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Review

Cholesterol Detection by Electrochemical Sensors: A Review

Ahmed Kareem Obaid Aldulaimi,[1,*] Alhussein Arkan Majhool,[2] Shakir Mahmood Saeed,[3] Mohaned Adil,[4] and Ayat Hussein Adhab[5]

[1]*College of Food Sciences, Al-Qasim Green University, Babylon, Iraq*
[2]*College of Applied Medical Sciences, University of Kerbala, Kerbala, Iraq*
[3]*Department of Pharmacy, Al-Noor University College, Nineveh, Iraq*
[4]*Medical Technical College, Al-Farahidi University, Iraq*
[5]*Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq*

*Corresponding Author, Tel.: +9647842555056
E-Mail: ahmedaldulaimi1@gmail.com

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Abstract- One of the important molecules in homeostasis, especially for hormone metabolism, cellular membrane production, and vitamin D synthesis is cholesterol. However, studies showed that increased levels of this molecule would be associated with increased incidence of cardiovascular diseases including heart failure and myocardial infarction (MI). Thus, the measurement of the blood level of cholesterol is an important step for early detection and prevention of several diseases. Electrochemical sensors with high accuracy could be useful for the detection of cholesterol in body fluids. Due to the outstanding chemical and physical properties that nanoparticles possess, they are perfectly suited for the development of new and improved sensing devices. In particular, electrochemical sensors and biosensors are two types of sensing devices that could benefit from the use of nanoparticles. Many different kinds of nanoparticles, such as metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles, and even composite nanoparticles, have found widespread application in electrochemical sensors and biosensors, respectively. This review has covered a variety of electrochemical biosensors for cholesterol detection, including conductometric, amperometric, and potentiometric-based biosensors, as well as their detection techniques and limitations.

Keywords- Electrochemical Biosensors; Cholesterol; Conductometry; Amperometry; Potentiometry

1. INTRODUCTION

Cardiovascular diseases (CVDs) are among the most common causes of mortality and morbidity in different communities. For instance, 17.8 million deaths occurred only in 2017, indicating the importance of CVDs [1]. One of the critical risk factors of CVDs is hypercholesterolemia. It leads to atherosclerosis and a further increase in myocardial infarction (MI) and other related disorders. Thus, the determination of cholesterol levels is one of the primary steps for the clinical approach and treatment of CVD patients [2]. However, cholesterol is also required for normal functions of the body. Contribution to the cell membrane, hormones, and vitamin D production indicates its essence for normal body function. This molecule and other fatty acids are required for homeostasis and several critical functions of the body such as cell membrane and hormone production are based on the existence of cholesterol and other lipids. A blood cholesterol level of less than 5.17 mmol/L (200 mg/dL) is considered a normal level, 5.17 (200 mg/dL) to 6.18 mmol/L (239 mg/dL) is borderline high, and more than 6.21 mmol/L (240 mg/dL) is considered high [3]. Diet and liver are the two main origins of cholesterol and due to their hydrophobic nature, their carriers are soluble particles called lipoproteins [4].

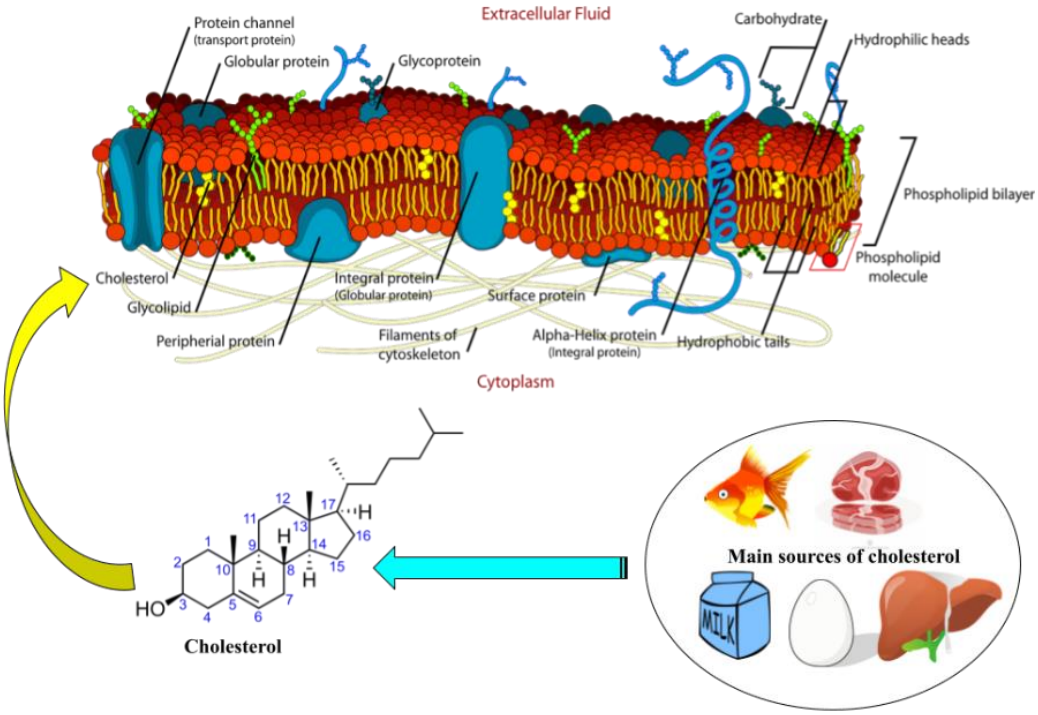

Figure 1. Schematic shows Cholesterol comes from two sources (The liver and from foods and animals). Cholesterol plays a pivotal role in maintaining the structural integrity and regulating the fluidity of cell membranes

Lipoproteins are composed of cholesterol esters, free cholesterol, phospholipids, triglycerides, and apolipoproteins. Seven classes of lipoproteins have been introduced by their

size, apolipoprotein type, and lipid compositions including chylomicrons, chylomicron remnants, IDL, VLDL, LDL, HDL, and Lp. Some of them such as LDL and VLDL are atherogenic and HDL is anti-atherogenic. Hypertension, arteriosclerosis, lipid metabolism dysfunction, nephrosis, jaundice, anemia, and several other diseases could be caused by high levels of cholesterol. Thus, the detection of cholesterol levels could be used as an effective tool for early prevention of CVDs. Due to the importance of this molecule, several analytical methods such as gas chromatography (GC), liquid chromatography (LC), classical chemical methods, mass spectrometry (MS), and enzymatic assays have been developed for its detection [5,6]. Biosensors could be used as an alternative method for the detection of cholesterol due to their high accuracy in the detection of both free and esterified cholesterol. However, their expensiveness, complexity, and time consumption have made their use a challenge [7,8].

2. ELECTROCHEMICAL SENSORS

Electrochemical sensors are valuable diagnostic instruments because of their small size, rapid response time, and low cost [9-18]. IUPAC defines a chemical sensor as "a device that converts chemical data, ranging from the concentration of a single sample component to complete composition analysis, into an analytically usable signal" [19]. Two essential function units are required for sensor function: its physicochemical transducer and receptor. A transducer is a convertor of the signal created by the interaction between receptor and analyte to a comprehensible value. Another part of sensors is their receptor. Receptors could be varied from surfaces to molecules which could interact with the analyte specifically. Biosensors are created by using biomolecules as receptors, such as DNA and antibodies [20,21]. Several categories have been introduced for electrochemical sensors such as electrogenerated chemiluminescence, amperometric, potentiometric, impedimetric, and photoelectrochemical sensors. For instance, in potentiometric sensors, Nernstian equilibrium is formed at the sensor interface due to the interaction between the sensor and analyte. Information about the concentration of the analyte has been obtained when no current is allowed to flow in the system. Amperometric sensors use a voltage applied across a reference electrode and a working electrode to start electrochemical oxidation or reduction, and then measure the resulting current as a quantitative indicator of the analyte's concentration. On the other hand, conductometric sensors, also known as impedimetric sensors, assess variations in the surface impedance to identify and quantify analyte-specific recognition events on the electrode [22]. Several studies showed the capacity of electrochemical sensors for cholesterol detection [23]. Low detection limit, portability, wide linear response reproducibility, and high sensitivity are some of the advantages of these sensors [23]. In light of the above, the purpose of this article is to provide a comparative review of the various electrochemical sensors for the analysis of Cholesterol in terms of their performance, including their detection range (LDR), detection limit (LOD), and electrode modification.

3. NANOPARTICLE APPLICATION IN ELECTROCHEMICAL SENSORS

Nanoparticles are ideally suited for the development of new and enhanced sensing devices, particularly electrochemical sensors and biosensors, due to their exceptional chemical and physical properties [10,18,24-31]. Nanoparticles with a high ratio of surface area to volume exhibit extraordinary properties that are in many cases distinct from bulk materials [32-40]. Due to the innovative evolution of nanostructures and their widespread applications in numerous scientific disciplines, a variety of nanostructure fabrication techniques, including sol-gel, co-precipitation, and solid-state, have been developed for their synthesis [33,36,41-48]. In electrochemical sensors and biosensors, numerous types of nanoparticles, including metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles, and even composite nanoparticles, have been widely utilized [14, 49-54].

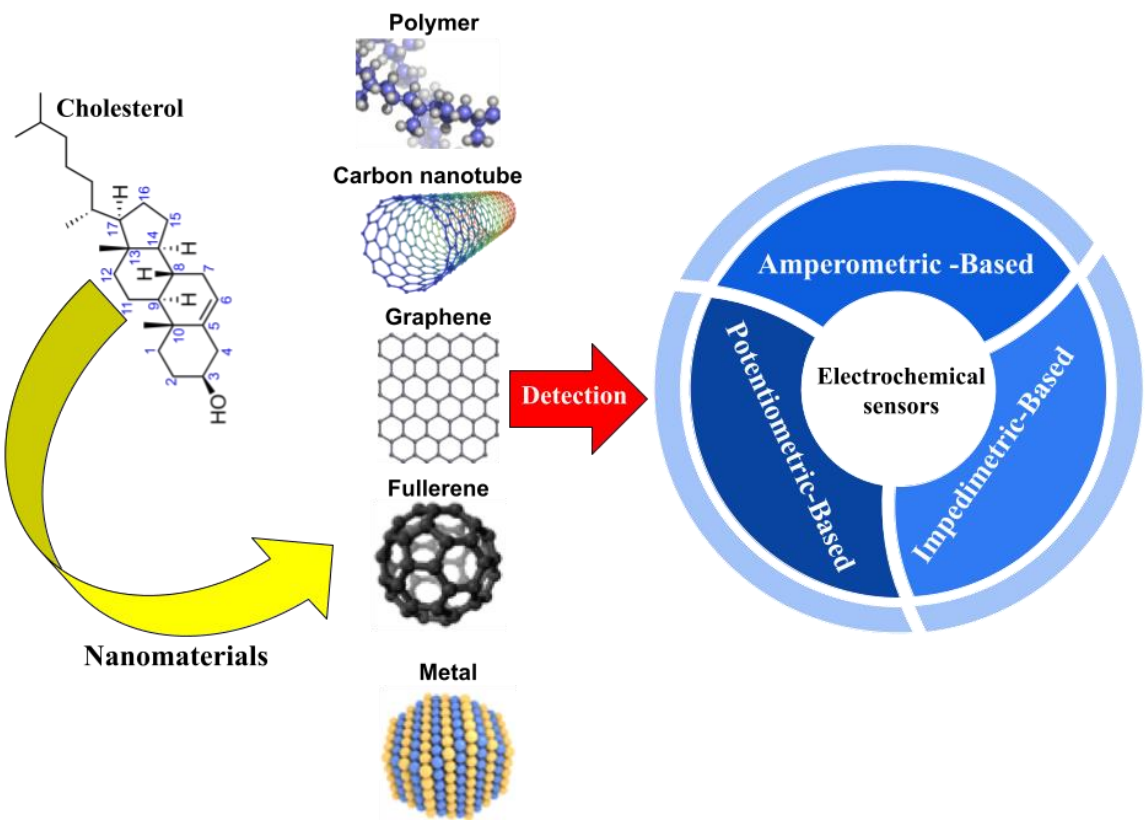

Figure 2. Nanomaterials and their application in the development of electrochemical cholesterol sensors

4. AMPEROMETRIC-BASED ELECTROCHEMICAL SENSORS FOR CHOLESTEROL DETECTION

As mentioned above, amperometric sensors measured analyte concentration based on redox reaction. To perform this measurement, a controlled-potential system is needed with an electrochemical cell with two or three electrodes placed in an electrolyte of appropriate

composition [55,56]. A more advanced design often used is the three-electrode cell, where one electrode acts as a reference electrode to keep the potential constant relative to the working electrode where the reaction occurs [57,58]. Graphite and platinum are appropriate materials for use as a third electrode. Moreover, appropriate electrolyte plays a crucial role in the decrease of solution resistance via inhibition of electromigration effects [18].

In a study, Dey et al. used biosensors for the detection of cholesterol and H_2O_2. Cholesterol oxidases, enzymes, graphene, and nanoscale Pt particles were used in these biosensors. First, they developed electrochemical sensors using Pt nanoparticles and graphene. Pt nanostructures had an average size of 12 nanometer randomly distributed on the surface of graphene. This sensor showed a linear response to H_2O_2 up to 12 mM and its detection limit was 0.5 nM. Pt nanoparticle catalytic activity along with the high conductivity of graphene increased H_2O_2 oxidation which further increased the accuracy of this electrochemical sensor. By adding cholesterol oxidase and cholesterol esterase to this material surface, they increased the sensitivity to about 2 $\mu A/\mu M/cm^2$ with a detection limit of 0.2 μM. They found that these biosensors were highly stable and other electroactive materials could not disturb their function [59]. In a study, researchers used cholesterol oxidase nanoparticles (ChOxNPs) aggregates, and glutaraldehyde cross-linked, functionalized cholesterol esterase nanoparticles (ChENPs), and Au as an electrode to develop a biosensor with improved amperometric determination of serum cholesterol. ChENPs and ChOxNPs had an average size of 35.40 nm and 56.97 nm, respectively. The detection range of biosensors was 10-700 mg/dl for cholesterol and it could measure low amounts of cholesterol (about 0.1 mg/dl). Additionally, they found a strong correlation between the current method and other conventional methods in measuring serum cholesterol levels [60,61]. Giri and colleagues emphasize the importance of controlled synthesis of materials with porous nanostructure for cholesterol sensing due to their good electron transport characteristics, high surface area, and unique optical and electrical properties. In this study, a microtubular ZnO@ZnS heterostructure through a simple aqueous chemical sulphidation process from corresponding ZnO microtubes was synthesized. Furthermore, ZnS microtubes were obtained by removing Zinc oxide from the ZnO@ZnS nanostructure by using acetic acid. Both ZnS and ZnO@ZnS microtubes had high surface areas of 68 and 56 m2 g−1, respectively, and modified electronic structures. Good amperometric cholesterol-sensing performance and sensitivity of 52.67 mA M^{-1} cm^{-2} with a low limit detection of 0.02 mM were measured in ZnO@ZnS microtubes. Additionally, in comparison with Au- or Pt-modified sensors, the ZnO@ZnS heterostructure had better performance due to the facilitated electron transport to the electrode and modified electronic structure [62].

In another study, a cholesterol biosensor was developed using cholesterol oxidase and glutaraldehyde crosslinking procedures. Electropolymerization of pyrrole and aniline on a Pt surface was done using cyclic voltammetry between 0.0 and 0.7 V. Identification of cholesterol was based on H_2O_2 oxidation and this biosensor showed a response time of 300 seconds.

Reproducibility and stability tests showed during 30 activities 82% of the response was retained and during 23 days 60% of the response was retained [63]. Hooda and Colleagues developed a biosensor which was an HRP-incorporated carbon paste-based amperometric biosensor that used cellulose acetate (CA) membrane, cholesterol oxidase, and cholesterol esterase. The incubation temperature was between 35°C to 50 °C and the optimum pH was 7.0. A linear relationship was seen between the amount of current, substrate, and cholesteryl acetate. In addition, the electrode retains its activity at 50% after storage in sodium phosphate buffer [64]. Chawla et al., prepared cholesterol oxidase NPs and used them for cholesterol amperometric detection. The desolvation method was used to create nanoparticle aggregates of commercial cholesterol oxidase (ChOx) and their formation and characteristics were studied using transmission electron microscopy and scanning electron microscopy. The NP aggregates were found to be longer-lasting, more active, and more stable than the free enzyme. The optimum pH and temperature were 6.0 and 35°C respectively. The low detection limit was 1.56 mg/dl in a linear range of 12.5–700 mg/dl. Moreover, by using 0.1 M sodium phosphate buffer at pH 7.0 and 4 °C the electrode retained 50% of its activity for 180 times over a period of 90 days [65].

In another study, Sharma et al. developed a versatile and highly sensitive biosensor platform. The platform is based on an electrochemical-enzymatic redox cycle induced by selective immobilization of enzymes on interdigitated nanoscale carbon electrodes (IDEs) decorated with gold nanoparticles (AuNPs). Without resorting to sophisticated nanofabrication technologies, they used wafer-level batch carbon microelectromechanical systems (C-MEMS) processes to fabricate 3D carbon IDEs in a reproducible, simple, and cost-effective way. Additionally, the AuNPs were selectively electrodeposited on specific carbon nanoelectrodes; The high surface-to-volume ratio and fast electron transfer capability of AuNPs enhanced the electrochemical signal through these carbon IDEs. To selectively detect cholesterol using AuNP/carbon IDE, ChOx was selectively immobilized by electrochemical reduction of the diazonium cation. The sensitivity of the AuNP/carbon IDE biosensor was ensured by efficient amplification of the ferricyanide and ferrocyanide redox mediators between the selectively immobilized enzyme sites and the two AuNP/carbon IDE peaks. The presented AuNP/carbon IDE-based cholesterol biosensor exhibited a wide sensing range (0.005–10 mM) and high sensitivity (~993.91 μA mM−1 cm−2; limit of detection (LOD) ~1.28 μM). In addition, the proposed cholesterol biosensor was found to be highly selective for cholesterol detection [66].

Phetsang and colleagues' study successfully demonstrated a simple method for the development of sensitive amperometric biosensors by electrochemically synthesizing a Pt/rGO/P3ABA nanocomposite (platinum/reduced graphene oxide/poly(3-aminobenzoic acid) film on a screen-printed carbon electrode (SPCE). Co-electrodeposition of rGO and Pt along with the reduction of GO to rGO and electropolymerization of P3ABA were used for the development of this platform. Investigators used this platform for the development of glucose

and cholesterol biosensors. Results showed high sensitivity of 15.94 and 22.01 μA mM^{-1} cm^{-2} and low detection limits (LODs) of 40.5 and 44.3 μM for cholesterol and glucose, respectively. Additionally, concentration ranges were from 0.25-6.00 mM for glucose and 0.25-4.00 mM for cholesterol [67].

Table 1. Nanomaterials and Electrochemical methods for Cholesterol detection

Nanocomposite	Cholesterol sample	Method	Linearity range	LOD	Reference
$CoCl_2$	model media	linear sweep voltammetry	25–200 μM	2 μM	[71]
positive bromine	model media	cyclic voltammetry	30–200 μM	3.2 μM	[72]
DPADGU	dairy products	differential pulse voltammetry	1 to 200 μM	1.5 μM	[73]
β-CD/MCNTs	serum	differential pulse voltammetry	1 nM–3μM	0.5 nM	[74]
MB-bound β-CD/ PNAANI/Gr	blood	pulse voltammetry	1–50 μM	0.5 μM	[75]
DATDAGUD	serum	voltammetry	0.1-50 μM	0.01 μM	[76]
MB-bound Gr- β-CD	model media	Differential Pulse Voltammetry	0.001–0.10 mM	1 μM	[77]
MWCNT@MIP	model media	cyclic voltammetry	10-300 nM	1 nM	[78]
PMO	blood	chronoamperometry and differential pulse voltammetry	0.001-15.5 M	0.00052 M	[79]
H^+ modified L-MMT	blood	cyclic Voltammetry	1–20 mM	1 mM	[80]
L-MMT	model media	cyclic voltammetry	1–20 mM	-	[81]
PAP/Au NP/ MWNT	model media	cyclic voltammetry and differential pulse voltammetry	1×10^{-13} – 1×10^{-9} M	3.3×10^{-14} M	[82]
NiO/Gr	milk	cyclic voltammetry	2–40 μM	0.13 μM	[83]
ZnO (NRs)	blood	cyclic voltammetry	1–9 mM	1.8 mM	[84]
CuO/Gr	model media	cyclic voltammetry	0.04–300 μM	9 nM	[85]
Cu_2O NP/TiO_2 nanotubes	serum	cyclic voltammetry	24.4–622 μM	0.05 μM	[86]
Cu_2S nanorodes (NRs)	model media	cyclic voltammetry	0.01-6.8 mM	0.1 μM	[87]
Ag NP/chitosan	blood	cyclic voltammetry	2.8×10^{-4} – 3.3×10^{-2} M	0.18 mM	[88]
Pt NP	model media	cyclic voltammetry	2-8 mM	-	[89]
Pt NP/CNT	model media	cyclic voltammetry	0.005-10 mM	0.0028 mM	[90]
Pt NP/macroporous Au	model media	cyclic voltammetry	up to 5 mM	0.015 mM	[91]
CNTs functionalized by nitric acid	serum and blood	cyclic voltammetry and differential pulse voltammetry	1–50 μM	0.017 μM	[92]

In Chauhan et al., study, a pencil graphite rod was used as a platform for cholesterol oxidase from Streptomycin sp. immobilization to further evaluate serum cholesterol. The method has the advantage over earlier amperometric methods in that it requires low potential to generate

electrons from H_2O_2, which does not allow ionization of serum substances. The optimum working conditions were a reaction time of 30 seconds, pH of 6.8, and a temperature of 25°C. the low detection limit was 0.09×10^{-3} M and the concentration range of cholesterol was from 1.29×10^{-3} to 10.33×10^{-3} M. Moreover, after restoration at 4°C, the enzyme electrode was reusable up to 200 times over a period of 25 days [68]. In another study, Pt/PoPD/ChOx (ChOx enzyme immobilized onto Pt/PoPD electrode surface) amperometric enzyme electrode was developed for detecting free cholesterol. Cyclic voltammetry in an acetonitrile-water solution containing o-phenylenediamine (oPD) monomer and (±)-10-camphor sulfonic acid (HCSA) in combination with poly(o-phenylenediamine) (PoPD) polymer film and Pt electrode was used for the development of this sensor with further ChOx immobilization. Optimal pH, temperature, and buffer concentration were calculated at 7.5, 40 °C, and 0.05 mol L^{-1}. With a response time of 150 s and a concentration range of 9.8×10^{-3}-11 μmol L^{-1}, this biosensor could be used for serum cholesterol determination [69]. The stability and sensitivity of biosensors for polyphenols, ascorbic acid, and uric acid have been improved by using epoxy resin membranes as a support for enzyme immobilization. The aim of Pundir et al., study was to create a better amperometric biosensor that could detect serum cholesterol levels, which is important in diagnosing certain medical conditions. To construct the biosensor, cholesterol oxidase was immobilized on an epoxy resin membrane mounted on a platinum electrode with a parafilm. Optimal response time of 25 seconds at 45°C and pH 7.0, minimum detection limit of 1.0 mM, and working range of 1.0 to 8.0 mM cholesterol were some of the features of this biosensor [70].

5. POTENTIOMETRIC-BASED ELECTROCHEMICAL SENSORS FOR CHOLESTEROL DETECTION

Potentiometric biosensors are based on the potential difference between working and reference electrodes. Its response is proportional to the concentration of the analyte relative to its activity with the reference electrode. Field-effect transistors (FETs), coated-wire electrodes (CWEs), and ion-selective electrodes (ISEs), are three important classes of this type of biosensors. ISE is further divided into liquid electrodes, solid electrodes, and glass electrodes. Analyzing anionic or cationic species and organic ions in oil industries, drug manufacturing, and effluents are some of the applications of ISEs. Potentiometric biosensors are low-cost and simple which has caused the spread of using them in different experiments [51-53]. Additionally, athletic performance and clinical medicine are other applications of potentiometric biosensors based on sweat analysis [93]. Israr and colleagues developed an electrochemical biosensor for potentiometric cholesterol determination using ZnO nanorods. Hexagonal-shaped ZnO nanorods were fabricated using 250 μm silver wire and low-temperature hydrochem. Like other biosensors mentioned before ChOx was used and immobilized onto ZnO nanorods. The working range of this biosensor was from 1×10^{-6} M to

1×10^{-2} M, its sensitivity was 35.2 mV per decade, and a stable output signal was obtained after 10 seconds [94]. A new cholesterol biosensor utilizing a stabilized polymeric lipid membrane immobilized on a graphene electrode was developed by Nikoleli et al. ChOx and a polymerization mixture (5 mg of phosphatidyl choline, 0.070 mL methacrylic acid, 0.8 mL ethylene glycol dimethacrylate, 8 mg 2,2′-azobis-(2-methylpropionitrile), and 1.0 mL acetonitrile)were used in this biosensor. The biosensor is highly reproducible, selective, and sensitive, and its slope curve was about 64 mV per decade. Its lipid membrane structure made it biocompatible with human biological fluids and other biological applications [95]. An invention described by Vanaja et al. presented a new sensor that uses molecular imprinting of cholesterol to estimate total cholesterol levels in human blood serum. In this work, the molecular imprint of cholesterol (MIPC) anchored into a silica matrix (SiO_2+Si_3N_4) as a sensing material was fabricated. They found that this invention could selectively detect cholesterol even in the presence of other organic compounds, a situation similar to the human blood [96].

Graphene nanosheets in another study were used for the development of potentiometric cholesterol biosensors. After suspending graphene in N-methylpyrrolidone, these nanosheets were exfoliated onto copper wires. This biosensor showed fast response time, high sensitivity, and wide range of cholesterol detection (approximately 82 mV/decade, 4 seconds and 1×10^{-6} M to 1×10^{-3} M respectively) [97]. Pandey and colleagues main goal were to create a new and enhanced support system using carbon nanotubes for detecting cholesterol via electrochemistry. They achieved this by using a cellulose acetate membrane containing single-walled carbon nanotubes (SWNT) for potentiometric sensing of cholesterol. ChOx was immobilized onto the membrane, resulting in uniform distribution as shown by scanning electron micrographs. The electrocatalytic activity of ChOx/CA-CNT was tested with cholesterol concentrations ranging from 10^{-3} M to 10^{-8} M. Compared to ChOx/CA, the ChOx/CA-CNT showed better electrocatalytic response to cholesterol, making it an effective sensor with a detection limit of 10^{-8} M and a range between 10^{-3} M and 10^{-8} M [98]. Another study introduces a new method for creating a hybrid functionalized electrode for use in a cholesterol biosensor. Pt-Au@ZnONRs and modified multi-walled carbon nanotubes (MWCNTs) were used for the development of this biosensor. The results after ChOx recruitment showed that Pt-Au@ZnONR and MWCNT improved the enzyme performance by creating an optimal environment. Its low detection limit, cholesterol concentration range, and sensitivity were 0.03 μM, 0.1-759.3 μM, and 26.8 μA mM^{-1} respectively [99]. In a similar study, A simple, disposable electrochemical biosensor has been developed to quickly and accurately detect cholesterol levels in saliva. By using a platinum nano-cluster (Pt-NC) with an optimized vertical structure and the right amount of enzyme immobilization, the biosensor can detect low concentrations of cholesterol in saliva. This biosensor had a limit of detection at approximately 2 μM, a sensitivity of 132 μA mM^{-1} cm^{-2}, and a detection range of 2-486 μM.

Furthermore, it could be used for the detection of dopamine, lactate, glucose, and several other substances. Thus, Pt-NC-based biosensors are promising nanostructures for further studies [100].

6. IMPEDIMETRIC-BASED ELECTROCHEMICAL SENSORS FOR CHOLESTEROL DETECTION

Studying electrochemical processes, electrode systems, and their quantitative parameters required specific tools such as electrochemical impedance spectroscopy (EIS). Interfacial properties could be changed after biorecognition activities and EIS could be used for measurement of these events. Biorecognition activities include whole-cell capturing, substrate-enzyme interaction, and antibody-antigen recognition. Impedimetric biosensors with their high sensitivity, stability, accuracy, and wide linear range could be used for the detection of cholesterol [101]. Pt black/Nafion composite and Pt black/PEDOT: PSS (poly(3,4-ethylenedioxythioxythiophene) polystyrene sulfonate) along with an enzyme-loaded nitrocellulose paper were used for the development of an impedimetric biosensor for detection of cholesterol in saliva. Results showed a sensitivity of 7.5%/decade at 1 kHz and a cholesterol detection range of 5-4000 ng/mL, thus this biosensor could be used for the detection of cholesterol in the saliva. Furthermore, BPt/Nafion composite had better results than BPt or BPt/PEDOT electrode [102]. In another study, polypyrrole and Pt nanoparticles were recruited to develop a multilayer nanocomposite electrode for an impedimetric biosensor. Investigators placed Pt Nps electrochemically between two layers of polypyrrole on indium tin oxide (ITO) glass plates which further resulted in a high-electroactive surface area. This area is used for the immobilization of ChOx and ChEt. Results showed a fast response, high sensitivity, and low detection limit for this biosensor (196 Ω/mM/cm^{-2}, 2.5×10^{-4} M/l, and 25 s, respectively) made it favorable for further studies [103]. Similarly, a biosensor for measuring total cholesterol has been created by depositing a film made of silver nanoparticles and polyaniline onto an indium tin oxide-coated glass plate. Cholesterol oxidase and cholesterol esterase were covalently immobilized in the sensor. The nanoelectrodes displayed an impedimetric response that had a fast response time, low Michaelis-Menten constant, low detection limit, wide linear range, high regression coefficient, and high sensitivity. The team successfully used the ChEt-ChOx/AgNP/PANI/ITO nanoelectrode to estimate total cholesterol in blood serum using the impedimetric technique [104]. Alagappan et al. have developed an electrochemical cholesterol biosensor using gold nanoparticles-functionalized multi-walled carbon nanotube-polypyrrole (Au NPs-f-MWCNT-PPy). Firstly, Au NPs-f-MWCNT was created via wet chemical methods. Then, pyrrole was electropolymerized to create PPy, a ChOx support matrix. High sensitivity (10.12 μA mM^{-1} cm^{-2}), the linear response from 2×10^{-3} to 8×10^{-3} M, and low detection limit (0.1×10^{-3} M) along with its validity and reproducibility in biological samples have indicated its advantages and further examinations [105]. In another study, Dhand et al. used nano-

structured polyaniline (PANI) colloidal suspension, indium-tin-oxide (ITO) glass plate, N-ethyl-N'-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to develop a platform for ChOx immobilization. Ultraviolet-visible spectroscopy (UV-vis), Fourier transform-infrared (FT-IR), impedance spectroscopy techniques, and scanning electron microscopy (SEM) were used for the analysis of PANI/ITO and ChOx/PANI/ITO electrodes. This biosensor had a detection limit of 25 $mgdL^{-1}$, sensitivity of 7.76×10^{-5}Abs $(mg/dL)^{-1}$, and linearity of 25 to 400 $mgdL^{-1}$ of cholesterol [106].

7. SUMMARY AND FUTURE PERSPECTIVE

This article provides a discussion of the numerous distinct electrochemical sensors that have been proposed to detect cholesterol. Using nanostructures in biosensors could be a useful approach to increase their sensitivity, and stability, and increase the detection range. Amperometric-based biosensors had better sensitivity and reproducibility in comparison with other types of biosensors. However, one of the main challenges for using these biosensors is their costs and an affordable, nonenzymatic, and simple approach would be needed. As this study has shown, nanomaterials could play a critical role in sensing cholesterol. The increase in electron transfer, biomolecule immobilization, and catalysis of electrochemical reactions are some of the main advantages of these materials. Further studies would be needed about these materials' biocompatibility and their function in the body environment. Furthermore, the development of tunable analytical biosensors with compact sizes would be needed.

Declarations of interest

The authors declare no conflict of interest in this reported work.

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