

When glutamate dehydrogenase (GDH) with nicotinamide-adenine dinucleotide (NAD) is the cofactor, NADH is produced in the place of H_2O_2 [14].

Evolution in Biosensor Technology

A wide variety of biosensor availability is seen today as the principle of working of biosensors is getting changed over the years.

1) First Generation Glucose Biosensors

The first proposed biosensor includes an oxygen electrode, GOx thin layer, semipermeable oxygen inner membrane, and an outer dialysis membrane. Natural oxygen substrate-based biosensors come under the first generation for the detection of the hydrogen peroxide produced as the process is simple but requires higher operation potential for high selectivity. The variation of the O_2 tension due to less solubility O_2 in biofluids is another drawback of these biosensors [1].

1.1) Electroactive Interferences

The hydrogen peroxide amperometric measurement at common working electrodes requires a relatively high potential. The co-existing [9] reducing species [11] like ascorbic acids, uric acids or acetaminophen may interfere

and affect the selectivity and sensitivity of the process. Moreover, other components existing in the sample which can undergo oxidation will also affect the accuracy.

To avoid this, attempts towards reduction of access to the surface of electrodes were made by using selective coating with multi and mixed polymer layers [9]. Their transport properties based on charge, size, or polarity can block the electroactive compounds and surface-active macromolecules. The surface is thus protected which results in higher stability. High selectivity is shown by electro-polymerized films, polyphenol, and over-oxidized polypyrrole by confining GOx onto the surface [11]. Multi-(overlaid) layers that have combining properties of different films can be used for additional advantages, *i.e.*, the intervention of neutral acetaminophen and negatively charged ascorbic and uric acid were eliminated by simultaneous alternate deposition of cellulose acetate and Nafion [9]. The use of metalized carbon transducers (Rh-C or Ru-C) also offers high selectivity through determining H_2O_2 at an optimal potential range of 0.0 V. The surface metal oxide film is converted to free metal using H_2O_2. An anodic current signal is produced when it is reoxidized electrochemically. By including a discriminative layer with metal to a Nafion film, additional improvements can be made. Horseradish peroxidase (HRP) is an enzyme that catalyzes peroxide oxidation thus offering a low potential selection detection of the GOx-generated H_2O_2. The carbon nanotube (CNT)-modified electrodes offer high selectivity towards glucose detection. On coupling CNT with platinum nanoparticles shows high efficiency as it has enhanced sensitivity and speed [11].

1.2) Oxygen Dependence

The errors due to the oxygen deficit prevailed in devices based on oxidase as they use oxygen as physiological acceptor of electrons. These errors result in the reduced upper limit of linearity. When normal O_2 concentration is lower, around 1 order of magnitude than the physiological level of glucose points out oxygen deficit. This constraint can be overcome by applying mass transport limiting films. Their usage will enhance the flux of the O_2 and glucose permeability ratio [11].

2) Second Generation Glucose Biosensors

2.1) Electron Transfer between GOx and Electrode Surfaces

The second-generation glucose sensors, redox mediators, replace oxygen. The transfer of electrons from enzymes to the surface of the working electrode will lead to further improvements [1].

$$\text{Glucose} + \text{GOx(ox)} \longrightarrow \text{Gluconic acid} + \text{GOx(red)}$$
$$\text{GOx(red)} + 2\text{M(ox)} \longrightarrow \text{GOx(ox)} + 2\text{M(red)} + 2\text{H}^{+}$$
$$2\text{M(red)} \longrightarrow 2\text{M(ox)} + 2\text{e}^{-}$$

Scheme 1.2 Mechanism of synthetic mediators electron transfer process, where $M_{(ox)}$ and $M_{(red)}$ are the oxidized and reduced forms, respectively.

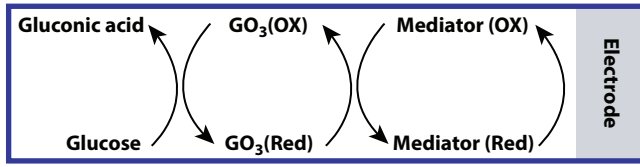


Figure 1.5 Event sequence in 'second generation' of mediator-based glucose biosensors [11].

2.2) Use of Non-Physiological Electron Acceptors

The synthetic mediators transport the electrons from the FAD center to the electrode surface, as shown in Scheme 1.2.

A current signal is produced when the reduced form is reoxidized at the electrode, regenerating the mediator's oxidized form. This is shown in Figure 1.5 [11].

Ferrocene, ferricyanide, quinines, tetrathiafulvalene (TTF), tetracyanoquinodimethane (TCNQ), thionine, methylene blue, and methyl viologen are some of the electron mediators which can improve the sensor performance. The advantages of ferrocenes include ferrocenes inertness towards oxygen, stability in both oxidized and reduced states, resilience across a wide range of pH levels, reacting rapidly with enzymes, and showing electron transfer kinetics [1], which enables them to perform effectively. The oxidation of endogenous species cannot be completely removed but minimized by low potential of most mediators. Further, additional errors will be led by consumption of mediators. For an extended continuous operation, mediated systems display low stability [11].

2.3) Wired Enzyme Electrodes

A redox polymer, coupled with enzyme wiring, enhances electrical conductivity between the redox center of GOx and electrode surfaces. Establishing a communication link between GOx and electrodes is the base of a non-diffusional route of biosensing. This connection is achieved

by tethering the enzyme to the surface using a long, flexible, hydrophilic polymer backbone.

To accomplish this, a dense array of covalently bonded osmium-complex electron relays, such as poly(vinylpyridine) or poly(vinyl imidazole)] are used. This arrangement forms a three-dimensional network that attaches to the surface, minimizing the distance between redox centers and FAD center of the enzyme. Electrons from the redox site of GOx are transported through the gel polymer network to the electrode, providing high current outputs, rapid response times, and stabilizing the mediator to the surface. Ultra small enzyme electrodes can be used with the aid of huge current densities. Wired enzyme electrons are thus particularly attractive for *in vivo* applications [11].

Main advantages are high current outputs, fast response, and stability. Ultra-small enzyme electrodes can be used with the aid of huge current densities. Wired enzyme electrons are thus particularly attractive for *in vivo* applications [11].

2.4) Modification of GOx with Electron Relays

Another approach to facilitate electron transfer between the GOx and the electrode surface involves chemically altering GOx with electron-relay species. The flavin center of GOx undergoes oxidation due to the covalent attachment of the ferrocene group and results in electron tunneling in a number of consecutive steps. Enzyme reconstitution process improves the efficiency of electrical communication with electrodes in the glucose biosensors. The fitment of electron-transfer relays at the boundary of enzymes is also considered in the case of short electron-transfer distances [11].

2.5) Nanomaterial Electrical Connectors

The application of nanomaterials in bioanalytical chemistry is a vast field. Nanomaterials offer an effective means for wiring redox enzymes, such as GOx, to electrodes. Gold nanoparticles and CNTs serve as efficient electrical connectors between the electrode and the redox center of Gox (Figure 1.6) [13]. Utilizing a dithiol linker, gold nanoparticles are immobilized onto the gold electrode, acting as nano plugs for electrical wiring to the redox-active center of GOx. This will result in a high electron-transfer turnover (around 5000/s). Moreover, additional nanomaterials can be tethered to enzymes via CNTs, facilitating favorable surface orientation and serving as electrical connectors between their redox center and the electrode surface. The activation of GOx by these requires over potential and this can be reduced, enhancing the contact between nanomaterials and the electrode [11].

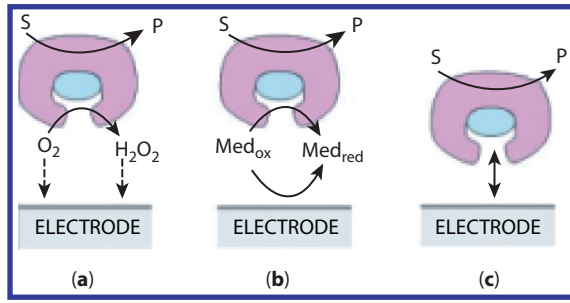


Figure 1.6 (a) Redox mediators, (b) Direct electron transfer, and (c) [11] Amperometric enzyme electrodes [10].

3) Third Generation Glucose Biosensors
They operate without reagents or mediators, utilizing a low potential similar to that of the enzyme's redox potential [11]. It is based on the enzyme's active site enabling direct transfer of an electron from glucose to the electrode [11]. Highly toxic mediators are avoided [1] due to very low operating potential and high selectivity is ensured [11]. Only few enzymes peroxidases [1] have been reported that can enable an effective electron transfer at conventional electrodes. Studies were done for new electrode materials as attempts for direct electron transfer of GOx to conventional electrons were futile.

The newly customized optimally designed electrode ensures minimal electron-transfer distance between the immobilized protein and the surface. One approach is by creating third-generation amperometric glucose biosensors utilizing conducting salt electrodes with charge-transfer complexes like TTF-TCNQ [11]. These complexes can facilitate the electrochemistry of pyrrole-quinoline quinone enzymes (GDH-PQQ) and flavoproteins (GOx) [1].

Classification of Glucose Biosensors and Their Properties
1) Based on Transducing Element
1.1) Electrochemical Glucose Biosensors
Detection of the electrochemical signal during a bio-interaction process is the basic working principle of electrochemical biosensors. Detectors can be classified into potentiometric, amperometric, or conductometric types based on their mechanism. The potentiometric sensor measures change in the charge density at the surface of the electrode and amperometric biosensor measures current liberated as a result of transfer of electrons between a biological system and electrode. The change in ionic conduction

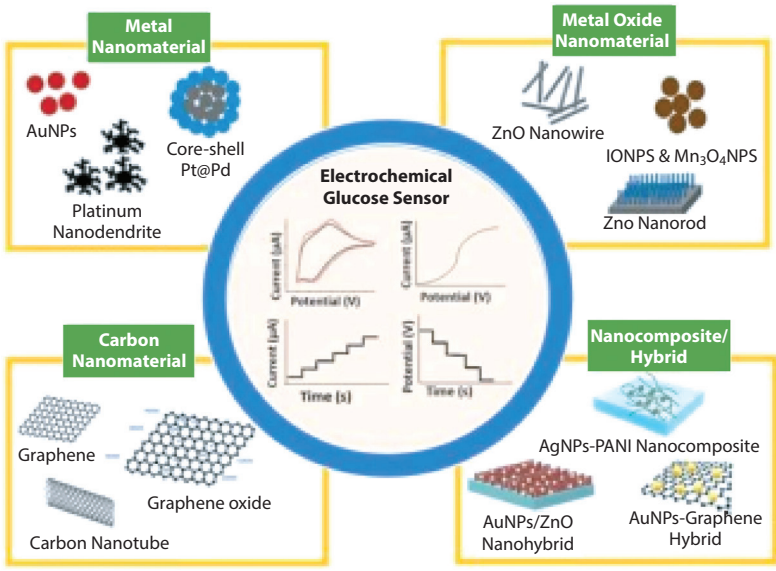


Figure 1.7 Schematic of nanomaterial-modified electrode for glucose biosensor cinnamic acid diazonium salt, which, in electrochemically reduced form, is used as the immobilization matrix for the glucose biosensor [15].

between metal electrodes is measured by the conductometric sensors. Miniaturization and simplification of the system are achieved by integrating an immobilized enzyme complex with an electrochemical sensor, thus enabling reagent-less glucose analysis. Modification of the working electrode with various nanomaterials is one of the current developments in glucose biosensors and is depicted in Figure 1.7. Nanomaterials possess unique characteristics, including a large surface area for enhanced reaction activity, excellent catalytic efficiency, and strong adsorption capabilities. This enables them to work as a matrix to modify the electrode surface. This also provides enzyme immobilization biocompatible areas.

The attachment of other biological agents are achieved by modifying the glassy carbon electrode (GCE) with the cinnamic acid group. Miniaturization is accomplished by attaching the enzyme to the self-assembled oligophenylethynylenethiol monolayer, serving as a crosslinker for immobilizing glucose oxidase to the gold electrode [7]. Out of the three most commonly used enzymes for glucose detection, *i.e.*, hexokinase, glucose oxidase (GO), and glucose-1-dehydrogenase, glucose oxidase (GOx) is widely regarded as a standard enzyme for biosensors because of its high selectivity, affordability, and capacity to endure elevated pH levels, temperatures, and ionic strengths. When glucose dehydrogenase is used

instead of GO, amperometric biosensing of glucose can also be carried out. Mediators, which are carriers that can be biologically active or synthetic, are used to enhance the connection between the redox enzymes and electrodes [7].

1.2) Optical Biosensors

Another way for glucose sensing includes the utilization of optical properties of compounds with intrinsic fluorescence and their coenzymes.

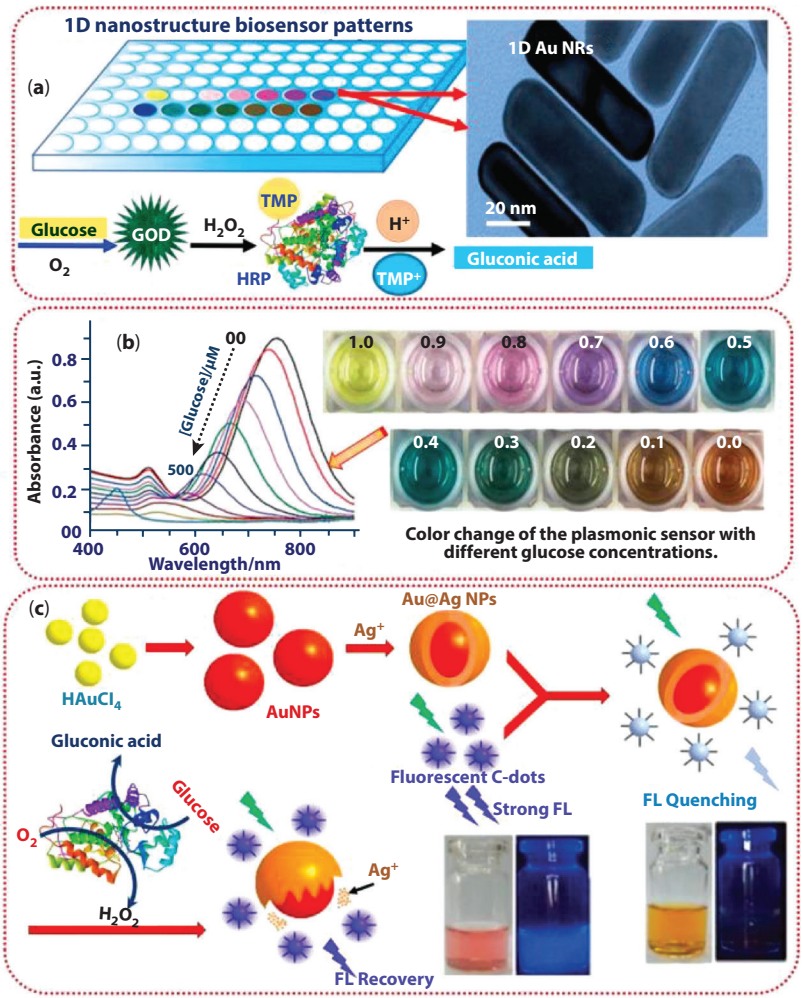


Figure 1.8 Disposable strips of optical glucose biosensor and wearable electronic devices [16].

This type of biosensor undergoes changes in its spectral properties when the binding of enzyme takes place. After the binding of the enzyme with the glucose, the fluorescence intensity change is seen for the protein part of the enzyme but no associated change is seen in the absorption spectra of the same. Co-enzymes can contribute to absorption and luminescence changes due to its interaction with glucose. The utilization of oxygen upon the interaction of the enzyme is measured to determine the glucose concentration using probes [7]. Nano/microscale device plays remarkable impacts in developing sensitive visualization assays, low-cost analyses, and home tests of diabetics (Figure 1.8).

Fluorescent-based glucose sensing is an exceptionally sensitive technique for detecting glucose at the molecular level. The sensing schemes include enzymes which include plant lectin, bacteria, or intrinsic cellular fluorescence. The smaller size of probes such as dyes and quantum dots enables biofunctionalization through diffusion rather than endocytosis.

Fluorescent probes are remotely interrogated using an external UV excitation source, and are capable of penetrating tissues to a depth of centimeters. This results in specific and configurable photoluminescence in quantum dots and dyes. Förster resonance energy transfer (FRET) is the key process behind the photoluminescence behavior. Notably, observations are made without interference from light scattering in tissues, and corrections are applied for errors due to photobleaching or fluorophore degradation.

Furthermore, FRET-based biosensors offer spatial resolution of target analytes, as the decay time of acceptor/donor fluorophores depends on the distance as $1/R^6$. When fluorophore-to-fluorophore distances reach above 10 nm, FRET signals attenuate rapidly. For non-invasive, *in vivo* sensing, and continuous monitoring, fluorescent-based glucose sensors are highly advantageous. Additionally, optical glucose sensors can accurately measure fluctuations in glucose level and associated biochemical pathways, including feedback cascade involving glucose catabolism, adenosine triphosphate (ATP) production and other biological processes [9].

A novel optical Prussian blue (PB)-based biosensor is developed which can detect H_2O_2. It evaluates the pH and acts as an optical transducer in pH-based biosensors. The redox species are detected based on the color change on its reduction. The used film is then renewed on introducing it to a flow injection system. The film system serves as the transducer for optical biosensors. The modified reduced film, combined with glucose oxidase, forms the basis of an optical biosensor. This type is mainly used for the glucose determination in urine samples. This sensor used for the determination of glucose in soft drinks is an optical fiber biosensor. Electroluminescence is the basic working principle of such sensors where