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Nanocomposite modified electrode for cholesterol estimation: A review

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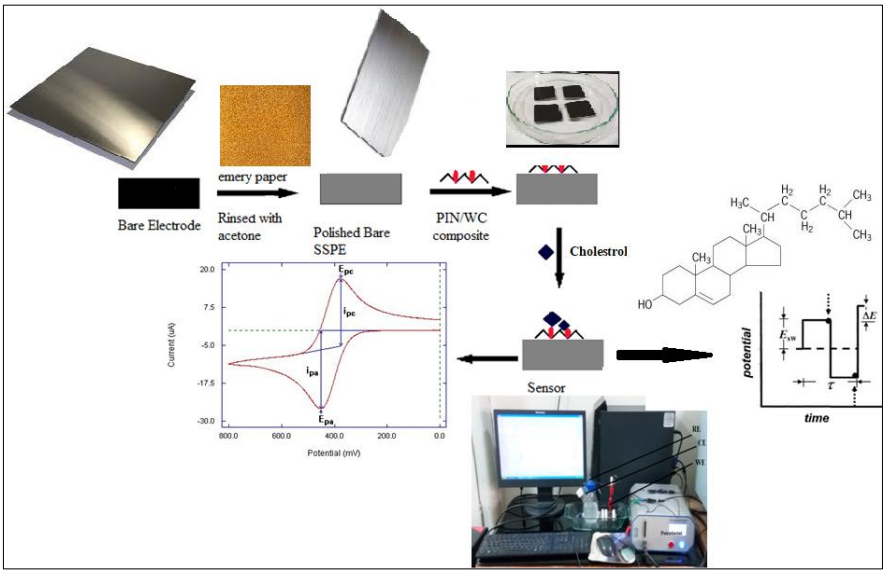
Abstract
Cholesterol is a waxy steroid of fat and it plays an important role in the initiation and progression of cardiovascular diseases, so its estimation in blood serum is very important. In this work we report, non-enzymatic electrochemical sensors for the determination of cholesterol with different nanocomposite coated working electrode The surface morphology and the property of nanocomposite were characterize by scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X- Ray Diffraction spectra (XRD) simultaneous TG-DTA-DTG. The electrochemical behavior of cholesterol was studied using cyclic voltammetry (CV) and Square wave voltammetry (SWV). This review is focusing on the synthesis of different type of coating materials for the working electrode surface modification. Present report also compiles the development of electrochemical sensor for trace level highly sensitive quantification of cholesterol.

Keywords: cholesterol, electrochemical sensor, cylicvoltammetry

Introduction
Cholesterol is a waxy steroid of fat, one of the essential components of mammalian cell membranes and is a precursor of other biological materials, such as bile acid, vitamin D, steroid hormones (glucocorticoids, estrogens, progesterones, androgens and aldosterone) [1]. The human blood contains 70% of cholesterol in ester form and 30% as free form, which together gives the total cholesterol level [2]. The normal concentration of free cholesterol in human serum is below 5.2×10^{-3} M, *i.e.* 200 mg/dL but if its level exceeds 6.2×10^{-3} M, *i.e.* 240 mg/dL, that could result in a serious health threat, such as coronary disease, arteriosclerosis, myocardial infarction, brain thrombosis, lipid metabolism dysfunction, hypertension, or cerebral thrombosis [3, 4]. The estimation of blood cholesterol is one of the most widely performed assays in biochemistry, since cholesterol plays a vital role in the initiation and progression of cardiovascular diseases.



Graphical Abstract

It makes the cell walls rigid and strong, protecting itself from the foreign bodies. The measurement of blood cholesterol concentration is a routine practice in medical screening or diagnosis.

Various methods have been reported for the analysis of cholesterol in biological fluids including colorimetric, spectrophotometric, high-performance liquid chromatography (HPLC) and electrochemical methods [5-7]. Among these, some methods often present certain disadvantages such as lack of specificity and selectivity, because of the interfering reactions and use of unstable and corrosive reagents [8]. Over various analytical methodologies, the electrochemical approach for sensing has gained momentum in past few decades, due to their high sensitivity, fast response time, simple handling and low cost. The electrochemical sensing of cholesterol has been performed by the enzymatic reaction of cholesterol with cholesterol oxidase, where the concentration of either H_2O_2 generated or oxygen consumed during the enzymatic reaction is being monitored [9]. The detection of cholesterol using enzyme is based on the reaction as follows [10].

$$\text{Cholesterol} + O_2 + \text{ChOx} \longrightarrow \text{Cholestene-3-one} + H_2O_2$$

The cholesterol generated from cholesterol ester is oxidized by oxygen in the presence of ChOx enzyme to cholestene-3-one and H_2O_2. The measurement of H_2O_2 by oxidation or reduction will be an indirect quantification of cholesterol levels. Although enzyme based sensors show good selectivity and high sensitivity, but the activity of the enzyme decreases with use, and the enzyme get easily denatured during its immobilization procedure, due to its intrinsic instability [11]. In order to overcome the disadvantage of enzymatic cholesterol sensors, non-enzymatic cholesterol sensor has been designed and fabricated, which has several attractive advantages such as stability, simple fabrication, reproducibility, low cost and freedom from oxygen limitations. The results emerged demonstrate that this method could hopefully be integrated into clinical labs, as this does not demand either any hazardous and costly chemicals or any complex synthetic routes. Organic conducting polymers have recently emerged as a new class of electroactive materials and are interesting subjects for research and development [12-14]. Conducting polymer nanocomposites has been developed with enhanced properties to overcome the inherent limitations like low sensitivity, poor selectivity of pure conducting polymers. Polymer nanocomposites (PNCs) have proved to be an excellent transducer in electro analytical sensors, due to their unique properties like low cost, enhanced electronic properties, rapid electrode kinetics, biocompatibility, and environmental stability [15, 16].

Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and Square Wave Voltammetry (SWV) methods are commonly employed for electrochemical monitoring cholesterol. These techniques consists of three electrodes system working, reference and auxillary electrodes. CV provides the capability for generating redox behavior of a species during the forward scan and then determining its outcome with the reverse scan or subsequent cycles, all within seconds and it also determine the kinetics for electrochemical (EC) reactions at electrode surface. The important parameter of a cyclic voltammogram are the magnitudes of anodic peak current (ipa), cathodic peak current (ipc), anodic peak potential (Epa) and cathodic peak potential (Epc). A cyclic voltammogram is performed by clicking the potential of a working electrode and measuring the electric current. The magnitude of this current is proportional to the concentration of the analytes in solution. A CV System consists of an electrolysis cell, a Potentiostat, a current-to-voltage converter, and a data acquisition system [17]. SWV is a powerful electrochemical technique suitable for analytical application, mechanistic study of electrode processes and electro kinetic measurement. The major advantage of SWV include they are very sensitive, often allowing direct analyses at the parts per billion (ppb) level and even the low parts per trillion (ppt) level when used in a stripping mode, requires less time per sweep than older techniques such as differential pulse polarography and the square-wave frequency can be used to differentiate between processes with fast and slow kinetics. In SWV the current at a (usually stationtary) working electrode is measured, while the potential between the working electrode and a reference electrode is swept linearly with time. The potential waveform can be viewed as a superposition of a regular square wave onto an underlying staircase. Capacitive and background current tend to hide the useful information brought by faradic current. The aim of the differential pulse voltammetry (DPV) is to minimize the contribution of both capacitive and background current to the measured signal. DPV response consists of a difference of currents plotted against the potential, namely the mean value between the potentials at which the two currents are sampled. The first current value is taken just before the application of the impulse and the second one just before the end of the impulse, which is long enough to allow the charging current to become negligible.

Nanocomposite modified cholesterol sensing and biosensing

The nanocomposite synthesized with different composition is utilized on stainless plate for the development of electrochemical sensors. EC sensors can be employed as cholesterol monitoring system for the blood serum of patients infested with high cholesterol. Other traditional techniques available for detection are costlier and have sophisticated instrumentation but proposed EC sensors is easy to design, low cost, and simple to handle. EC sensors are highly efficient for determination of cholesterol depending upon the surface of working electrode with different electrode coating materials.

Cholesterol sensor based on metal composite

The colloidal gold for molecular sensing of cholesterol for its detection. GNPs was functionalized with tomatine, a glycoalkaloid found in the leaves and stem of tomato plants and was characterized by a blue shift in Plasmon absorption spectra followed by reduction in the particle size [18]. The electrochemical non-enzymatic route of sensing by chemically converting graphene modifying with β-CD for cholesterol detection using Methylene Blue as redox indicator. Methylene Blue (MB) forms an inclusion complex with Grp-β-CD and emerges as a cholesterol sensing matrix. Cholesterol molecule replaces the MB molecule and moves out in the buffer solution, hence, detects electrochemically using Differential Pulse Voltammetric (DPV) technique. The sensing matrix is characterized using FT-IR and Raman spectroscopy. Themorphology of functionalized graphene sheets was studied by Transmission Electron Microscopy [19].

For cholesterol detection, an EC sensor was developed bynano-sized carbon inter-digitated electrodes (IDEs) coated with gold nanoparticles (AuNPs) with the help of CV. The

surface morphology was characterized by scanning electron microscopy (SEM). It exhibit a sensing range of (0.005–10 mM) with L.O.D. of ~1.28M) [20].

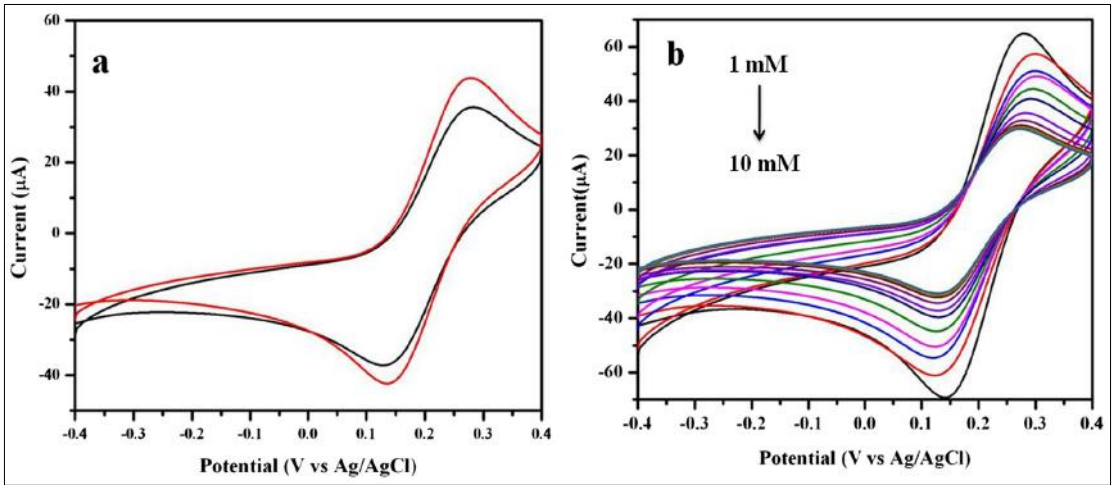


Fig 1: (a) CV curves recorded for (-)f-MWCNT-Au-PPy/GCE and (-)f-MWCNT-Au-PPy-ChOx/GCE from a solution of 0.1 M PBS, 5 mM of $K_4[Fe(CN)_6]$ and 1 mM of cholesterol and (b) CV curves for varying concentration of cholesterol from 1 mM to 10 mM on f-MWCNT-Au-PPy-ChOx/GCE. Scan rate = 100 mV s^{-1} [21].

Fabricated gold nanoparticles-decorated multiwalled carbon nanotubes (GNPs-MWCNTs) immobilized by electron transfer of cholesterol oxidase (ChOx). GNPs-MWCNTs were prepared based on the reduction of $HAuCl_4$ in the presence of carboxyl group functionalized MWCNTs rounded gold nanoparticles with diameters of 6-10 nm decorated on carbon nanotube surfaces were studied by Transmission electron microscopy image. A linear response in the cholesterol concentration range from 0.01 to 5.00 $mmolL^{-1}$ with a detection limit of 4.3 $mmolL^{-1}$ estimate data signal-to-noise ratio of 3 was observed under optimized conditions. The apparent Michaelis–Menten constant was measured to be 0.29 $mmolL^{-1}$, indicating that the immobilized ChOx on GNPs-MWCNTs matrix retained its native activity. The concentration of free cholesterol in a human serum sample is well determined by the well-established spectrophotometric method [22]. The fabrication of gold/platinum hybrid functionalized ZnO nanorods (Pt-Au@ZnONRs) and multiwalled carbon nanotubes (MWCNTs) modified electrode. Its application for cholesterol biosensor is investigated. The prepared Pt-Au@ZnONRs suspension was dropped on the MWCNTs modified glass carbon electrode, and followed with cholesterol oxidase (ChOx) immobilization by the adsorbing interaction between the nano material and ChOx as well as the electrostatic interaction between ZnONRs and ChOx molecules. The resulted biosensor exhibited a linear response to cholesterol in the wide range of 0.1-759.3 μM with a low detection limit of 0.03 μM and a high sensitivity of 26.8 μA mM^{-1} [23].

Developed highly sensitive biosensor by selective enzyme immobilization on nano-sized carbon interdigitated electrodes (IDEs) decorated with gold nanoparticles (AuNPs). Use of wafer-level carbon Micro electromechanical systems (C-MEMS) processes was done processes to fabricate 3D carbon IDEs reproducibly, simply, and cost effectively. The 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 M).

Fabricated sensitive and selective electrochemical sensor by using a screen printed carbon electrode (SPCE), multi-walled carbon nanotubes (MWCNTs) and β-cyclodextrin (β-CD) for detecting cholesterol. β-CD was immobilized on functionalized MWCNTs modified SPCE which acts as a host to recognize guest (cholesterol) molecule specifically. The sensor was able to detect cholesterol level ranges from 1 nM to 3 μM with a detection limit of 0.5 nM and using differential pulse voltammetry (DPV). Cholesterol was confirmed in the presence of common interfering species including glucose, uric acid and ascorbic acid [24].

Cholesterol sensor based on metal oxide

The cholesterol determination cholesterol oxidase (ChOx) and catalase (CAT) were co-immobilized on a graphene ionic liquid-modified glassy carbon electrode (GR–IL/GCE) to develop a highly sensitive amperometric cholesterol biosensor. H_2O_2 generated during the enzymatic reaction reduced electro catalytically by immobilized CAT to obtain a sensitive amperometric response to cholesterol. The proposed biosensor show cased an excellent sensitivity of 4.163 mA $mM^{-1}cm^{-2}$, a response time less than 6s, and a linear range of 0.25–215 μM [25]. Synthesized $NiFe_2O_4$/CuO/FeO chitosan nanocomposite as a cholesterol biosensor. The phase identification, morphology and particle size were characterized by X-ray diffraction pattern (XRD), scanning electron microscopy (SEM), high resolution transmission electron microscope (HR-TEM) and Fourier transform infrared (FTIR) spectroscopy. The quantification of cholesterol was efficiently done by immobilizing cholesterol oxidase (ChOx) onto a chitosan-$NiFe_2O_4$/CuO/FeO nanocomposite ($NiFe_2O_4$/CuO/FeO-CHNC) deposited onto an indium-tin-oxide (ITO) glass substrate via the sol–gel technique. The electrochemical study results in good linearity (50–5000 mg/L), a low detection limit (313 mg/L), high sensitivity 0.043 μA (mg/Lcm^{-2}) a fast response time 10 s and a shelf-life of 3 months [26]. The cost effective sensor material prepared from modified graphene oxide based molecular imprinted polymer (GO-MIP). graphene oxide (GO) prepared from modified Hummers method undergoes acid fictionalization to form GO-COOH leading to surface modification with glycidyl methacrylate (GMA) together with N,N'-dicyclohexyl carbodiimide (DCC), dimethyl amino pyridine (DMAP) and dimethyl sulfoxide (DMSO) to form GO-CH=CH_2. The obtained GO-CH=CH_2 undergoes molecular imprinting process with monomer (methacrylic

acid) and initiator (α, α'-azobisiso butyro nitrile) and template molecule (cholesterol). The formation of different preparatory stages of materials were characterized by FTIR, XRD, Raman and TEM techniques. The obtained GO-MIP demonstrated a lower limit detection of 0.1nM and response time of ~2 min with maximum sensing performance at pH 5.0 [27].

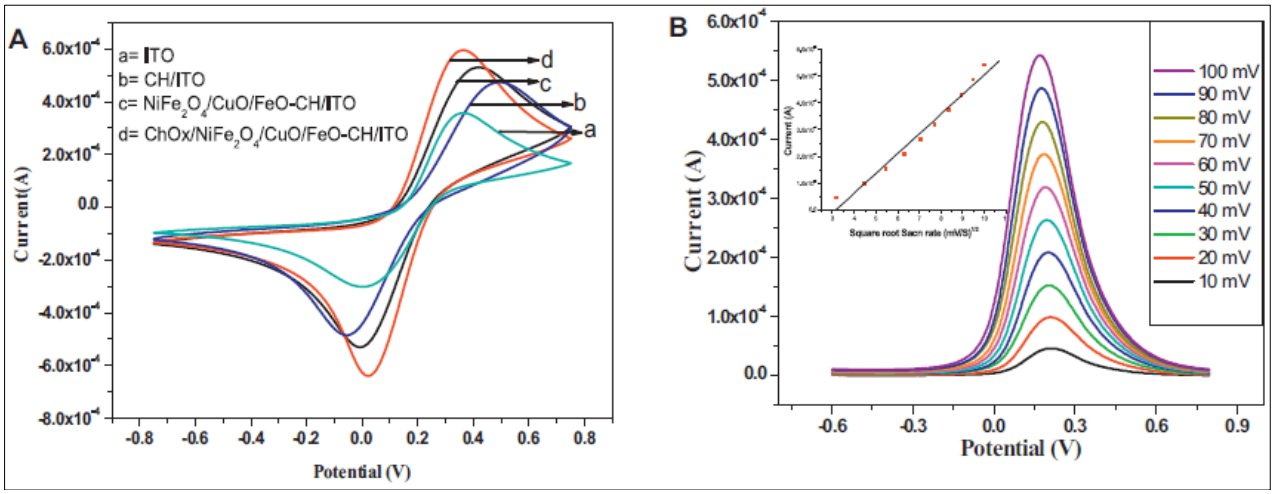


Fig 2: Cyclic voltammogram (CV) of a bare ITO electrode (a), the CH/ITO electrode (b), the $NiFe_2O_4$/CuO/FeO-CH/ITO electrode (c) and the ChOx/$NiFe_2O_4$/CuO/FeO-CH/ITO bioelectrode (d) DPV of the ChOx/$NiFe_2O_4$/CuO/FeO-CH/ITO bioelectrode using an increasing scan rate of 10-100 mV/s. In the inset, the magnitude of the current vs. potential difference is a function of the square root of the scan rate (10–100 mV/s) [26]

Cholesterol sensor based on conducting polymer

Developed a sensor for ultra-trace cholesterol (CHO) detection based on electropolymerized aminothiophenol (ATP) molecularly imprinted polymer (MIP) on a glassy carbon electrode (GCE) modified with dopamine and graphene (DGr) and bio inspired Au microflowers. Scanning electron microscopy (SEM) and atomic force microscope (AFM) were used for characterization of morphology of the MIP modified electrode. The hydrogen bonding interaction between templates and monomers was characterized by ultraviolet spectroscopy. The sensor's linear response range was between 10^{-18} and 10^{-13} M with a detection limit of 3.3×10^{-19} M [28].

Prepared A nanocomposite of graphene (G), polyvinylpyrrolidone (PVP) and polyaniline (PANI) and used for the modification of paper-based biosensors via electro spraying. The droplet-like nanostructures of G/PVP/PANI-modified electrodes of average size 16071.02nm are obtained. The presence of small amount of PVP (2 $mgmL^{-1}$) in the nano composites substantially improve the dispersibility of G and increase the electrochemical conductivity of electrodes which enhances the sensitivity of the biosensor. Furthermore, cholesterol oxidase (ChOx) is attached to G/PVP/PANI-modified electrode for the amperometric determination of cholesterol. The proposed system can be applied for the determination of cholesterol in a complex biological fluid (i.e. human serum). The linear range of 50 µM to10mM is achieved and the limit of detection is found to be 1 µM for cholesterol [29].

A cholesterol oxidase (ChOx) immobilized PANI/Au/chitosan nanocomposite for cholesterol detection. The enzyme-substrate kinetics for the nanocomposite biosensor showed that it facilitates enzymatic reaction and activity. PANI nanocomposites with CNTs and/or graphene are also extensively used for cholesterol detection. A ChOx immobilized graphene/PVP/PANI modified paper-based electrode has shown a low detection limit of 1 µM2. The presence of PVP was reported to enhance the sensitivity of the biosensor. It has often been observed that these biosensors show a high shelf life when stored at low temperature. PANI/Ag nanocomposite too was employed as cholesterol sensing. The biosensor gave a fast response towards cholesterol and showed uniform activity for up to 50 days when stored at low temperatures [30].

A ceramic carbon electrode CCE based on monolithic molecular imprinting sensor. For determination of cholesterol the incorporated MWCNT@MIP in CCEs functioned as a recognition element. For the confirmation of presence or absence of cholesterol MWCNT@MIP-CCEs were tested by linear sweep Voltammetry and cyclic Voltammetry. The sensitivity with a linear range of 10-300 nM and a detection limit of 1nM(S/N¼3) of cholesterol sensor was observed [31].

Table 1: A comparison of available nanocomposite cholesterol sensors and their characteristics properties

S. No.	Electrode matrix	Detection technique	Enzymatic/ Non-enzymatic	Detection limit	Linearity	Sensitivity	Ref.
1.	$NiFe_2O_4$/CuO/FeO-CH/ChOx	Amperometry	Enzymatic	313 mg/L	50–5000 mg/L	0.043µA /mg/L cm^{-2}	[12]
2.	Nafion/ChOx/GNPs-CNTs/GCE	CV	Enzymatic	4.3 µM	0.01–5.0 mM	-	[22]
3.	G/PVP/PANI nanocomposites	Amperometry	Enzymatic	1 µM	0.05-10 mM	34.77 μAmM^{-1}	[29]
4.	ChOx/CH–NanoCeO_2	CV and DPV	Enzymatic	5 mg/dL	-	47 $\mu A/mg\ dL^{-1}\ cm^{-2}$	[32]
5.	RTIL/NH2 MWCNTs	Amperometry	Enzymatic	12×10^{-9} M	26×10^{-9} - 3.4×10^{-6} M	540 μAmM^{-1}	[33]
6.	Au-f-MWCNT-PPy	Amperometry	Enzymatic	0.1 10^{-3} M	$2\text{-}8\times10^{-3}$ M	-	[21]
7.	Pt NP/(CNT)	Chronoamperometry	Non-enzymatic	-	0.005 mM - 10 mM	8.7 $\mu A\ mM^{-1}\ cm^{-2}$	[34]
8.	Grp/β CD/Methylene Blue	DPV	Non-enzymatic	0.001-0.10 mM	-	0.01µA/µM	[19]
9.	ChOx/AuNP/carbon IDE	Chronoamperometry	Enzymatic	~1.28 µM	1-10 mM	~993.91µA $Mm^{-1}cm^{-2}$	[20]
10	CS-SnO_2/ITO	CV	Enzymatic	0.13 mM	-	34.7µA/$mgdL^{-1}\ cm^{-2}$	[35]

Conclusion

Now a days, great advances of nanomaterials have been achieved in electrochemical sensing by modifying working electrode surface. This review, addresses the important examples of metal composite, metal oxide and conducting polymer based nanocomposite synthesis, their fabrication methods for electrochemical construction with particular emphasis on cholesterol monitoring. The unique properties of nanocomposites based EC sensors are they offer low cost, more selective and sensitive electrodes for cholesterol monitoring in biological samples. The development of new nanocomposites might lead to great potential for further improvement in bio analytical performance characteristics of electrochemical integrated detection devices.

References

1. Arya SK, Datta M, Malhotra BD. Recent advances in cholesterol biosensor. Biosensors and Bioelectronics. 2008; 23(7):1083-1100.
2. Schwartz CC, Vanden Broek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output *in vivo* in humans. Journal of lipid research. 2004; 45(9):1594-1607.
3. Wang C, Tan X, Chen S, Yuan R, Hu F, Yuan D *et al*. Highly-sensitive cholesterol biosensor based on platinum–gold hybrid functionalized Zno nanorods. Talanta. 2012; 94:263-270.
4. Zhu L, Xu L, Tan L, Tan H, Yang S, Yao S. Direct electrochemistry of cholesterol oxidase immobilized on gold nanoparticles-decorated multiwalled carbon nanotubes and cholesterol sensing. Talanta. 2013; 106:192-199.
5. Krug A, Göbel R, Kellner R. Flow-injection analysis for total cholesterol with photometric detection. Analytica Chimica Acta. 1994; 287(1-2):59-64.
6. Nakaminami T, Kuwabata S, Yoneyama H. Electrochemical oxidation of cholesterol catalyzed by cholesterol oxidase with use of an artificial electron mediator. Analytical Chemistry. 1997; 69(13):2367-2372.
7. Hojo K, Hakamata H, Kusu F. Simultaneous determination of serum lath sterol and cholesterol by semi-micro high-performance liquid chromatography with electrochemical detection. Journal of Chromatography B. 2011; 879(11-12):751-755.
8. Brahim S, Narinesingh D, Guiseppi-Elie A. Amperometric determination of cholesterol in serum using a biosensor of cholesterol oxidase contained with in a polypyrrole–hydrogel membrane. Analytica Chimica Acta. 2001; 448(1-2):27-36.
9. Karube I, Hara K, Matsuoka H, Suzuki S. Amperometric determination of total cholesterol in serum with use of immobilized cholesterol esterase and cholesterol oxidase. Analytica Chimica Acta. 1982; 139:127-132.
10. Yıldırımoğlu F, Arslan F, Çete S, Yaşar A. Preparation of a polypyrrole-polyvinyl sulphonate composite film biosensor for determination of cholesterol based on entrapment of cholesterol oxidase. Sensors. 2009; 9(8):6435-6445.
11. Park S, Boo H, Chung TD. Electrochemical non-enzymatic glucose sensors. Analytica chimica acta. 2006; 556(1):46-57.
12. Miller JS. Conducting polymers-materials of commerce. Advanced Materials. 1993; 5(9):671-676.
13. Foulds NC, Lowe CR. Immobilization of glucose oxidase in ferrocene-modified pyrrole polymers. Analytical Chemistry. 1988; 60(22):2473-2478.
14. Cooper JC, Hall EAH. Electrochemical response of an enzyme-loaded polyaniline film. Biosensors and Bioelectronics. 1992; 7(7):473-485.
15. Wallace GG, Smyth M, Zhao H. Conducting electroactive polymer-based biosensors. Trends in Analytical Chemistry. 1999; 18(4):245-251.
16. Bagheri H, Ayazi Z, Naderi M. Conductive polymer-based micro extraction methods: A review. Analytica Chimica acta. 2013; 767:1-13.
17. Harnisch F, Freguia S. A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biofilms. Asian Journal of Chemistry. 2012; 7(3):466-475.
18. Raj V, Johnson T, Joseph K. Cholesterol aided etching of tomatine gold nanoparticles: A non-enzymatic blood cholesterol monitor. Biosensors and Bioelectronics. 2014; 60:191-194.
19. Agnihotri N, Chowdhury AD, De A. Non-enzymatic electrochemical detection of cholesterol using β-cyclo dextrin functionalized graphene. Biosensors and Bioelectronics. 2015; 63:212-217.
20. Sharma D, Lee J, Seo J, Shin H. Development of a sensitive electrochemical enzymatic reaction-based cholesterol biosensor using nano-sized carbon interdigitated electrodes decorated with gold nanoparticles. Sensors. 2017; 17(9):2128.
21. Alagappan M, Immanuel S, Sivasubramanian R, Kandaswamy A. Development of cholesterol biosensor using Au nanoparticles decorated f-MWCNT covered with polypyrrole network. Arabian Journal of Chemistry, 2018.
22. Zhu L, Xu L, Tan L, Tan H, Yang S, Yao S. Direct electrochemistry of cholesterol oxidase immobilized on gold nanoparticles-decorated multiwalled carbon nanotubes and cholesterol sensing. Talanta. 2013; 106:192-199.
23. Wang C, Tan X, Chen S, Yuan R, Hu F, Yuan D *et al*. Highly-sensitive cholesterol biosensor based on platinum–gold hybrid functionalized ZnO nanorods. Talanta. 2012; 94:263-270.
24. Nawaz MAH, Majdinasab M, Latif U, Nasir M, Gokce G, Anwar MW *et al*. Development of a disposable electrochemical sensor for detection of cholesterol using differential pulse voltammetry. Journal of pharmaceutical and biomedical analysis. 2018; 159:398-405.
25. Gholivand MB, Khodadadian M. Amperometric cholesterol biosensor based on the direct electrochemistry of cholesterol oxidase and catalase on a graphene/ionic liquid-modified glassy carbon electrode. Biosensors and Bioelectronics. 2014; 53:472-478.
26. Singh J, Srivastava M, Kalita P, Malhotra BD. A novel ternary NiFe2O4/CuO/FeO-chitosan nanocomposite as a cholesterol biosensor. Process biochemistry. 2012; 47(12): 2189-2198.
27. Alexander S, Baraneedharan P, Balasubrahmanyan S, Ramaprabhu S. Modified graphene based molecular imprinted polymer for electrochemical non-enzymatic cholesterol biosensor. European Polymer Journal. 2017; 86:106-116.
28. Yang H, Li L, Ding Y, Ye D, Wang Y, Cui S *et al*. Molecularly imprinted electrochemical sensor based on

bioinspired Au microflowers for ultra-trace cholesterol assay. Biosensors and Bioelectronics. 2017; 92:748-754.
29. Ruecha N, Rangkupan R, Rodthongkum N, Chailapakul O. Novel paper-based cholesterol biosensor using graphene/polyvinylpyrrolidone/polyaniline nanocomposite. Biosensors and Bioelectronics. 2014; 52:13-19.
30. Shrivastava S, Jadon N, Jain R. Next-generation polymer nanocomposite-based electrochemical sensors and biosensors: A review. Trends in Analytical Chemistry. 2016; 82:55-67.
31. Tong Y, Li H, Guan H, Zhao J, Majeed S, Anjum S *et al*. Electrochemical cholesterol sensor based on carbon nanotube@ molecularly imprinted polymer modified ceramic carbon electrode. Biosensors and Bioelectronics. 2013; 47:553-558.
32. Malhotra BD, Kaushik A. Metal oxide–chitosan based nanocomposite for cholesterol biosensor. Thin Solid Films. 2009; 518(2):614-620.
33. Karimi S, Ghourchian H, Rahimi P, Rafiee-Pour HA. A nanocomposite based biosensor for cholesterol determination. Analytical methods. 2012; 4(10):3225-3231.
34. Yang J, Lee H, Cho M, Nam J, Lee Y. Nonenzymatic cholesterol sensor based on spontaneous deposition of platinum nanoparticles on layer-by-layer assembled CNT thin film. Sensors and Actuators B: Chemical. 2012; 171:374-379.
35. Ansari AA, Kaushik A, Solanki PR, Malhotra BE. Electrochemical cholesterol sensor based on tin oxide-chitosan nanobiocomposite film. *Electroanalysis:* An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis. 2009; 21(8):965-972.
36. Raj V, Jaime R, Astruc D, Sreenivasan K. Detection of cholesterol by digitonin conjugated gold nanoparticles. Biosensors and Bioelectronics. 2011; 27(1):197-200.