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A CRITICAL REVIEW ON RECENT ADVANCEMENTS AND CRUCIAL ASPECTS OF ENZYMATIC AND NON-ENZYMATIC CHOLESTEROL BIOSENSORS

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ABSTRACT: This work cites the recent advancements in the enzymatic and non-enzymatic lipid profile, specifically the sensing of low-density lipoprotein (LDL), high-density lipoprotein (HDL) triglycerides (TG). Moreover, this paper dealt with total cholesterol sensors using nanomaterials. Research papers with different synthesizing techniques and materials were reviewed. Accordingly, recent trends and methods are discussed. Furthermore, cholesterol sensors' review reveals that specific cholesterol detection is still to be improved to avoid coronary heart disease—detection techniques to tackle this dilemma overviewed. Future perspectives to modify the Cholesterol sensors using nanomaterials are also discussed.

Keywords: Cholesterol; Bio-sensors; Nanomaterials; Metal-oxide; Non-enzymatic

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1.INTRODUCTION

Biosensors are often labeled as a three-component system comprising a bioreceptor, transducer, and signal-processing unit [1]. The interaction of the target analyte with the bioreceptor results in a quantifiable signal. Sensors have been used for analytes spanning the areas of food testing [2], medicine [3], industry, and environmental sensing [4]. The sensors have been industrialized to substitute traditional testing dealings and often technical methods, demanding precise expertise and time, thus representing a vital role in their respective industries [5,6]. The better and efficient determination of biological recognition elements came into effect by introducing the term "Biosensor." The blossoming of biosensors demands the combination of Physics, Chemistry,

Biology. The international union of pure & applied chemistry (IUPAC) defined biosensors as a device that uses precise biochemical reactions arbitrated by isolated enzymes, immune systems, tissues, organelles, or cells to spot chemical compounds ordinarily by electrical, optical, or thermal signals [7–10]. The signs of progress in nanotechnology enhance biosensor's development in compact form, and achievements in engineering result in inexpensive biosensors. The Coupling of biological elements similar to tissues, cells, antibodies, enzymes, etc., to a transducer results in the proper detection of the signal, finally feeding to the display unit succeeded by processing and amplification. This integrated electronic device is called a biosensor [10,11]. "Biosensor," the term was formulated by Cammann [8–11]. The invention of an oxygen detection sensor was titled Leland C. Clark Jr as the father of biosensors [11,12]. Biosensors have vast applications in medical diagnosis because of their extreme selectivity towards biomolecules. The processing of power of modern electronics, mainly in micro and optoelectronics, enhanced biosensors' utilization. First, the target analyte interacts with the biorecognition element resulting in some physiochemical changes. Then the physiochemical change, which is detected and measured by the transducer. This change will be converted into an electronic signal by the transducer and displayed in analog or digital format. The signal engendered is relative to the total analyte in the sample solution so that analyte quantity can be detected smartly [12,13]. The organic molecule cholesterol is a type of lipid which is an inevitable structural component in animal cell membranes, and these carriers of cholesterol in the blood are lipoproteins. This lipid (fat) and protein combination are conventional of three types viz: low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG) [14,15]. The Framingham study portraits that the non-fasting high lipoprotein and total cholesterol levels in the blood are the leading cause of coronary heart diseases in men and women aged 49 years and above [16]. Exposure to the accretion in individuals' cholesterol levels periodically helps lessen the death rate due to coronary heart diseases. The concentration value of cholesterol less than 200 mg/dL is desirable and depresses the peril for coronary heart issues. The cholesterol level overhead of 200 mg/dL increases the risk. The borderline high range of cholesterol levels is 200-239 mg/dL and 240 mg/dL. A higher level of blood cholesterol doubles the chance of coronary heart difficulties than a standard value person. The minimum range of cholesterol in the body is measured ideal has ranged from 200 - 180 mg/dL with altering humans' lifestyle. Table.1 gives the desirable, borderline, and high values of total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride according to the national cholesterol education guidelines program (NCEP). With collective happenings of stroke and cardiac arrest around us and after spotting the source behind them, more precise and quicker estimation of cholesterol in the serum has gained common concern. To quantify the Cholesterol level, various methods like the fluorimetric method [17], a colorimetric method [18], enzymatic [19], spectrophotometric [20] are used. A method of free enzymes with electrochemical processes has also been used [21].

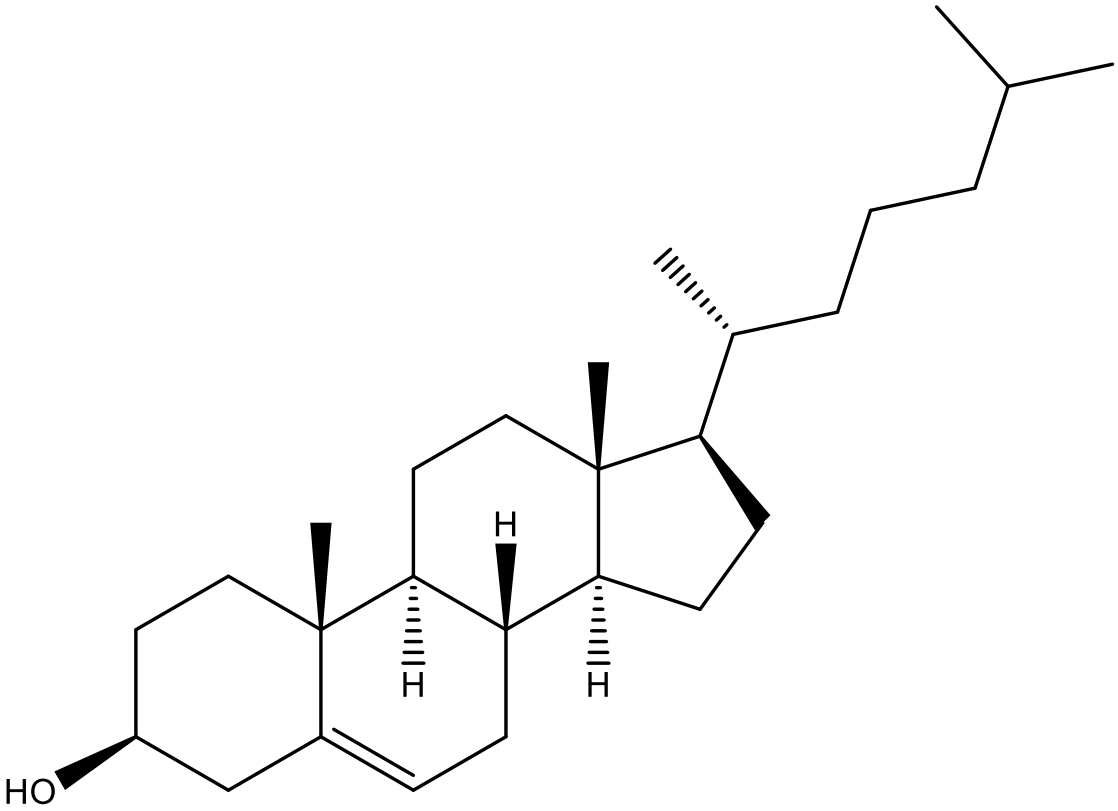


Figure 1 Structure of Cholesterol

But the issues with the mentioned methods are mainly classified as a) they require pre-treatment of the sample, b) time-consuming, c) tedious, d) need to be trained human resources. Detection of Cholesterol via free enzymes is over-priced, too, as it is disposed of after use or parting of free enzymes is again time-consuming. The immobilization process of enzymes permits its recycling. Biosensors with immobilized sensing elements are getting attracted due to their property to give a sensitive and rapid response, reproducible nature, and compact size. These advantages have found various applications that can aid us in our day-to-day life, from health care to environmental monitoring and food analysis to medical diagnostics [22].

Table 1 detailing the range of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides [12]

Cholesterol Type	Desirable(mg/dL)	Border line(mg/dL)	High(mg/dL)
Total Cholesterol	>200	200-239	≤ 240
LDL Cholesterol	>130	130-159	≤ 160
HDL Cholesterol	≤50	40-49	> 40
Triglycerides	>200	200-399	≤ 400

2. Reported review articles in Cholesterol sensors

Table 2 represents the critical review articles reported in recent years

Year	No. of paper	Title of the paper	Contributions	Reference
2020	2	An Overview of Electrochemical Determination of Cholesterol	Various methods, principles, and recent advances in electrochemical cholesterol sensors are reviewed	[23]
		Non-enzymatic electrochemical approaches to cholesterol determination	Reviewed non-enzymatic cholesterol detection in detail	[24]
2019	3	Cholesterol biosensors: A review	The article reviewed various cholesterol-sensing techniques.	[14]
		Electrochemical amperometric biosensor applications of nanostructured metal oxides: A review	Detailed the progress in the nanomaterials enabled sensor, viz. glucose, urea, and cholesterol.	[25]
		The imperative role of polymers in enzymatic cholesterol biosensors- an overview	Analyzed the use of various polymers, biopolymers, and its composites for cholesterol sensing applications	[26]
2018	1	Recent approaches to ameliorate selectivity and sensitivity of enzyme-based cholesterol biosensors: a review	Stages in the evolution of cholesterol sensors and several methods for immobilization of enzymes are discussed in detail	[27]
2017	-	-	-	-
2016	1	Nanomaterials Towards Fabrication of Cholesterol Biosensors: Key Roles and Design Approaches	Reviewed the effect of nanomaterials on the performance of cholesterol biosensors	[28]
2015	-	-	-	-

The present work emphasizes the chemistry and principles behind the process of cholesterol sensing. This review discourses topical advances alongside provides detailing on approaches for enhancing their performance. Finally, this work outlines critical challenges and changes in their further progress and highlights research after 2015 so far.

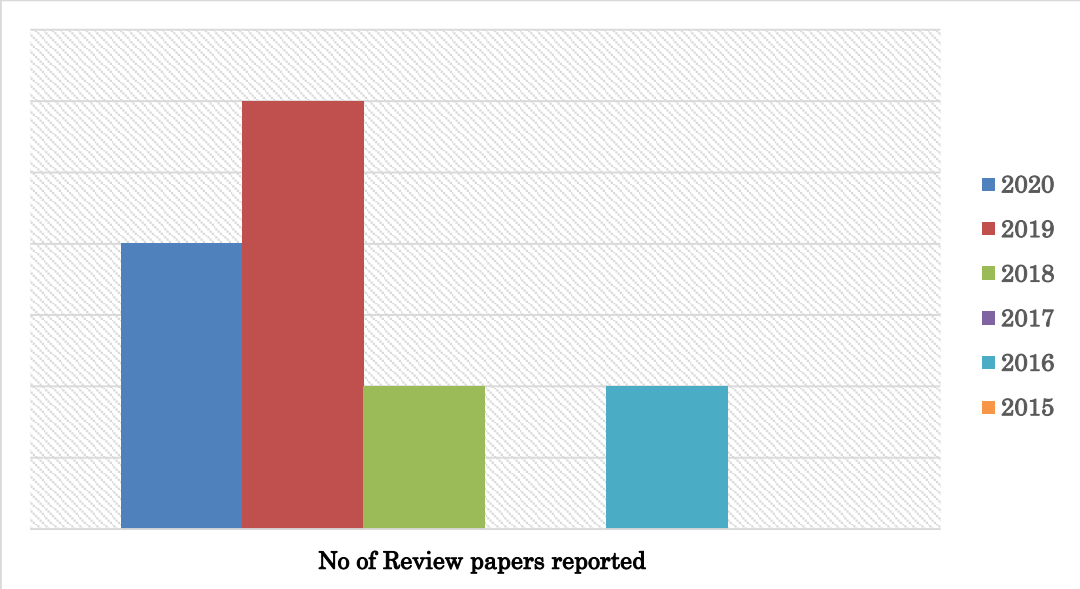


Figure 2 represents the number of review articles reported in recent years

3. Conceptualization of Biosensor

An analytical setup or device that senses the biochemical variations developed due to a particular analyte is termed a biosensor. The first idea leading to the sensing technology is the correlation between the produced signal and the analyte concentration. The primary assembly comprises the biorecognition entity, transducer, and signal processor. The biorecognition entity, the major sensing element in the biosensor, comprises specific biological components: an enzyme, antibody, a cell, receptor molecule, or DNA. The recognition layer is immobilized, directly on the transducer or some other support existing in its immediate contact. The transducer functions as a translator that detects the physicochemical variations (pH change, intake or release of gases, mass changes, electron transfer) and transforms it into an electronic signal. Further, the processor converts it into measurable digital output. The schematic representation of the biosensor has been depicted in Figure.3. In the case of the Cholesterol sensor, the analyte is nothing but cholesterol, more precisely LDL, HDL, or TG. The enzymes Cholesterol oxidase or Cholesterol esterase is the common receptors for Cholesterol detecting biosensors [15,29]. The transducers can be any electrodes, thermistors, photodiodes, etc. This transducer converts the biorecognition entity into some measurable signal and is then fed to the display unit. Depending upon the transducer , biosensors are classified into different categories, namely electrochemical [10,29–32], ion-sensitive [10,33,34], optical [4,35,36], piezoelectric [10,37–39] biosensors. Among these, Electrochemical approaches take a step ahead in terms of desirable selectivity, easiness in preparations of the sample, required economic instrumentation facilities, simplicity, and a platform to fabricate cholesterol sensors with appreciable performance.

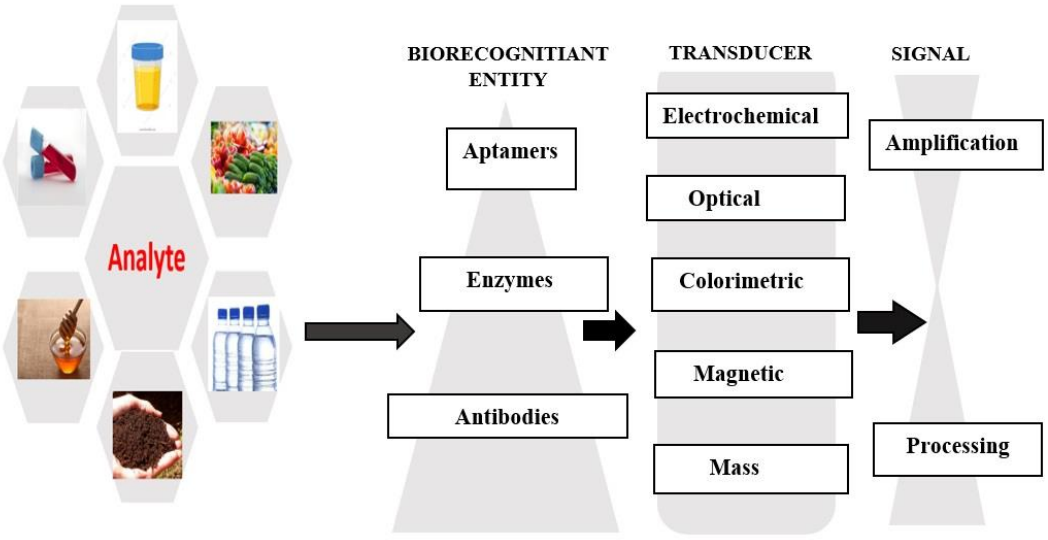


Figure 3 Schematic representation of the biosensing mechanism

3.1 The electrochemistry behind the Cholesterol sensing mechanism

The proficient approaches behind cholesterol sensing are mainly classified into enzymatic and non-enzymatic. The most used recognition elements in the enzymatic cholesterol sensors are cholesterol oxidase (ChOx) and cholesterol esterase (ChE). Moreover, a new method to lessen interference is introduced, using Horseradish peroxidase (HRP). HRP comprises a redox center connected with ferroheme /ferriheme pair, and it facilitates the conversion of reduced state into oxidized and vice-versa via the direct electron transfer. Over these advantages, cholesterol's enzymatic detection stuck on a few disadvantages, mainly related to the stability of enzymes such as ChOx and ChE, which get altered by exposure to the factors, namely temperature, moisture, pH, *etc.* To overcome the disadvantages of enzymatic detection alongside enhanced sensor parameters, the non-enzymatic detection method was introduced. In addition to stability and reproducibility, non-enzymatic detection comprises several advantages, such as the facile fabrication method, cost-effective [40]. Thus, considerable efforts have been attentive to fabricate cholesterol sensors for non-enzymatic detection. Furthermore, due to the sturdy catalytic capability and facile incorporation of metal nanoparticles to the electrode surface, non-enzymatic detection strategies are getting attention [41].

3.2 The enzymatic interaction of Cholesterol molecules with cholesterol oxidase (ChOx)

In this approach, the cholesterol concentration level in the sample was assessed by measuring the amount of hydrogen peroxide (H_2O_2) produced over the enzymatic process. The enzyme ChOx is regularly used to sense cholesterol since it smoothly catalyzes and enhances cholesterol's oxidation process. The oxidation of cholesterol in the presence of ChOx results in the products cholest-4-3-one and H_2O_2 [42]. When the Cholesterol oxidase reacts with the cholesterol in the blood sample, the final product results in hydrogen peroxide. The reaction is as follows,

Cholesterol + O_2+ ChOx ⟶ cholestene-3-one + H_2O_2 ⟶ (1)

Cholesterol ester + water ⟶ cholesterol + fatty acids ⟶ (2)

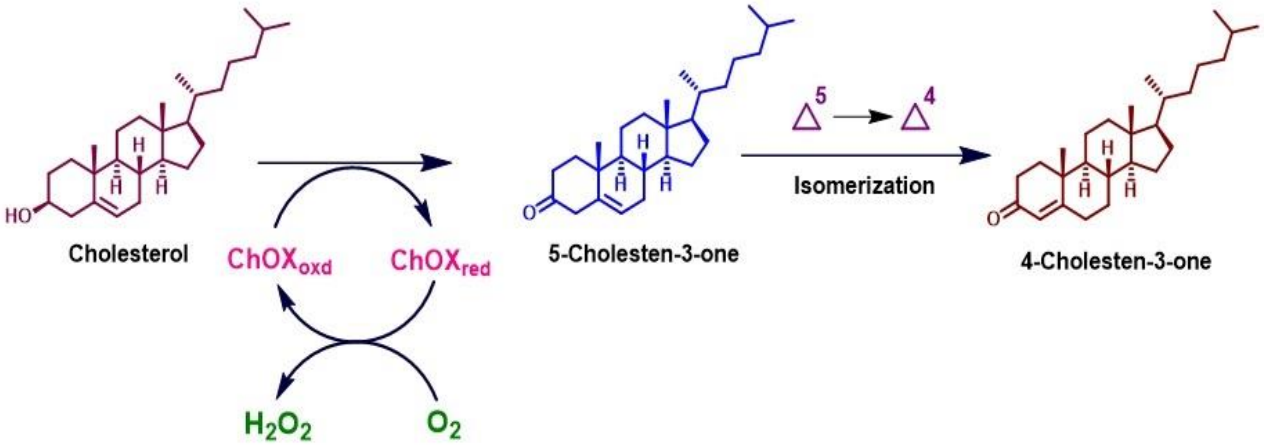


Figure 4 The pathway of cholesterol oxidase enzyme reaction [43]

In the existence of the ChOx enzyme, oxygen oxidizes the cholesterol produced from cholesterol ester to cholestane-3-one and H_2O_2 [40,44]. Figure.4 shows the pathway of Cholesterol oxidase enzyme reaction. This review article highlights new approaches in the nanomaterials for cholesterol biosensor designs.

Furthermore, H_2O_2 can be followed by the qualitative interaction with homovanilic acid, catalyzed by HRP, or Trinder's reaction [45]. Cholesterol is generally found in the blood in its esterified form. To observe the total amount of blood cholesterol, the cholesterol esters must be hydrolyzed via ChEt before the examination [46]. Dey et al. described a cholesterol sensor utilizing the enzymes ChOx and ChEt. The latter hydrolyzes the cholesterol ester to cholesterol, and the former catalyzes the oxidation process of cholesterol. The amount of generated H_2O_2 was sensed by the Platinum nanoparticles [47].

3.2.1 Enzyme immobilization techniques

Enzyme immobilization results in the physical confinement and localization of the enzymes in a precisely defined space without hindering their catalytic nature, supporting repeated and continuous use. The use of immobilized enzymes is appreciable in organic synthesis procedures. It enables the ability to achieve the technical and economic profits of isolated enzymes-based biocatalysts alongside enhances its specificity, thereby reducing product inhibition [49]. The idea of stabilization has been a motive force for the concept of enzyme immobilization [49]. The enzyme immobilization on the electrode surface has advantages like cost-effective enzyme preparation and supports repeat usages. The mode of enzyme immobilization is the utmost vital part that chooses the biosensor's sensitivity range and stability [50] .

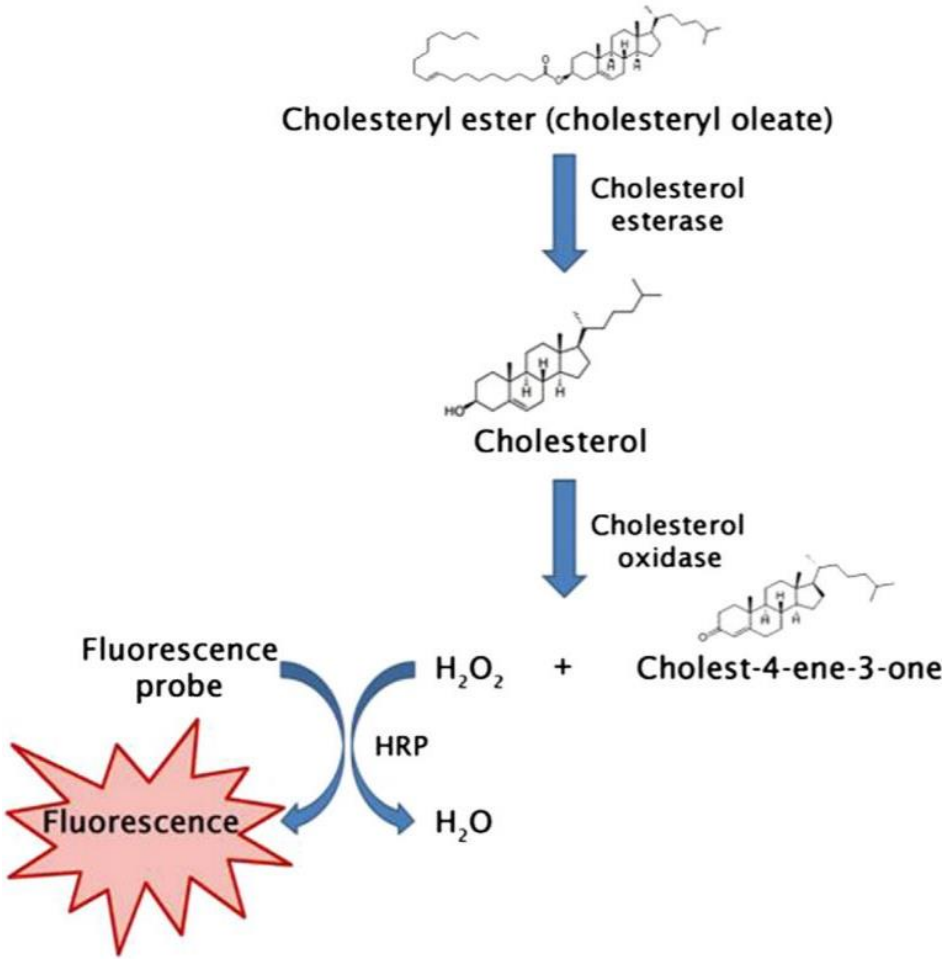


Figure 5 represents the illustration of the cholesterol detection principle[48]

The enzyme can be immobilized using different strategies that are adsorption, covalence, entrapment, c ross-linking, or affinity, which is illustrated in Figure.6, and Table.3 describes the methodologies in detail [29,51]

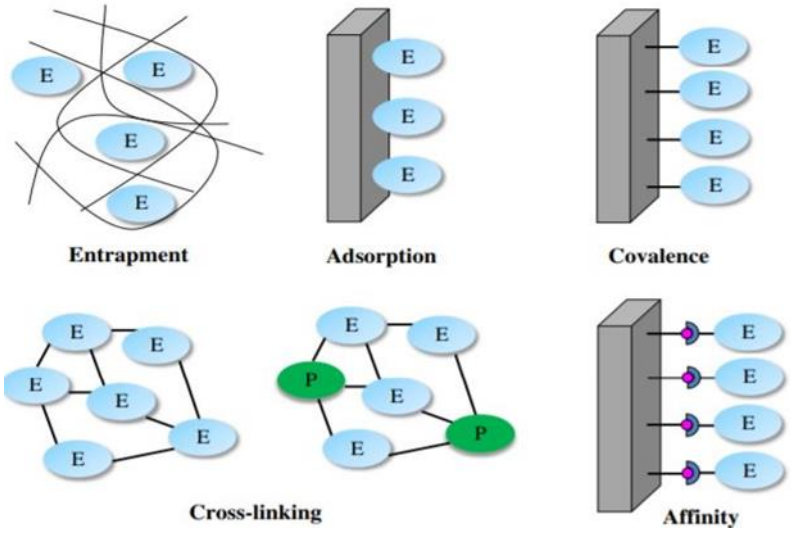


Figure 6 Illustrates different immobilization techniques[51]

Table 3 describes different immobilization techniques [51]

Immobilization technique	Binding Method	Benefits	Drawbacks
Non-covalent Adsorption	Weak forces or bonds	Facile, Simple, Less loss on enzyme activity	Desorption, Non-specific adsorption
Covalent Coupling	Interaction between the functional groups in the enzyme and the support matrix	Absence of diffusion barrier, Stable, Short response time	Matrix not regenerable Coupling with a toxic product
Crosslinking	Covalent bonding between the molecules of an enzyme via poly-functional reagents	Facile	High enzyme activity loss
Entrapment	Capturing the enzymes in gels or polymers	Interaction between the enzyme& monomer that could affect the activity Different enzymes can be immobilized within the same polymer	Diffusion barrier, Enzyme leakage, High concentrations of enzyme and monomer needed
Affinity	Affinity bonds between a functional group on a support and affinity tag on a protein sequence	Controlled & oriented	The necessity of the presence of a specific group in the enzyme

3.2.2 Study on enzymatic Cholesterol sensor

Enzymatic detection of Cholesterol level in the samples get much attention among the various sensing mechanisms.

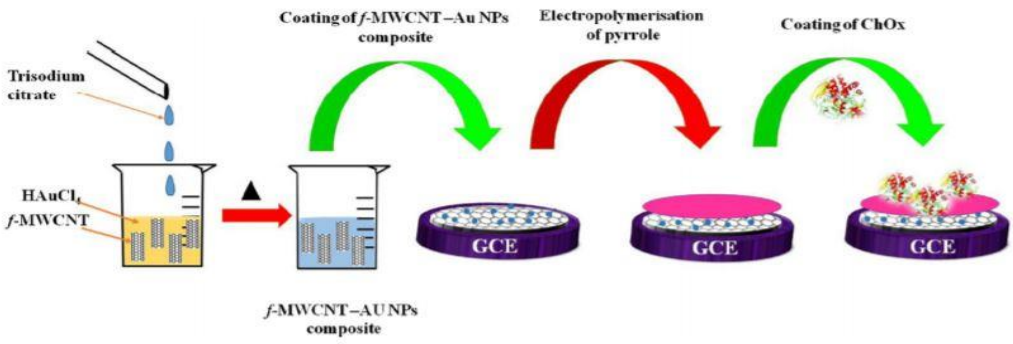


Figure 7 Schematic representation of the manufacture of the Au-f-MWCNT-PPy-ChOx/GCE sensor[48]

Table.4 details a few reported works on some sensors' sensing characteristics based on ChOx, ChE, HRP. M Alagappan et al. reported a novel electrochemical cholesterol sensor using AuNPs-f-MWCNT-PPy-ChOx/GCE as a sensing element. Figure.7 represents the fabrication process of the electrochemical senor using AuNPs-f-MWCNT-PPy-ChOx/GCE as a sensing element.

Table 4 shows a detailed study on the sensing ability of various enzymatic cholesterol sensors

S. No	Enzyme	Immobilization method	Technique involved	Sensing element	DL (µM)	Sensitivity (µA mM^{-1} cm^{-2})	LR *(µM)*	Ref
1.	ChOx	Entrapment	Amperometry	Au-f-MWCNT-PPy	0.1	10.12	2-8	[44]
2.	ChOx	Entrapment	Amperometry	MoS_2-AuNPs/GCE	0.26	4460	0.5-48	[52]
3.	ChOx	Entrapment	Optical	Ag/GO/AgNPs/ChOx	1131	5.14	0-10	[53]
4.	ChOx	Entrapment	Electrochemical	ChOx/CS–GR/GCE	0.715	-	5-1000	[54]
5.	ChOx	Adsorption	Fluorescence	AF-MSN-QD@ZIF-8-ChOx	2.39	-	5.17-258.6	[55]
6.	ChOx	Adsorption	Ratiometric	AF-MSN-QD@ZIF-8-ChOx	0.923 µg/mL	-	2-100	[55]
7.	ChOx	Adsorption	Photometric	PB/MWCNT	3	-	4-100	[56]
8.	ChOx	Entrapment	Fluorescence	ChOx-MOF/AgNC/MoS_2-NS	0.03	-	0.06–15	[57]
9.	ChOx	Electrodepositio	Amperomet	SPE/PB/	11	23.8	36.6-	[58]

		n with PDA and ChOx	ry	MWCNT ox/PDA @ChOx			400	
10.	ChOx	Electrodepositio n with PDA and ChOx	Amperomet ry	SPE/PB/ MWCNT ox/PDA @ChOx	1.5	32.14	5-100	[58]
11.	ChOx	Electrodepositio n with PDA and ChOx	FIA	SPE/PB/ MWCNT ox/PDA @ChOx	-	18.09	100-400	[58]
12.	ChOx ChE	Adsorption	Amperomet ry	ChENPs +ChOxN Ps/Au	8.84	-	10-700	[59]
13.	Apo-ChOx	Entrapment	Amperomet ry	PTBA/F AD/apo-ChOx	0.22	0.21	0.8-4.8	[60]
14.	Apo-ChOx	Entrapment	Amperomet ry	PABA/F AD/apo-ChOx	0.32	0.022	0.8-5.6	[60]
15.	ChOx HRP	Adsorption Entrapment	Colorimetry	PCN-333/ChO x&HRP	0.6	-	0.0-40.0	[61]

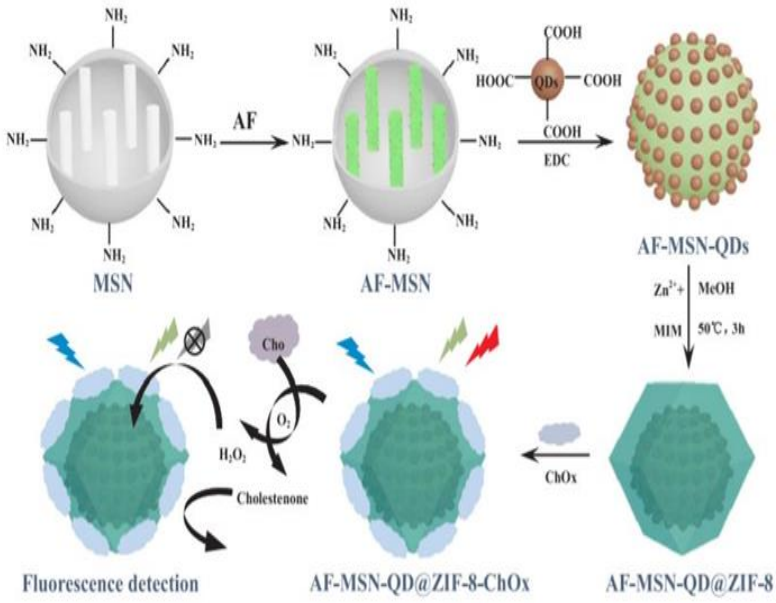


Figure 8 Illustrates the fluorescence detection method proposed by Ke Wang et al. [55]

3.3 Enzyme-free direct biocatalytic oxidation of cholesterol.

The practical difficulty in enzymatic cholesterol detection, limiting the sensor characteristics, paved the way for innovation to non-enzymatic sensors. The variations in attributes with the exposure to temperature and pH alongside the less stability of enzymes are also marked as disadvantages of enzymatic over non-enzymatic biosensors. Moreover, the immobilization procedures, storage, and high cost of enzymes also stand as a significant reason for the idea of non-enzymatic detection of cholesterol sensors [62]. Since cholesterol molecules' non-electroactive nature on the simple monotonous electrodes, sensing via electrochemical methods is supported by either direct or indirect cholesterol oxidation [63]. The incorporation of nanostructures directed the sensors to exhibit enhanced characteristics. Nanotubes, nanowires as well as nanoparticles are exploited the surface modification and recuperating the efficiency of biosensors.

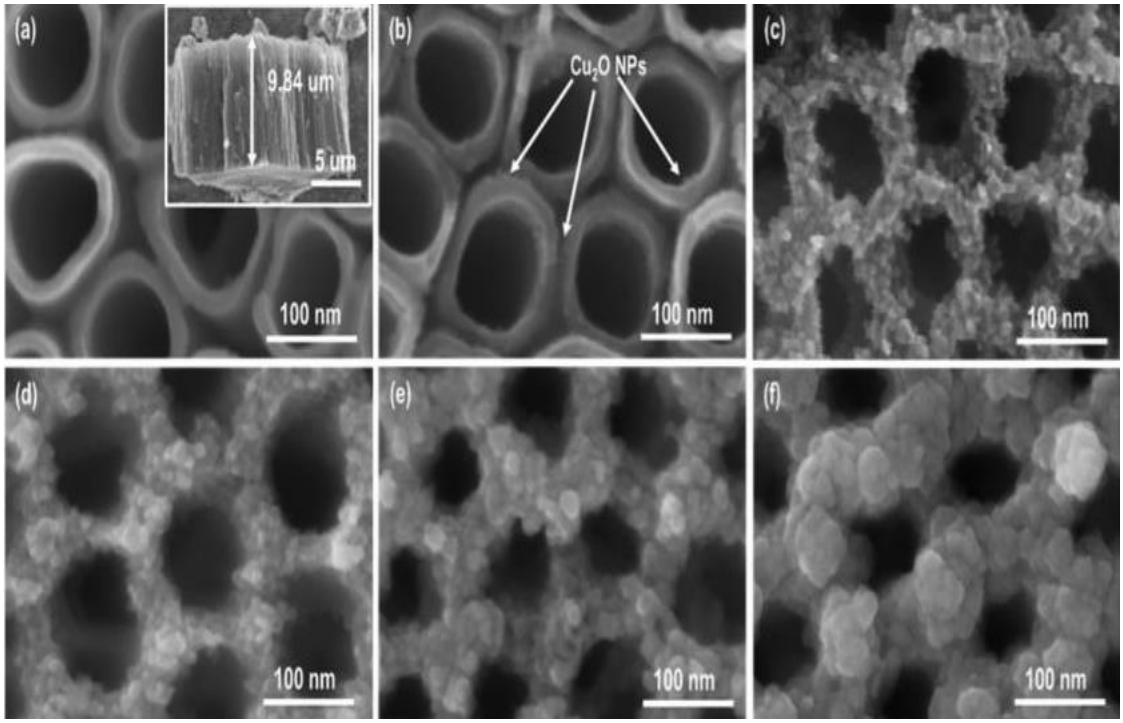


Figure 9 SEM images showing the top view of (a) pristine TNTs, and TNTs decorated with Cu_2O NPs for (b) 5, (c) 10, (d) 20, (e) 30, and (f) 40 cycles. Inset showing pristine TNTs film thickness [65]**.**

V. Gautam et al. proposed a PANI/MWCNTs/Starch decorated carbon paste electrode to detect cholesterol effectively. Specific binding sites improved electrocatalytic features, and speedy electron transfer enabled the cholesterol molecules' oxidation process. The suggested nano morphology promises appreciable sensor characteristics in the absence of biocatalyst and analyzed real-time samples [64]. N.Khaliq et al. disclosed a non-enzymatic cholesterol sensor depending on Cu_2O nanoparticle's decorated TNTs electrode. The boundaries between TNTs and Cu_2O nanostructure offers active sites for the effective oxidation process of cholesterol molecules and significantly enhanced the charge transfer rate. The fabricated electrode provides long-term

stability, highly sensitive to pH and thermal variations, and appreciable sensor characteristics. Figure.9 explains the SEM images of the synthesized material. The reduction in the interference of cholesterol in the presence of common species due to the low redox potential of the electrode results in the practical clinical use of the fabricated electrode(65).

Table 5 shows a detailed study on the sensing ability of various non-enzymatic cholesterol sensors

S.No	Technique involved	Sensing element	DL (mM)	Sensitivity ($\mu A\ mM^{-1}\ c\ m^{-2}$)	LR *(mM)*	RP (s)	Ref
1.	Electrochemical	PANI/MWCNTs/starch	0.01	800	0.032 – 5	4-6	[64]
2.	Amperometric	Cu_2O NPs/TNTs	0.00005	6034.04	0.0244–.622	3	[65]
3.	CV method	GO-MIP	$0.1*10^{-7}$	-	$100-1*10^{-7}$	-	[66]
4.	DPVmetric method	Grp/β-CD/Methylene Blue	0.001	0.01	0.001–0.10	-	[67]
5.	Electrochemical	NiO/CVD-grown graphene	0.00013	40.6	0.002-0.04	5	[68]
6.	DPV	Ru-Pi-Ppy/CFP electrode	540×10^{-10}	19988	$1.6*10^{-7}$-.00002	-	[69]
7.	Electrochemical	ZnO nanorods	1.78	4.2	1-9	-	[70]
8.	Electrochemical	Ag NPs-ZnO nanorods	0.184	135.5	1-9	-	[70]
9.	Electrochemical	PVIM-Co_5POM/MNC Composite	1×10^{-18}	210,64	$0.5*10^{-3}$-5	5	[71]
10.	Colorimetric	CuO: GNS composite	0.078	-	0.1 –0.8	-	[72]
11.	Electrochemical	NiO	0.1	27	0.12–10.23	-	[73]
12	Electrochemical	NiO	0.5	63	1-12	-	[73]

Agnihotri et al. suggested a non-enzymatic sensing method for cholesterol using β-CD functionalized graphene and achieved an enhanced sensor characteristic. The work unveils the properties of graphene after β-CD functionalization, especially in terms of solubility. The sensor can easily detect cholesterol using the DPV technique, where cholesterol molecule is replacing MB molecule and forming the inclusion complex within the hydrophobic core of Grp-β-CD [67].

4. Classification based on types of cholesterol

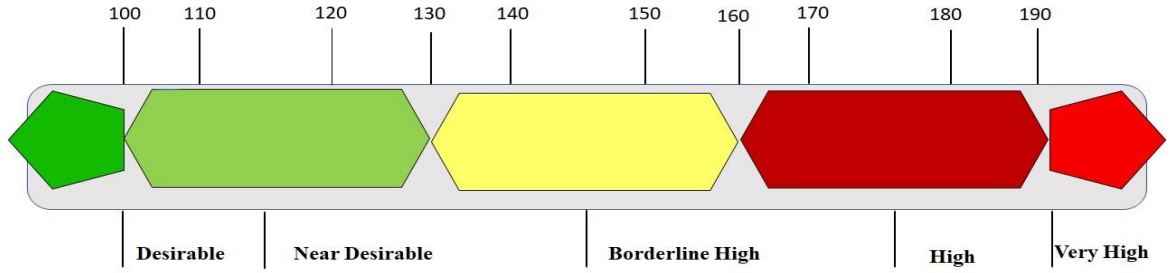
The feasibility of Cholesterol sensing, namely as low-density lipoprotein, high-density lipoprotein, triglycerides, and total cholesterol especially using nanomaterials, has been looked into with another, thoroughly, for further progressive studies and applications. The demand for inspecting cholesterol levels for each category of cholesterol is explicitly mentioned in this critical analysis. The development of handy cholesterol monitors for patients to check the blood cholesterol level is one of the main challenges and scope. The imminent changes in this area are rooted in nanomaterial-based sensors; "a compact, reasonable, efficient and pocket-sized cholesterol sensing device". Various cholesterol devices are overviewed, featuring the relevance and fruitfulness of cholesterol sensors utilizing nanomaterials

4.1 Low-Density Lipoprotein (LDL) Cholesterol Sensor

Cholesterol is transported from the liver to different parts of the body with a low-density lipoprotein carrier, which is too acknowledged as bad cholesterol. Various studies revealed that LDL-C is the primary factor for coronary heart diseases. Figure.10 displayed a desirable range of LDL-C. By good check-up, one can recognize the cholesterol level in the blood to avoid coronary heart diseases to an extent [14–16,29]. Suticha Chunta et al. disclosed the first low-density lipoprotein sensor (LDL-C) form on molecularly imprinted lipoprotein (MIP's). This work favors the development of non-fasting blood testing equipment for the LDL-C clinical diagnosis. In the procedure, the screening of the ratios of monomers acrylic acid (AA), methacrylic acid (MAA), and N-vinylpyrrolidone (VP), respectively, were done by using 10 MHz dual-electrode quartz crystal microbalances (QCM). The unique way of mixing VP and MAA in the ratio 2:3 disclosed the sensor's linearity to LDL-C from 4 to 400 mg/dL in 100 mM Phosphate Buffer solution beyond significant interference. The reported LDL-MIP sensor system reveals 95-96% analytical accuracy at a 95% confidence interval with accuracy at 6-15%, correspondingly. Human serum diluted 1:2 with Phosphate buffer solution was analyzed by LDL-MIP sensors and confirmed its applicability to real-life samples. The sensor responses excellently correlated to the standard technique's results, namely a homogeneous enzymatic assay (R_2=0.97). This correlation corroborates that the system applies to the human serum to track down the LDL cholesterol level. The selectivity of LDL-C in a high rate than HDL-C or Triglycerides is one of the highlights of this work, and the other in this system is helpful for the patients so that they can avoid 12 hrs fasting and for the physicians they can obtain results faster than the current techniques [74]. Guifen Jie et al. reported an LDL-C sensor

hinge on the electrochemiluminescence (ECL) of CdS nanocomposites. In this work, the ECL biosensor, which is not under any label, detects LDL-C, developed using self-assembly, and Ag nanoparticle amplification techniques. The LDL concentration was measured through the decline in ECL intensity rise from the specific binding of LDL to apoB-100.

(a)



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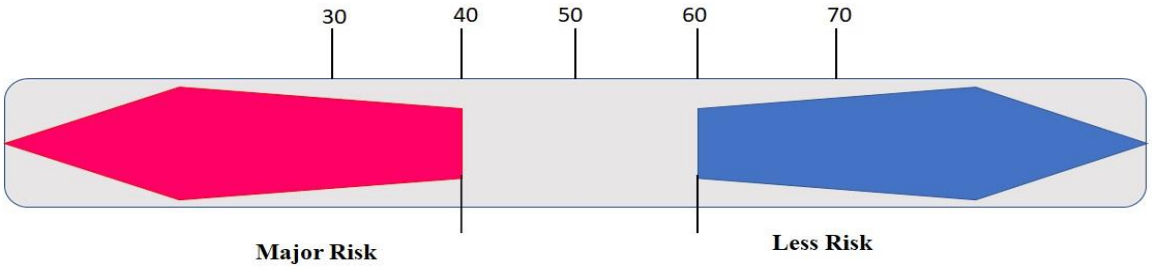

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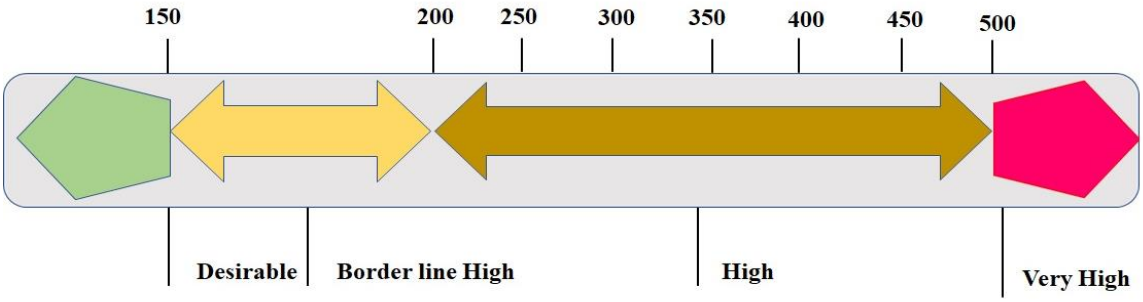

Figure.10 The range of low-density lipoprotein, high-density lipoprotein, triglycerides in (mg/dL) [75]

The sensor's ECL peak intensity declined linearly with LDL concentration in the range of 0.025-16 mg mL^{-1} with a detection limit of 0.006 mg mL^{-1}. The CdS nanocomposites not only showed high ECL intensity and good biocompatibility but could also provide more binding sites for apoB-100 loading. Besides, the gold nanoparticle strengthening for protein ECL analysis was applied to enhancing the detection sensitivity. The nanocomposites are an effective conjugate to provide a sufficient number of sites for binding apoB-100 molecules. The Ag nanoparticle amplification for protein ECL analysis was used to improve the detection sensitivity. The combination of the high sensitivity of nanocomposites ECL detection with the specificity of ligand-receptor binding is one

of the main returns of this biosensor [75] Roli Verma et al. reported Surface Plasmon Resonance sensors using optical fibers compact in size, economical, useful for remote sensing, and high lending accuracy. Also, the sensor offers high sensitivity and biocompatibility. The sensitivity is found by analyzing the spectra and is obtained as 0.18387 nm per mg/dl concentration of LDL. The detection accuracy was calculated from the inverse of the full width at half minimum (FWHM) of the SPR spectra for solutions of different LDL concentrations. The response of the sensor reported in their study is faster (about 2 min) than the previously reported sensors as the reaction and regeneration times are minimal. One of the highlights of their work is the reusability of the sensor. The possibility of online monitoring is an additional advantageous feature of the present sensor [76]

4.2 High-Density Lipoprotein (HDL) Cholesterol Sensor

High-density lipoprotein (HDL) is considered a good Cholesterol because it prevents arterial diseases. Increased HDL-C rate beyond 60 mg dL-1 is deemed a protective role in cardiovascular diseases, while low HDL-C rate > 40 mg dL^{-1} is mediated to an increase in heart diseases. Fig.3shows this desirable range of HDL-C. Consequently, the NCEP gives priority to the frequent measurement of HDL -C levels in the bloodstream [14–16,29,77] Termeh Ahmadraji et al. communicated a novel HDL-C sensor-related to a homogeneous analysis method to determine HDL-C with a printed electrochemical sensor for measuring the reduction of H_2O_2 at an Ag coated electrode. The sensitivity and selectivity are enhanced by the usage of Emulgen (B-66). The biosensor had a linear response of $4.49*10^{-8}$ A mM^{-1} between 0.5- and 4-mM HDL-C with an average r.s.d. of 7.0% [78] Suticha Chunta et al. reported the synthesis of a sensor that can selectively bind the HDL-C with the help of a molecularly imprinted polymer (MIP). Hence, it serves as an artificial biomimetic sensor coating. In the ratio of 2:3, the enhanced polymer contains methacrylic acid and N-vinylpyrrolidone, which is cross-linked with the ethylene glycol dimethacrylate. The dual-electrode quartz crystal microbalance (10MHz) is used in this sensor. Without substantial intrusion, the detection range towards HDL standards in the clinically relevant ranges is 2-250 mg/dL HDL-C in 10 mM phosphate-buffered saline (PBS, pH = 7.4). The sensor discloses recovery rates between 94 and 104% at a 95% confidence interval with an accuracy of 2.3–7.7% and shows a considerable correlation with the enzymatic colorimetric assay, which is the standard in clinical tests. Our sensor's repeatability at HDL-C solution at concentrations of 20, 50, and 100 mg/dL gives a coefficient of variation (CV) of 7.7, 2.3, and 3.4%, respectively, which agrees with the NCEP standard values. One of the highlights of this work is the sample pre-treatment, which is not tedious, but instead, diluting it is sufficient. This MIP sensor's other advantages are significant selectivity, long life term, less time of measurement, and it does not have complex assays [79].

4.3 Triglycerides Cholesterol Sensor

Estimation of triglycerides is crucial since its high concentration can cause hyperlipidemia. The usual triglycerides range is 40-160 mg/dl in men and 35-135mg/dl in women [80–83].

Hyperlipidemia is associated with several disorders, such as diabetes mellitus, nephrosis, etc. [84]. Fig.3 reveals the desirable values of TG-C. Frequent updation about the triglyceride cholesterol level in blood is essential to avoid coronary diseases and the diseases related to hyperlipidemia. Pratima R. Solanki et al. developed a triglyceride cholesterol sensor using Cerium oxide nanoparticles derived using a sol-gel technique coated on ITO glass plates. The enzyme used was lipase, which is extracted from Candilla Rugosa CLEA. This material raised the essential characteristics of TG-C biosensors, linearity as 50–500 mg/dL, a low detection limit of 32.8 mg/dL, response time of the 20s, shelf life of 3 months, sensitivity of 0.195 mA/mg dL cm^2 with linear regression coefficient as 0.998 and standard deviation as 0.0021 mA/mg dL^{-1}. This work gives scope that this electrode can be used to detect other biomarkers, including lipoproteins, uric acid, etc. [85]. C.S. Pundir et al. communicated the construction of triglyceride cholesterol sensors using PVA membrane-bound enzymes. This amperometric biosensor used for TG's measurement was constructed by mounting a PVA membrane having immobilized lipase, GK, GPO, and HRP onto the Platinum electrode with a parafilm. This acts as a working electrode. The reference electrode used is Ag/AgCl and the Cu wire as an auxiliary electrode. The optimization of working conditions done precisely and reports the essential sensor characteristics. At pH 7.0 and 25ºC, this sensor showed optimum response within 2s, and the minimum detection limit was 0.21mM. The current (mA) was in proportion to TG's concentration in the range 0.56–2.25 mM. The minimum detection limit of the method was 0.21 mM [86]. Jagriti Narang et al. communicated the development of triglyceride cholesterol sensor using PVC membrane-bound lipase, glycerol-kinase, glycerol-3-phosphate oxidase, horseradish peroxidase on a Pt electrode along with Ag/AgCl reference electrode and Cu auxiliary electrode. The biosensor measures the electrons generated from H_2O_2 at 0.4 V. The concentration of TG-C was directly proportional to the current measured. A linear relationship was obtained between triglyceride concentration ranging from 0.56 to 2.25 mM and current. This method's detection limit was 0.11 mM. The advantage of the electrode used in this work can be operated 100 times within 40 days, which should be kept at 4^O C [87]. Wei-Yin Liao et al. developed a biosensor with iridium nanoparticles modified carbon to detect triglyceride cholesterol. The amount of enzymatically produced hydrogen peroxide is the detection technique used in this biosensor. A linear response to glyceryl, a short chain of triglyceride (tributyrate) in the concentration range of 0 to 10 mM, and a sensitivity of 7.5 nA mM^{-1} in bovine serum 7.0 nA mM^{-1} in human serum were observed experimentally. The iridium nanoparticle modified working electrode-based biosensor provided a relatively simple means for determining Triglycerides in human serum [88]. Kunal Mondal et al. reported the enzymatic biosensors for the detection of triglycerides with Highly Sensitive Porous Carbon and Metal/Carbon Conducting Nanofiber; Electrospun carbon (CNF) and silver/carbon nanofibers were used to detect these biomolecules. Ag nanoparticles improved electrical conductivity and graphitization to carbon nanofibres. Oxygen

plasma promoted enhanced immobilization of the enzyme on nanofiber. Ag nano-particles' presence increased the sensitivity into fourfold higher than the CNF bioelectrode in the range of 1.232 μA mg/dL^{-1} cm^{-2} and 0.33 μA mg/dL^{-1} cm$^{-2,}$ respectively. The sensor's detection range is 25–500 mg/dL, and it exhibits a faster response (10s). Excellent selectivity, good reproducibility is some of the merits of this triglyceride cholesterol sensor [89]. A. Phongphut et al. presents Triglyceride biosensor based on Au/PEDOT-PSS nanocomposite. They are inkjet-printed and later onto screen-printed carbon electrodes (SPCEs). Lipase, glycerol kinase, and glycerol-3-phosphate oxidase are the enzymes used in this biosensor for immobilization. The presence of Gold nanoparticles enhanced the electrical responses of the sensor. The operating potential is optimized as 0.4 V in 0.1 m NaOH buffer pH of 7.0. The sensor exhibits the characteristics of a wide dynamic range of 0-531mg/dL; sensitivity is 2.66A/Mm, and the response time is the 30s. Moreover, the detection limit of this Triglyceride cholesterol sensor is 7.88mg/D. The reported sensor's advantages are low interference, high reproducibility, and 40% lifetime [90].

4.4. Cholesterol Sensors Using Nano-particles

4.4.1 Metal oxide Nanoparticles

Cholesterol sensing using nanomaterials is more efficient because it provides a large surface-to-volume ratio [91–93]. The preparation methods of nanomaterials also enhance sensing performance. Regular check-up for total cholesterol is inevitable to avoid coronary heart diseases. Thus, increasing the efficiency of sensors also became one of the challenges. Some of the techniques used to synthesize nanomaterials are mentioned in Table.7. Ahmad Umar et al. reported a novel sensor for the detection of cholesterol. The sensor is based on the immobilization of cholesterol oxidase (ChOx) onto the ZnO nanoparticles. This ultrasensitive cholesterol sensor shows a very high and reproducible sensitivity of 23.71A mM^{-1} cm^{-2}. The detection limit 0.37 ± 0.02 nM and the response time less than 5s reflect the increased efficiency of this Cholesterol amperometric sensor. The sensor shows linearity in the range 1.0 to 500.0. A relatively small range of the enzyme's kinetic parameter ~4.7 mM has been obtained, which indicates the enhanced enzymatic affinity to ChOx to Cholesterol [94]. Meihe Zhang et al. developed a cholesterol biosensor based on the electrogenerated chemiluminescence property. The matrix used for the enzyme's immobilization is cerium oxide - graphene composite (CeO_2–graphene). The experiment result reveals that the presence of CeO_2–graphene could catalyze the electron generated chemiluminescence of a luminol hydrogen peroxide system to amplify the luminol ECL signal greatly. Moreover, the use of CeO_2–graphene contributes a unique biocompatible microenvironment for the cholesterol oxidase enzyme, which results in better stability and a long lifetime of this biosensor. This cholesterol sensor's linearity ranges from 12μm to 7.2 mM, with a detection limit of 4.0 μM (signal/noise = 3). Outstanding reproducibility, long-term stability, and selectivity are some of the highlights of cerium-oxide graphene-based sensors [95]. Bansi D. Malhotra et al. communicated the immobilized ChOx enzyme carried out a

biosensor based on CeO2 nanoparticles incorporated within the CH matrix and the cholesterol detection on the matrix. The experimental result reveals that ChOx/CH–NanoCeO2/ITO bioelectrode could catalyze the catalytic property to cholesterol because the nanocomposites provide more active sites which offer a friendly environment for the immobilization of enzymes. The immobilization of ChOx results in increased electron transport between the analyte and CH–$NanoCeO_2$/ITO electrode surface. This bioelectrode exhibits sensing characteristics such as a detection range of 5mg/dL. The ChOx/CH–$NanoCeO_2$/ITO bioelectrode exhibits exciting features such as a detection range of 10–400 mg/dL, the detection limit of 5 mg/dL, response time of 10s, low Km value of 3.5 mg/dL, the value of regression coefficient of 0.994 and the sensor exhibits a high sensitivity of 47 μA/mg dL^{-1} cm^{-2} [96].

Table 6 The comparisons for a different category of Cholesterol biosensors

Cholesterol type	Linear range	Detection limit	Sensitivity	Response Time	pH	Reference
LDL	0.10- 10.34 mmol/L	0.2-410 mg/Dl	-	-	-	[75]
LDL	0.025-16 $ngmL^{-1}$	0.006 ngmL	-	-	7.4	[76]
LDL	0-190 mg/dL	-	0.18387	2 min	7.4	[77]
HDL	0.5-4 mm	-	-	-	6.8	[79]
HDL	-	2-250 mg/dL	-	-	7.4	[80]
TG	50-500 mg/dL	32.8 mg/dL	10-195 Ma/mgdL	20 s	6.5	[85]
TG	0.56-2.25 mM	0.21 mM	-	2 s	7	[86]
TG	0.56-2.25 mM	0.11 mM	-	-	7	[87]
TG	0-10 mM	-	7-7.5 $nAmM^{-1}$	-	-	[88]
TG	-	-	1.232 μAmg/cm dL^{-1}	10 s	7	[89]

TG	0-531 mg/dL	7.88 mg/dL	2.66 A/mm	30 s	7	[90]
C	1-500	37	23.71 $AmM^{-1}cm^{-2}$	>5 s	7.4	[94]
C	12 µm-7.2 Mm	-	40 µm	-	7.4	[95]
C	10-400 mg/dL	5 mg/dL	-	10 s	7.4	[96]

Table 7 Various sensors based on nanomaterials synthesized by different techniques

Nanomaterial	**Synthesis method**	**Key parameters**	**Sensitivity**	**Detection limit**	**Response time**	**Ref**
$NiFe_2O_4$/CuO/FeO-chitosan	Sol gel method	Drying & Preheating temperature, Annealing time	0.043 A/mg/L cm^{-2}	313 mg/L	10s	[97]
SPE/Au	Electro deposition	Substrate temperature, Anode potential, Working pressure, Deposition time, DC: applied voltage, RF: used power impedance matching	-	3.0 µg/mL	-	[98,99]
Inkjet-printed PB	Co-precipitation	Thermal decomposition, both the injection rate of precursors over the hot solvent and the temperature homogeneity	2.1µA mM^{-1} cm^{-2}	.2mM	200s	[99–102]
PB	sol gel	Drying & Preheating temperature, Annealing time	88.51n AmM^{-1}	12.4µm	-	[103]

5. Future perspectives and trends

In this article, several approaches implemented to detect cholesterol are reviewed. A comprehensive analysis was carried out by analyzing various sensing methodologies and sensor characteristics. A comparative study of enzymatic and non-enzymatic based on the synthesis techniques, level of complexity, performance under varying sensor parameters for different types of cholesterol was also

presented. Further, some factual inferences on each methodology were also shown based on the comparative study carried out. Besides, the advantages and limitations of all these methods are vividly stated in this paper. Based on the analysis done on various literature research, the following points can be summarized.

- Even though the advantages over the non-enzymatic detection technique were proven, cholesterol detection using different methods and materials was not reported much. When comparing the sensing techniques, the electrochemical process is the only method reported frequently followed by colorimetric techniques. The research gap in this field on other techniques such as optical, piezoelectric, etc., is still there to enhance the sensing characteristics.

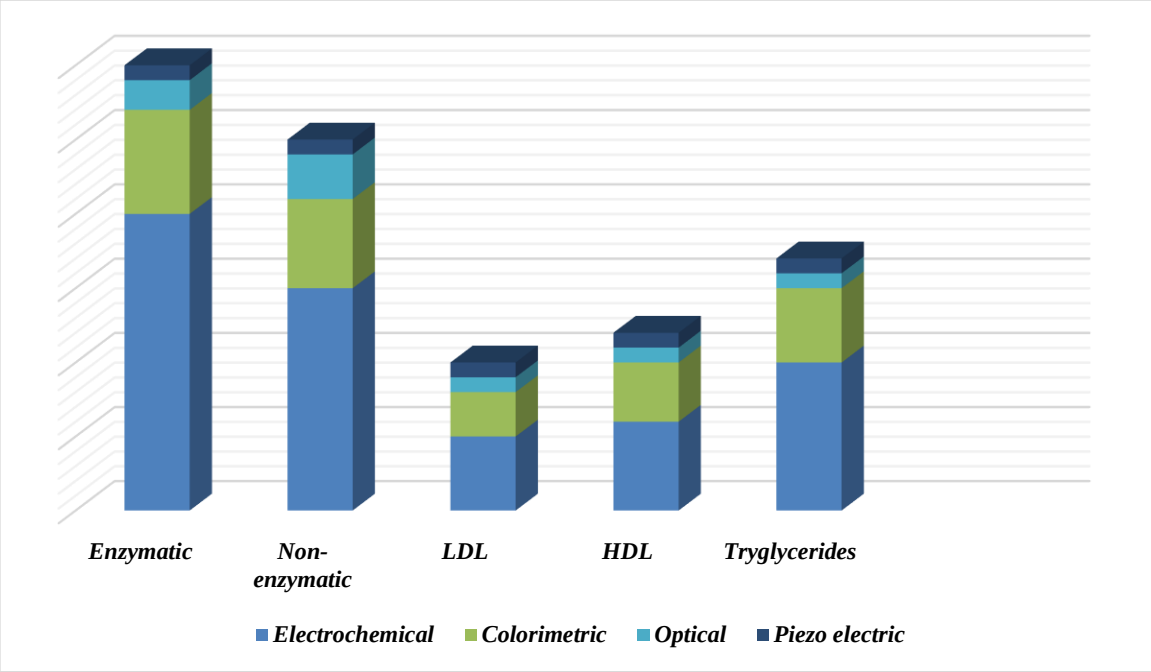

Figure 11 Comparison of techniques used in cholesterol sensing

- The use of metal-oxide nanomaterials for cholesterol sensing on both enzymatic and non-enzymatic sensors was comparatively less in number than other reported biosensors. The current cholesterol sensors' current trends via metal-oxides, namely NiO, CuO, and ZnO, offer enhanced sensing characteristics for biosensors. The upcoming researchers have plenty of chances to work on cholesterol sensors based on metal-oxide based nanomaterials.
- Among the reported articles on LDL, HDL, and triglycerides, a few works are written for LDL and HDL sensing in particular. The requirement of sensing each type of cholesterol is inevitable for a healthy life, whereas the research gap is still on.
- Even if several cholesterol sensors are discussed in the literature, none of them are substantially capable of achieving all the sensor characteristics in the utmost in comparison with other biosensors. However, the efforts that have been made so far in this regard require, at most, appreciation. Furthermore, it should be noted that cholesterol sensors are still in the developmental phase having remarkable research space to carry out further researches.

2. CONCLUSION

This review summarized various approaches for nanomaterials-based cholesterol biosensors. In addition, the work cites multiple studies on cholesterol sensors carried out using different synthesis methods and sensing techniques. From the detailed analysis, the paper confirms the imminent position of metal-oxide-based nanomaterials in achieving appreciable sensor characteristics alongside the effectiveness of the electrochemical strategy in the biosensing application. However, the practical application of non-enzymatic cholesterol sensing with enhanced features has to emerge to the level precisely in terms of cost, stability, and reproducibility. Furthermore, different facile methods have to evolve beside the electrochemical sensing technique, specifically optical, piezoelectric methods, etc., to develop handy and highly sensitive cholesterol biosensors. Finally, the work gives a detailed insight for the researchers with a note of all the possibilities and prospects of cholesterol sensors for identifying the research gaps.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, and all the reproductions were done with the publishers' permission.

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