Chapter 1

Introduction
1. Introduction

1.1 Need for the study

Diagnosis of diseases demands the qualitative and quantitative analysis of various biomolecules and pathogens. Number of analytical methods and tools are available for the same. Due to the socioeconomic development and increased awareness large number of people want to diagnose and prognose diseases at an early stage. Currently people depend on the clinical laboratories and hospitals which host sophisticated instruments and trained technicians. But this is accessible only to urban people and the cost of the analyses is very high, which necessitates the low cost point of care diagnosis devises. Biosensors are emerging as the simplest and accessible POC device.

Diabetes mellitus is a chronic disorder characterized by insulin deficiency and has complications affecting eye sight (diabetic retinopathy), kidney (diabetic nephropathy), neural and cardiac functioning. Insulin secreted by pancreas is required by the cells to produce energy. Thus in diabetics a decrease in glucose oxidation in cells and increase in blood glucose levels occur. This chronic disease affecting an estimated 250 million people worldwide and the number is expected to double in the next 20 years [1]. Hence the determination of blood glucose concentration is highly important for the monitoring control of diabetes.

Abnormal levels of cholesterol in the body result in heart disease, hypertension, arteriosclerosis, coronary artery disease, cerebral thrombosis etc. This stimulated the public awareness about the monitoring of blood cholesterol. The safe level of total cholesterol in healthy human blood is 200 mg dL$^{-1}$ (5.16 mM), borderline value 200-239 mg dL$^{-1}$ (5.16-6.18 mM), and the high level is above 240 mg dL$^{-1}$ ($\geq$ 6.21 mM). Thus precise determination of cholesterol in body fluids is highly important in clinical diagnosis.

1.2 Biosensors

IUPAC defines biosensor as "an independently integrated receptor transducer device, which is capable of providing selective, quantitative or semi-quantitative analytical information using a biological recognition element" [2, 3]. Biosensor is first introduced by Leland C Clark Jr. in 1956 and he is considered to be the father of biosensors. The simple operational procedure and low cost enable biosensors to be
used as a tool by the common man for self monitoring of disease conditions. Currently these are widely applied in diabetic monitoring, cardiac monitoring, drug discovery, agriculture, environmental, food industry and biodefense applications [4-11]. Pioneering companies like Abbot Point of Care Inc., Medtronic Inc., Lifescan Inc., F. Hoffmann-La Roche Ltd., AgaMatrix Inc., Siemens Healthcare and Nova Biomedical Corporation hold the maximum share of biosensor market.

Figure 1.1 Biosensors- structure, classification and applications

Biosensors monitor the analytical signal derived from the biochemical reaction between the analyte and the biorecognition element (enzymes, antibodies, DNA, aptamer etc.) as a function of analyte concentration. Suitable transducers and microelectronics were employed for the processing of the signals [12]. Tremendous amount of research work is focused towards improving the performance of the existing sensors and for the detection of new biomolecules. Figure 1.1 presents the general structure, applications and classification of biosensors in a nutshell.
Out of the different transducers used in biosensors electrochemical methods are playing prominent role due to the capability to fabricate robust, portable, miniaturized devices, tailor made for particular applications. Moreover they are economical and user friendly. These excellent properties exhibited by electrochemical biosensors made them suitable for many commercial applications. These sensors monitor the change in electrical parameters such as electrode potential, current, charge transfer impedance or capacitance as a function of analyte concentration [13]. Based on the signal monitored they are classified as amperometric [14], potentiometric [15, 16], voltammetric [17], impedimetric or conductometric [18-22] and capacitive [23, 24] biosensors. Among these, amperometric sensors which relate the current as a function of analyte concentration is the popular analytical tool since its detection limit can go down to nanomolar concentrations [25].

The common electrochemical methods for the determination of glucose and cholesterol are based on enzymatic oxidation followed by the sensing of the byproducts formed. These sensors possess good specificity and sensitivity the lack of storage stability and effect of experimental parameters such as pH, ionic strength, chemical inhibitors, and temperature on the protein structure of enzymes impedes their development [26]. Nonenzymatic sensors for glucose are well studied but that on cholesterol is still in infancy stage [27, 28]. Hence the discussion on glucose sensors is restricted only to nonenzymatic methods, since the enzyme based glucose sensors are well studied and reviewed. But for cholesterol detailed discussion on the development of enzymatic and nonenzymatic electrochemical sensors is presented here since the reports on the determination are scanty.

1.3 Electrochemical nonenzymatic glucose sensors

Nonenzymatic glucose sensors can be considered as the fourth generation electrochemical glucose sensors [29]. This is a practical application of oxidation of glucose reported on lead electrodes by Loeb which came long before the report by Clark’s enzymatic oxygen electrode [30]. The key idea behind the nonenzymatic glucose determination is the fabrication of suitable catalytic electrode material to oxidize glucose. This is performed by modifying the electrode surface with suitable electrocatalysts by selective etching, alloying, dealloying, electrodeposition, electrochemical anodization etc. The electrocatalysts can be in variety of forms,
specifically metals, alloys and bimetallic systems, carbon-based materials, metal-metal oxide heterogeneous nanocomposites and layered double hydroxides. In most of these materials except in carbon electrode the oxidation centre is a metal involving the d-orbitals and its electrons. A schematic representation of the classification of materials used for the fabrication of nonenzymatic glucose sensors is given in Figure 1.2 [29]. A brief review of these electrocatalysts and the possible mechanism of oxidation are presented here.

![Figure 1.2: Different electrocatalysts employed for the nonenzymatic glucose determination (not quantitative).](image)

**1.3.1 Mechanism for direct electrooxidation of glucose**

![Figure 1.3: Glucose anomers in a solution of pH 7 (relative ratio of α, β and γ is 37:63:0.003)](image)

At physiological condition glucose exists in two hemiacetal forms, α, β and with its acid catalysed hydrolysis product γ-glucose (aldehyde form) in an equilibrium ratio 37:63:0.003 (shown in Fig. 1.3). In α- and β-glucose the hydrogen atom attached
to the C1 carbon gets activated because of the high acidity of hemiacetalic OH group (pKa = 12.3), which is stronger than alcoholic OH (pKa = 16). Thus the glucose oxidation of both α and β forms produces glucono-δ-lactone, which gets easily hydrolysed to gluconic acid with the half life of 10 minutes and a rate constant of $10^{-3}$ s$^{-1}$ at pH 7.5. Regardless of the formation of gluconolactone the final product of glucose oxidation is reported to be gluconic acid after the two electron oxidation [27]. Other mechanisms with multiple bond rupture to produce low molecular weight fragments from glucose are reported. Baldwin group studied the oxidation of glucose and other carbohydrate species in highly alkaline medium. Coulometric studies and the product analysis with NMR proved that there is a complete rupture of bonds in glucose to produce 12 electrons. The major product identified was formate ion [31].

![Scheme 1.1: Twelve electron electrooxidation mechanism for glucose.](image)

There are two generalized models suggested for explaining the direct electrooxidation on metal electrode surface.

### 1.3.1.1 Chemisorption theory

According to this theory oxidation occurs via adsorption of glucose on the electrode followed by electrooxidation. The adsorption process presumably involves the d-electrons and d-orbitals of the metal substrate that allows it to form a suitable bond with the adsorbate [32]. The hydroxides formed on electrode surface are not
considered in this case. The adsorption model for glucose on the electrode follows concerted mechanism, in which the adsorption of glucose occurs simultaneously with hydrogen abstraction. Then the oxidation occurs and forms gluconolactone. In most of the electrooxidation cases the removal of the hemiacetal hydrogen atom is supposed to be the rate limiting step. This mechanism is depicted in scheme 1.2 [33].

Scheme 1.2: Adsorption of glucose on the metallic electrode

1.3.1.2 IHOAM Model

Another mechanism is known as ‘Incipient Hydrous Oxide Adatom Mediator’ (IHOAM) model for electrooxidation of different molecules on metal surface. This is based on the observation that ‘active’ surface metal atoms undergo a premonolayer oxidation step that forms an incipient hydrous oxide layer of reactive OH$_{\text{ads}}$. The adsorbed hydrous oxide mediates the oxidation and inhibits reduction of kinetically slow electrode reactions. The active sites of the electrode surface are considered to have a low lattice co-ordination number (LCN), and lacks in normal lattice stabilization energy.

Scheme 1.3: Pictorial representation of IHOAM model
Due to the low stability they are more reactive, and thus undergo premonolayer oxidation at lower potentials than thermodynamic surface oxidation products. The catalytic importance of the active OH$_{ads}$ layer is proved with respect to small organic compound oxidation, as the formation of the hydrous species was recognized as a fast, preoxidation step following chemisorption of the glucose molecule. The hydrous premonolayer then mediates oxidation of the adsorbed species at a lower potential [33].

1.3.2 Metal based electrodes

Metallic electrodes such as platinum, gold and palladium are well reported for glucose sensing application in different media. These electrocatalysts in the form of nanomaterials are found to have increased sensor characteristics. A brief account on the glucose sensors fabricated with metal nanomaterials is presented below.

1.3.2.1 Platinum

Electrocatalytic oxidation of glucose on platinum electrodes is reported in alkaline, neutral and acidic media. It was seen that regardless of pH, glucose oxidation on platinum yields glucono-δ-lactone which then hydrolyses to form gluconic acid [33]. Both IHOAM and chemisorption models were proposed for glucose oxidation on Pt by various authors. Since glucose oxidation on Pt is a surface confined process, the easy saturation, adsorption and poisoning of reactive intermediates cause nonreproducible results. In particular amino acids, other blood based proteins, electroactive compounds such as uric acid (UA), ascorbic acid (AA) and acetaminophen (AP) also strongly affect the specificity. It is found that the electrocatalytic activity of Pt is highly dependent on the size and shape of the particles. Detailed reviews are available on mesoporous platinum and platinum nanowires for glucose electrocatalysis [27, 33].

Nanomaterials of platinum in various forms and with different incorporation matrix are reported to solve the inherent drawbacks of conventional platinum electrodes. Guo et.al prepared amperometric glucose sensor with platinum nanoflowers on gold electrode which can sense up to 16 mM at +0.03 V potential [34]. Interference free, highly stable sensitive sensor for glucose is designed by Xhou et al. with platinum modified polyglutamic acid on glassy carbon electrode [35].
TiO$_2$ nanotube arrays modified with Pt nanoparticles are employed as a self-cleaning glucose sensor that could work at an applied potential of -0.5 V and could detect up to 15 mM glucose [36]. Nanoporous gold electrode modified with platinum is found to have a sensitivity of 145 µA mM$^{-1}$ cm$^{-2}$ and linear up to 10 mM [37]. Three dimensional platinum nanoparticles on silicon wafer respond to glucose up to a linear range of 15 mM at +0.4 V with good sensitivity in presence of large quantity of chloride ions [38]. Chang et al. developed platinum nanoclusters-graphene composite modified GC electrode for glucose sensing in PBS [39]. Platinum nanoparticles modified graphene hydrogel based sensor detects glucose up to 20 mM with a sensitivity of 137.4 µA mM$^{-1}$ cm$^{-2}$ [40]. Glucose sensor with two linear ranges of detections with maximum up to 20.3 mM concentrations with good sensitivity is reported by Wu et al. [41]. Pt nanoparticles encapsulated in carbon microspheres [42], nanoparticles of Pt on CNT [43,44], Pt nanotubules [45], mesoporous platinum [46], nanoporous Pt incorporated in microfluidics [47], Pt nanoparticles on mesoporous carbon [48], Pt nanoparticle [49], are among the platinum nanomaterials reported for glucose sensors.

1.3.2.2 Gold

The oxidation potential of glucose in neutral and alkaline medium is more negative on gold compared to the other metals such as palladium and platinum. Various forms of gold nanostructures are widely studied for biosensor applications [50,51]. They exhibit superior poison resistivity towards electrooxidation of glucose due to the enhanced surface area and the presence of highly active binding sites on the surface of particles. Chen et al. reported the sensor with nanoporous gold electrode with average pore size of 18 nm prepared by dealloying was used to detect glucose [52]. Prehn et al. fabricated gold micro pillar electrode using photolithographic and electrodeposition procedure [53]. Wide detection range up to 15 mM glucose concentration was achieved on gold nanoparticles modified ITO electrode by Fu et al. [54]. Electrodeposited gold nanostructure on glassy carbon electrode is employed for detecting glucose with a good sensitivity and selectivity [55]. Cooray et al. investigated the use of polymer stabilized gold nanoparticles for sensing glucose. A linear range up to 50 mM was achieved but found to suffer from interference of uric acid [56]. Graphene oxide nanoribbon in combination with gold nanoparticles is used
by Ismail et al. Nafion-polypyrrole film is used to avoid chloride interference in the study [57]. Highly porous gold electrode was fabricated by hydrogen bubble assisted electrodeposition and used for glucose estimation [58]. The use of gold nanoparticles-CNT composite electrode for the sensing of glucose was investigated voltammetrically by Gougis et al. The sensor detects linearly up to 50 mM glucose at -0.28 V with good sensitivity [59]. Wide linear detection range up to 16 mM glucose is achieved by Liu et al. using gold nanoparticles graphene composite electrode [60]. Gold nanoparticles modified GC electrode could detect glucose up to a concentration of 30 mM with good sensitivity and selectivity [61]. Gold in the form of nanowires [62], nanoparticles [62-64], 3D gold film [65], gold cluster films [66], lamellar ridge gold [67], Au urchin [68] were reported for the nonenzymatic glucose oxidation.

Different mechanisms were suggested for glucose oxidation on gold electrode. The earlier mechanism proposed for oxidation of glucose on gold proceeds via the formation of a surface oxide film which catalyses the oxidation of glucose as below,

\[
\text{Glucopyranose + Au}_nO_m \rightarrow \text{Gluconolactone + Au}_nO_{m-1}
\]

\[
\text{Gluconolactone + H}_2\text{O} \rightarrow \text{Gluconic acid}
\]

The gold oxides easily get regenerated by the electrolyte.

\[
\text{Au}_nO_{m-1} + 2\text{OH}^- \rightarrow \text{Au}_nO_m + \text{H}_2\text{O} + 2e^-
\]

Later this mechanism was disproved and the one accepted was the chemisorption mechanism proposed on platinum electrode [69].

1.3.2.3 Palladium

Palladium based nanomaterials have also showed excellent electrocatalytic activity for glucose oxidation. The mechanism of electrooxidation of glucose on Pd electrode involves initial chemisorptions of glucose by C\textsubscript{1} carbon abstraction. Then at a higher applied potential Pd-OH species are formed which oxidize the adsorbed glucose molecules and the Pd surface is regenerated [70]. The reactions are represented as below.

\[
Pd + x\text{OH}^- \rightarrow Pd(OH)_x + xe^-
\]

\[
Pd(OH)_x + \text{intermediates} \rightarrow Pd + \text{gluconolactone or gluconic acid}
\]

\[
Pd(OH)_x + \text{glucose} \rightarrow Pd + \text{gluconolactone or gluconic acid}
\]
The synergy between multiwalled carbon nanotubes and palladium nanoparticles is employed for the fabrication of glucose sensor with excellent sensor characteristics. The selectivity and precision of the sensor in blood glucose with conventional meters are commendable [70,71]. Palladium nanocube prepared by chemical reduction method is reported to sense glucose up to 10 mM by Ye et al. [72]. Improved detection range up to 50 mM is achieved for functionalized carbon nanotube modified with Pd nanoparticles [73]. The glucose sensor with palladium nanoparticles and nafion modified electrode was reported by Wang et al. and discussed the electrooxidation mechanism [74]. Another interesting coulometric determination of glucose with wide linear range and selectivity is reported on Pd nanoparticles modified epoxy silver electrodes [75]. Nanohybrid composite material of graphene and palladium was used for the nonenzymatic determination of glucose that could detect glucose up to 5 mM with a lower detection limit of 1 µM [76]. Pd nanoparticles on graphene oxide [74], Pd nanoparticles on graphene [76], Pd nanorod [77], are among the recently reported Pd metal based glucose sensors.

1.3.3 Metal oxide based sensors

The search for more economical electrocatalyst for glucose sensors without compromising on the sensitivity and selectivity gave birth to transition metal oxide based glucose sensors. These oxides are mainly of cheaper metals such as copper, nickel, zinc and cobalt, which possess excellent electrocatalytic ability towards glucose oxidation in alkaline medium. These materials in nano dimension were earlier used for the incorporation of enzymes because of their enhanced surface area. Moreover the oxide nanostructures are found free from poisoning and free of interference when compared with their metal counterpart. Their application in enzymatic and non-enzymatic electrochemical determination of glucose was well reviewed [78,79]. Given below a brief review on the metal oxide nanomaterials employed for the electrocatalysis of glucose. The most recent literatures are mainly considered for this review.

1.3.3.1 Cupric oxide based glucose sensors

Copper oxide, a p-type semiconductor with a narrow band gap of 1.2 eV has been widely studied for its electrocatalytic applications [80,81]. Nonenzymatic
glucose biosensors using copper oxide (CuO) gain great attention due to excellent stability, ease of synthesis and post synthesis handling, low cost and different redox properties of the oxide at different reaction conditions. The catalytic properties of CuO in alkaline medium are due to the formation of thermodynamically unstable Cu(III) species. The species responsible is CuOOH formed from CuO that catalyses the oxidation of glucose molecules [82]. The reported oxidation peak for copper near to 0.6 V in alkaline medium is due to copper(II)/(III) transition [83]. The voltammetric analysis also shows corresponding reduction peak for the metal oxide. Glucose oxidation occurs at the same region and the fact that during the cycle the reduction peak current decreases on increase in concentration of glucose suggests the Cu(III) is consumed during the oxidation. The other redox peaks for copper are clearly defined with the oxidation to Cu(I) and Cu(II) states, and the corresponding reduction, evident in voltammetric studies, which does not alter in the presence of glucose, suggests they are not involved in glucose oxidation. The oxidation can be the complete breakage of C-C bonds in glucose to get \( 12e^- \) was demonstrated by coulometric technique [31]. This could be the reason for the high faradaic current density obtained from nonenzymatic glucose sensors. The \( 12e^- \) oxidation proposed by Kano et al. is shown in scheme 1.1.

Variety of nanostructures of CuO such as nanowires, nanoparticles, nanoporous structures was employed to enhance the sensor characteristics such as sensitivity, selectivity and the linear range. Highly sensitive response at 0.35 V is obtained for a glucose sensor fabricated with CuO and CNT on GC electrode by Alizadeha and Mirzagholipur [84]. The synergetic effect between CuO and sulphur doped reduced graphene electrode was able to detect glucose up to 10.5 mM with a lower detection limit of 80 nM [85]. Dong et al. fabricated glucose sensor using CuO on zeolite that detects glucose with a detection limit of 0.37 µM [86]. The enhanced surface area of nanowires of CuO is utilized for the sensing of glucose by Zhang et al. [87]. CuO nanoparticles modified carbon spheres are found to have a sensitivity of 2981 µA mM\(^{-1}\) cm\(^{-2}\) and good selectivity to glucose [88]. Uniformly decorated CuO nanosheets on copper electrode showed enhanced glucose sensor response in basic medium [89].

CuO nanoparticles on glassy carbon [90], copper nanowires [91], CuO on carbon nanofibres [92], CuO nanospindle on CNT [93], CuO nanowires on Cu [94],
copper at carbon coaxial nanowires [95], mesoporous CuO nanospheres [96],
different CuO structures [82,97-100] are some among the numerous reports on CuO
based glucose sensors. Numerous earlier works on the CuO based glucose sensors are
well reviewed [101].

1.3.3.2 Nickel oxide glucose sensors

![Scheme 1.4: Nonenzymatic sensing of glucose on NiO modified electrodes.](image)

Nickel electrode was extensively studied for its catalytic properties towards
the electrooxidation of different organic molecules including glucose in basic
medium. Similar to the electrooxidation of glucose on copper oxide, nickel oxide also
catalyses the oxidation by Ni(II)/(III) couple [102]. Ni(II) gets electrooxidised to
Ni(III) in alkaline medium which releases electrons resulting in a peak current. At this
region glucose gets oxidized to gluconic acid by Ni(III), which gets reduced to Ni(II)
at the same time. Ni(III) species rapidly activates the nickel surface and acts as a
strong oxidant, with suitable empty d-orbitals to rapidly adsorb glucose molecule.
Glucose undergoes hydrogen abstraction at the surface to form radical intermediate
and reforming the Ni(OH)\(_2\) species. The hydroxyl ions in the electrolyte rapidly
convert the radical intermediate to gluconolactone. The sensing scheme is presented
in scheme 1.4.

Tremendous amount of work has been reported on glucose sensors based on
nickel oxide nanostructures for the enhancement of the sensor characteristics. NiO
modified carbon microspheres are found to have a sensitivity of 30.19 mA mM\(^{-1}\) cm\(^{-2}\),
that is one among the highest reported. The linear detection range is only up to
1.279 mM and does not meet the physiological range of glucose levels [103].
Yang et al. obtained high sensitivity response to glucose with nanocomposite of NiO and SiC modified GC electrode in basic medium [104]. NiO nanoparticles modified graphene showed enhanced capacitance behavior and glucose sensing properties [105]. Zhang et al. studied the sensing ability of electrospun NiO nanofibres on graphene and found that it detects glucose with good sensitivity and selectivity [102]. NiO and Pt modified reduced graphene oxide is found to be excellent materials for the fabrication of glucose sensors [106].

NiO nanostructures by Mu et al. [107], hedgehog NiO nanoparticles [108], NiO hollow spheres [109], porous nickel nanostructures on screen printed electrodes [110], Ni foam [111], NiO on mesoporous carbon [112], NiO on MWCNT [113] are among the other developments in glucose sensors based on NiO electrocatalyst.

### 1.3.3.3 Oxide of cobalt for glucose detection

Cobalt is found to be an excellent electrocatalyst due to its different oxidation states in alkaline medium. Various reports discuss the electrooxidation of glucose on cobalt oxide based electrodes in alkaline conditions. Glucose electrooxidation on cobalt also follows similar mechanism that on copper and nickel. The electrocatalyst formed is CoOOH which further gets oxidized to CoO$_2$ and mediates oxidation reaction [114]. At an applied potential, Co forms CoOOH, which gets further oxidized to CoO$_2$ (Co(III) to Co(IV)). This Co(III)/(IV) redox couple catalyses the glucose oxidation. The mechanism of electrooxidation of glucose on cobalt electrode is represented below.

\[
\begin{align*}
\text{Co} + 2\text{OH}^- & \rightarrow \text{Co(OH)$_2$} + 2e^- \\
\text{Co(OH)$_2$} + \text{OH}^- & \rightarrow \text{CoOOH} + \text{H}_2\text{O} + e^- \\
\text{CoOOH} + \text{OH}^- & \rightarrow \text{CoO}_2 + \text{H}_2\text{O} + e^- \\
2\text{CoO}_2 + \text{glucose} & \rightarrow 2\text{CoOOH} + \text{gluconolactone}
\end{align*}
\]

Cobalt oxide nanomaterials incorporated on various platforms are reported for the highly sensitive and selective detection of glucose. CoO-graphene composite is used for detection of glucose with a sensitivity of 669 $\mu$A mM$^{-1}$ cm$^{-2}$ and detection up to 8 mM by Ci et al. [115]. Electrodeposited CoOOH on Ti platform exhibited high sensitivity of 526 $\mu$A mM$^{-1}$ cm$^{-2}$ and high stability. The macroporous structures
surrounded by the nanosheets and the mesopores in the nanosheets lead the electrodes to show hierarchical mass transport channels and adequate active sites for detecting glucose with high sensitivity [116]. Well oriented CoOOH nanosheets prepared on cobalt substrate produce highly sensitive glucose sensor [117]. Cobalt oxide nanoparticles with reduced graphene detect glucose with high sensitivity [118]. Cobalt oxide on 3D graphene [119], electrospun Co$_3$O$_4$ nanofibres [120], Co$_3$O$_4$ nanoparticles, cobalt oxide acicular nanorods [121] are among the reports on Co based nonenzymatic electrochemical sensors for glucose.

1.3.3.4 Other metal oxides

Transition metal oxides of Fe, Mn, are reported for the nonenzymatic glucose oxidation. Manganese oxide is found to be sensing glucose at very low overpotentials because of its different oxidation states in alkaline medium. It is found that Mn shows oxidation states Mn$^{II}$(OH)$_2$, which can further oxidize to Mn$^{IV}$O$_2$ and then to Mn$^{VI}$O$_4^{2-}$. These transitions Mn(II) to Mn(VI) can not only act as an electron transfer mediator but also as a catalyst for glucose oxidation. Multiwalled carbon nanotubes electrochemically modified with MnO$_2$ are used for sensing glucose at a lower applied potential [122]. Carbon nanotubes-MnO$_2$ nanocomposit is reported to sense glucose with good sensitivity [123]. Mn$_3$O$_4$ on 3D graphene detects glucose up to 8 mM with a sensitivity of 360 µA mM$^{-1}$ cm$^{-2}$ [124]. FeOOH modified electrodes mediate the heterogeneous redox reactions and the Fe(III) species get regenerated subsequently which mimic the enzymatic oxidation reactions [125]. The proposed mechanism of glucose oxidation on FeOOH is,

$$2\text{Fe(III)} + \text{Glucose} \rightarrow 2\text{Fe(II)} + \text{Gluconolactone} + \text{H}_2\text{O}$$

$$\text{Gluconolactone} + \text{H}_2\text{O} \rightarrow 2\text{H}^+ + \text{Gluconate}$$

$$2\text{Fe(II)} \rightarrow 2\text{Fe(III)} + 2e^-$$

1.3.4 Carbon electrodes

Carbon nanomaterials are extensively used for biosensor construction. They include zero dimensional fullerenes, carbon dot, nano diamond and conducting carbon black, one dimensional carbon nanotubes (CNT), 2D material graphene and 3D materials such as diamond and graphite [29]. The high surface area, acceptable
biocompatibility, chemical and electrochemical stability and good electrical conductivity made them the best sensing material.

Glucose sensors with carbon are either constructed based on direct electrochemical oxidation of glucose on carbon or on specific electrocatalytic materials incorporated with carbon. Direct electrooxidation of glucose on ordered mesoporous carbon electrode was reported for the sensing of glucose in aqueous KOH medium and to show good sensitivity and selectivity. It is found that the increased surface area of the mesoporous carbon causes the electrocatalytic oxidation [126]. Unmodified multiwalled carbon nanotubes were used for the direct electrooxidation of glucose at a lower overpotential [127]. An impedance based glucose sensor was fabricated by Vlandas et.al with boronic acid on CNT incorporated in microfluidic channels [128]. Direct electrooxidation of glucose in alkaline medium using freestanding single walled carbon nanotube films and its biosensing application was reported by Wang et al. [129].

CNT and graphene [130] are having good electronic conductivity, thermal and mechanical stability. Enzymatic sensors with these materials are well reviewed by various authors [131-135]. A large number of research articles describe incorporation of various metals and metal oxides on carbon nanomaterials for glucose sensor fabrication [136-138] and these sensors are found to have enhanced sensor characteristics. Graphene modified with electrocatalysts such as copper [139,140], NiO [102,105], Pd nanofibres [76], Pt-Ni nanoparticles [141], CuO [142,143], Pt nanoflowers [41], cobalt oxide [119,144], Pt-Au/MnO$_2$ composite [145] exhibit good sensor characteristics. Carbon nanotubes modified with CuO [1,80,146], Ni [147,148], Pt-Pd [147], Pt [149], NiO [113], various bimetalics of Pt [150], Pd-Au [151], MnO$_2$ [152] were reported for nonenzymatic glucose sensing.

1.3.5 Layered double hydroxides (LDH)

These are layered anionic clays generally expressed by formula $[\text{M}^{\text{II}}_{1-x}\text{M}^{\text{III}}_x(\text{OH})_2]^z+(\text{A}^{n-})_{n/z}\cdot y\text{H}_2\text{O}$ (where $\text{M}^{\text{II}}$ and $\text{M}^{\text{III}}$ are divalent and trivalent metals respectively; $\text{A}^{n-}$ is the interlayer anion). Because of the specific layered structure and nano size, LDH materials are used for amperometric and potentiometric sensors. These are having advantages of low toxicity, high stability and biocompatibility with many functional molecules. Magnetic field assisted layer by layer formation of double
hydroxides of Co and Fe was reported for glucose sensor fabrication [153]. The mechanism of electrooxidation involves the central metal Co that acts as the electrocatalyst as shown below,

\[
\text{LDH-Co}^{\text{II}} + \text{OH}^- \rightarrow \text{LDH(OH)}^{\text{II}}\text{Co}^{\text{III}} + e^-
\]

\[
\text{LDH(OH)}^{\text{II}}\text{Co}^{\text{III}} + \text{glucose} \rightarrow \text{LDH-Co}^{\text{II}} + \text{gluconolactone}
\]

Zhao et al. fabricated two dimensional inorganic matrix for incorporation of gold nanoparticles and used for nonenzymatic glucose sensing [154]. Nonenzymatic glucose sensor with gold nanoparticles modified LDH of Ni and Al is reported where the nickel metal centre acts as the catalytic species via its Ni(II) to Ni(III) redox reaction [155].

1.3.6 Alloys and bimetallic systems

In order to overcome the limitations faced by the metal electrodes due to the poisoning and interference, combinations of metals such as alloys and bimetallic systems for the determination of glucose have been reported. The platinum based alloys received great attention due to the improved sensitivity, selectivity and anti-poisoning properties. Alloys of copper, nickel and cobalt, which are of low cost with improved sensor characteristics are reported in alkaline medium [156]. The properties of alloys and bimetallic nanomaterials are widely explored for the fabrication of glucose sensors with excellent sensor characteristics. A brief outline of various reports on glucose sensors based on alloys is given below.

1.3.6.1 Alloys of precious metals

Pt-containing bimetallic nanomaterials such as Pt-Ni [141], Pt-Pd [157], Pt-Au [158] have been demonstrated with enhanced electrocatalytic properties towards glucose oxidation. Li et al. compared the electrocatalytic properties of two different nanostructures, Pt-Pd nanowires and nanotubes for glucose sensor application and achieved good linear response and specificity [159]. Xu et al. fabricated Pt-Co alloy electrode by dealloying process for the sensing of glucose with good selectivity [160]. Pt-Pb alloy [161,162], Pt-Ru nanoparticles on CNT [163], Pt-Ir nanoparticles [164], nanoporous Pt-Ag, Pt-Cu alloys [165] and Pt-Au alloy [166], Pt-Co/C composite
are found to have enhanced sensor characteristics. Pt-Ni composite on rGO [168], Pt/Ni nanowire on GCE [169], Pt/Ni–Co nanowire modified GCE [170], Pt-Cu nanochain decorated GCE [171], Pt-Ni/C nanostructures [172] were also reported for efficient glucose sensing.

Pt based alloys are the most reported ones for glucose sensing, alloys of Pd and Au also possess very good sensor characteristics. Alloy of Pd with Fe was used for the nonenzymatic detection of glucose and \( \text{H}_2\text{O}_2 \) [173]. Pd-Cd network structures [174], Pd-Cu nanoparticles on graphene hydrogel [175], Pd-Ni 3D nanomaterial [176], Pd-Ni nanoparticles modified silicon nanowires [177], Pd–Au bimetallic cluster [178] were among this category. Nanoporous Au-Cu alloy [179] Au-Ag alloy with different metal ratios [180] and Pt-Au/C based nanocomposite [181] are some of the bimetallic systems containing Au for glucose sensing.

1.3.6.2 Bimetallic and alloys of copper, nickel and cobalt

Nickel-cobalt hydroxide on screen printed electrodes [182], Cu-Ni alloy on TiO\(_2\) nanotube arrays [183], Co-Cu alloy on graphene [184] were reported to have improved sensor characteristics. Ni-Cr alloy was recently reported for the detection of carbohydrates with good sensitivity [185]. Different oxidation states of these metals in alkaline medium help in the electrocatalytic oxidation of glucose. The synergistic effect between the metals in combination is found to enhance the sensor characteristics when compared with the individual metals. The low cost, ease of preparation and the high storage stability show the potential ability of these electrocatalysts for the fabrication of commercial glucose sensors. Mn-Cu alloy on graphene detects glucose at very low applied potential of -0.05 V with linearity up to 32.0 mM [186]. Electrodeposited MnO\(_2\) and copper on GC electrode detect glucose in alkaline medium with good reproducibility and sensitivity [187]. Co\(_3\)O\(_4\)/PbO\(_2\) core–shell nanorod arrays [188], NiO/Pt/erGO ternary composite [189] NiO–CdO nanofiber [190], Co-Cu dendritic alloy [191], palladium doped electro spun CuO nanofibres [192], NiO–Cu [171], CuO and Ag nano fibers [193] were reported for highly sensitive and selective glucose sensing. The synergy between Ag and Ni for better nonenzymatic determination of glucose was also reported [194].

This brief review clearly indicates that the development of glucose sensors is focused towards increasing the sensitivity and detection range. This is achieved by
suitable electrocatalysts and by the selection of suitable immobilization platform. Considerable amount of work is still in progress towards the enzyme free glucose sensors and their commercialization. These sensors can drastically decrease the cost of glucose point of care diagnosis systems and will be affordable for the people in third world countries. Low cost glucose sensors will also be useful in food analysis and the fabrication of fuel cells.

1.4 Nanoporous electrodes in biosensing

Nanoporous and nanotubular structures of carbon [132,195,196] and oxides of metals such as Zr [197], Ti [198], W [199], Nb [200], Al [201] and Ta [202,203] possess enhanced surface area and high aspect ratio that are suitable for electrocatalysis with increased response [50,78,204,205]. The uniqueness of nanoporous electrochemistry encompasses the geometrical effect on discriminative amplification of faradaic reactions. This allows selective amperometric detection, in which the selectivity and sensitivity can be tuned by the depth, shape, and interconnectivity of the nanopores. The nano-confinement effect is also caused by the concave geometry on the nano-scale of the electrode surface, involving characteristic molecular dynamics and stochastic behavior. Nanoporous electrodes provide extremely low interfacial electrical impedance and the capability of responding to sensor signals very quickly owing to their enlarged surface area and quantum confinement effect [206]. They can act as a template for the deposition of nanomaterials of different metal or alloy electrocatalysts. This can improve the selectivity and sensitivity of the sensors [82,207-211]. Titanium and tantalum oxide nanotube arrays have been investigated for their biosensing capabilities due to their high dielectric strength, wide band gap and excellent biocompatibility. Electrochemical anodization is one of the most commonly employed methods for the synthesis of these metal oxide nanotube arrays [212-220]. A brief review of the synthesis and biosensor applications of TiO$_2$ and Ta$_2$O$_5$ one dimensional nanostructures is given here.

1.4.1 TiO$_2$ nanotube arrays

TiO$_2$ nanotubes (NTs), one of the most extensively studied nanostructured oxides, possess remarkable physical and chemical properties and diverse applications
that include, but not limited to, photocatalysis [221,222], solar cells [223,224], photoelectrochemical water splitting [225], supercapacitors, sensors, drug delivery, and biological coatings [226-228]. These are of great interest in biomedical applications because of their excellent biocompatibility and catalytic properties [209,210,229-231]. Different methods are adopted for the synthesis of TiO$_2$ nanotubular arrays, which include, template-assisted processes [232,233], sol–gel method, hydro/solvothermal means, [234-236] and electrochemical anodization [237-239]. Out of these electrochemical anodization of titanium in fluoride medium was well accepted for the formation of TiO$_2$ nanotube arrays [229,240]. Moreover anodization procedure provides excellent tunability for pore size, length and wall thickness of the oxide nanotubes. This is done precisely tailoring the electrochemical parameters applied for the synthesis such as potential, current density, temperature, pH, water content and electrolyte composition. The mechanism of formation and growth of nanotube arrays in fluoride electrolyte was well studied and reported in literature. The most accepted mechanism involves the field assisted formation and dissolution of oxide film. The detailed mechanism involves the initial formation of oxide film by the reaction between Ti metal and O$^2-$ ion or OH$^-$ ions (formed by the electrolytic deprotonation of water).

At anode,

\[
\text{Ti} \rightarrow \text{Ti}^{4+} + 4\text{e}^{-}
\]

\[
\text{Ti}^{4+} + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+
\]

The net reaction is,

\[
\text{Ti} + \text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{H}^+ + 4\text{e}^{-}
\]

At cathode,

\[
4\text{H}_2\text{O} + 4\text{e}^- \rightarrow 2\text{H}_2 + 4\text{OH}^-
\]

Under the applied electric field there occurs the initial formation of oxide layer. Then the O$^2-$ ion diffuses through the oxide layer and reaches the metal oxide-metal interface and there further oxidation occurs. The Ti$^{4+}$ ions formed on metal oxide-metal interface diffuse to the metal oxide-electrolyte interface. Thus in the absence of fluoride ions there will only be the formation of a compact oxide layer which decreases the current flowing through the cell due to the decreased electrical conductivity. In the presence of fluoride ions the oxide film was etched or attacked by
the F\(^-\) ions and form water soluble \([\text{TiF}_6]^{2-}\) complex ion. The mobile Ti\(^{4+}\) ions also react with F\(^-\) ions to form soluble \([\text{TiF}_6]^{2-}\) and the reactions are,

\[
\begin{align*}
\text{TiO}_2 + 6\text{F}^- + 4\text{H}^+ & \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} \\
\text{Ti}^{4+} + 6\text{F}^- & \rightarrow [\text{TiF}_6]^{2-}
\end{align*}
\]

This mechanism was supported by the current density variation during the electrolysis, in which there is a current decrease due to the formation of oxide film at the initial stage and then the current rises due to the tube formation. Later the tube growth becomes constant which gives a constant current flowing through the circuit. At the base of the pore/tubes, the electric field is stronger resulting in enhanced field-assisted oxide growth and oxide dissolution. The overall rate in the steady-state phase is limited by the transport (diffusion) of F\(^-\) inside the channel from the solution to the growing TiO\(_2\) and the transport of \([\text{TiF}_6]^{2-}\) in the opposite direction. The concentration of fluoride ions, electrolyte pH, water content, electrolyte viscosity, anodization voltage, anodization time and temperature decide the diameter, length, and wall thickness of the tubes [198, 241-247]. Directly grown TiO\(_2\) nanotube arrays on Ti substrate by anodization helps good adherence of oxide film to the substrate, which avoids the inevitable loss in electrochemical activity for continuous operation [247]. The Schottky type contact to the metal surface enhances the rapid transport of surface reaction electrons to metal surface [227].

1.4.2 TiO\(_2\) nanotube arrays in biosensors

TiO\(_2\) nanotube arrays are widely employed in enzymatic sensors for the incorporation of enzymes. Due to the enhanced surface area, these sensors will have high sensitivity. Since the present work is on nonenzymatic biosensors, the discussion here is restricted to nonenzymatic sensors based on TiO\(_2\) nanotube arrays. Similar to the CNT counterpart [82,248] metal and metal oxide nanostructures modified TiO\(_2\) nanotube arrays were reported for the sensing of glucose [209,249] and ascorbic acid [205,250]. Different transition metal catalysts such as copper, nickel, platinum, cobalt and their alloys were used for the modification [209,251-253]. The increased surface area provided by the TiO\(_2\) nanotube arrays could incorporate more amounts of electrocatalyst so as to yield high sensitivity and selectivity.
1.4.3 Nanoporous tantalum oxide structures

Tantalum oxide (Ta$_2$O$_5$) nanoporous materials have been investigated for biomedical and electronics applications due to their high dielectric strength, wide band gap (4.4 eV) and excellent biocompatibility [254]. Ta$_2$O$_5$ is mainly used for protective coating, catalytic applications and for fabricating resistors and capacitors. Because of the high aspect ratio these are having potential applicability in electrocatalysis as in the case of TiO$_2$ nanotube arrays.

The anodization of tantalum normally yields thick oxide layer which is soluble in strong HF solution forming tantalum fluoride [254]. Recently some efforts have been successfully made to fabricate nanoporous Ta$_2$O$_5$ structures by anodization in fluoride electrolyte. Since oxides of tantalum are the most stable among valve metals, highly acidic medium is normally employed for dissolution. The mechanism of growth of nanostructures is similar to that of the formation for TiO$_2$ nanotube arrays, in which field assisted formation and dissolution of Ta$_2$O$_5$ occurs. Strong acidic medium of H$_2$SO$_4$ and HF supported the formation of highly oriented nanodimples [255]. Ta$_2$O$_5$ nanopore formation by electrochemical oxidation of Ta in glycerol and sulfuric acid medium has been reported recently [256,257].

The mechanism of formation and growth of Ta$_2$O$_5$ nanotube arrays in a medium containing concentrated H$_2$SO$_4$ and HF can be as follows [203],

$$2\text{Ta} + 5\text{H}_2\text{O} \rightarrow \text{Ta}_2\text{O}_5 + 10\text{H}^+ + 10\text{e}^-$$
$$\text{Ta}_2\text{O}_5 + 10\text{H}^+ + 14\text{F}^- \rightarrow 2\,[\text{TaF}_7]^{2-} + 5\text{H}_2\text{O}$$

The first reaction mainly occurs at the pore base and creates a local acidic gradient due to the generation of protons where the thickness of oxide film is less when compared to the pore walls. The chemical process as given in the second reaction occurs both at the pore base and walls, but the effective applied electric field will be more at the pore base resulting in an enhanced field assisted dissolution compared to walls of the nanopores. The effective fluoride ion concentration in the electrolyte is controlled by the strong acidic medium. Also, the highly viscous electrolyte reduces the diffusion rate of fluoride as well as locally formed H$^+$ ions at the pore base. Thus the dissolution will be more at the pore base [209].
1.5 Cholesterol biosensors

The major sensing procedures adopted for blood cholesterol utilize spectrophotometric and electrochemical principles. The spectrophotometric estimation is based on the colored product formed between cholesterol and different chromogens. The historical method for the determination of cholesterol is the nonenzymatic chemical oxidation of cholesterol to produce colored products based on Liebermann-Burchard and FeCl$_3$ reactions [258]. In Liebermann-Burchard reaction, cholesterol in chloroform is treated with concentrated sulfuric acid and acetic unhydride to get a greenish colour product [259-261]. Another important reaction for cholesterol estimation is the reaction of cholesterol with digitonin, a white precipitate of cholesterol digitonide is formed when an ethanolic solution of cholesterol is treated with ethanolic solution of digitonin [262-264]. A critical review on different stages of developments in cholesterol estimation was presented by Zak [265,266]. Spectrophotometric estimation by employing enzymatic oxidation of cholesterol to produce H$_2$O$_2$ was well studied. H$_2$O$_2$ reacts with various chromogens to form colored products. Since cholesterol in body is present in free form as well as in the esterified form, cholesterol ester hydrolase (ChEt) enzyme is used for free cholesterol release. The enzymatic oxidation of cholesterol and its determination by chromogens can be represented as,

Cholesterol ester $\xrightarrow{\text{ChEt}}$ Cholesterol + Fatty acids  
Cholesterol $\xrightarrow{\text{ChOx}}$ Cholestenone + H$_2$O$_2$  
H$_2$O$_2$ + Chromogen $\xrightarrow{\text{Peroxidase}}$ Colored products

Determination of cholesterol in gall stone and in serum is based on the above procedure using 4-aminoantipyridine and phenol as the chromogen [267-270]. Homovanilic acid [271], orthodianisidine dihydrochloride [272], 4-aminophenazone [273], 4-hydroxy benzoate/4-aminophenazone [274], o-dianisidine [275] are among the various chromogens reported for estimation of cholesterol.

But these methods have limitations such as lack of specificity, instability of coloring agents, and variability of color yield. Electrochemical estimation of the H$_2$O$_2$
produced then become popular and was being commercialized. A brief account of these sensors is given in the following section.

1.5.1 Electrochemical enzymatic cholesterol sensors

Electrochemical determination of cholesterol by enzymatic oxidation is depicted in scheme 1.5. Since most of the cholesterol present in the body is in the esterified form it was initially hydrolysed by cholesterol ester hydrolase enzyme. Peroxide oxidation reaction can bring about by peroxidase enzyme or by the electrode itself. Numerous reports are available on the development of cholesterol sensors with this three enzyme methodology. The exploration for the suitable transducer material for the effective incorporation of the enzymes on the electrode is the subject of many research articles [276-279].

![Scheme 1.5: Schematic representation of working of enzymatic electrochemical cholesterol sensor.](image)

Conducting polymers, metal and metal oxide structures were employed as the platform for the incorporation of the enzymes. Dong et al. reported an amperometric sensor in which electrodeposited palladium nanoparticles were used as the immobilization platform [280]. Amperometric enzyme microsensor was reported by Motonoka [281], a potentiometric ion selective electrode by Papastathopoulos et.al [282] and amperometric sensor with carbon paste electrode modified with enzyme [283] was reported by Charpentier et al. Haemoglobin was used as the alternative for peroxidase enzyme [284] for the sensing of cholesterol. Conducting polymers like electrospun polyaniline [285] and polypyrrole hydrogel membrane [286] were employed for enzyme immobilization [287]. Enzyme immobilized on α-Fe₂O₃ sensor developed by Umar et al. was efficient to detect cholesterol up to 8 mM without the
interference from ascorbic acid, uric acid and glucose [288]. Gold nanoparticles on carbon nanotubes were used as an electrochemical platform for cholesterol with a lower detection limit of 4 µM [289]. Cholesterol detection up to 16 mM was achieved by Ahmed et al. with zinc oxide nanorods on silver electrode [290]. Brahim et al. developed polypyrrole hydrogel platform for the oxidase enzyme which could detect cholesterol up to a level of 15 mM with a lower detection limit of 120 µM [286]. The immobilization matrix developed by Singh et al. using polypyrrole could detect cholesterol up to 8 mM concentrations in presence of glucose, lactate and uric acid [291]. Gold nanoparticles decorated graphene was used as an efficient platform for cholesterol oxidase to measure cholesterol in presence of glucose, ascorbic acid and uric acid [292]. Flow injection cholesterol sensor was developed by oxidase enzyme immobilized in polypyrrole matrix on platinum electrode [293]. CNT-chitosan based cholesterol sensor operates at +0.4 V was not affected by ascorbic acid, uric acid and acetamidophenol [294]. Cholesterol oxidase enzyme incorporated on nano ZnO and chitosan film [295] and spectrophotometric and electrochemical detection of cholesterol using tetraethyl orthosilicate sol-gel containing enzyme [296] showed enhanced sensor characteristics. Self assembled thiol on gold or platinum [297], activated polyvinyl chloride [298], chitosan-SiO$_2$-CNT composite [299], 2-aminoethyl-3-aminopropyl-trimethoxy silane on ITO [275], Pt-ZnO [300], graphene-cerium oxide [301] are among the different enzyme immobilization platforms used to construct highly efficient cholesterol sensors.

The enzymatic sensors possess excellent sensor characteristics; they have a few drawbacks such as long term stability and higher cost. In order to avoid these drawbacks nonenzymatic platform such as molecularly imprinted polymers and procedure like direct electrooxidation on various electrodes are the emerging trend in cholesterol estimation. A brief review regarding the development of these sensors is presented here.

1.5.2 Molecularly imprinted polymers for cholesterol sensing

Molecular imprinting is a comparatively fast and straightforward possibility to design three-dimensional cavities with tailored recognition properties or artificial bioreceptors. In this technique the analyte acts as ‘*template*’ and is mixed with monomers forming a highly cross-linked polymeric matrix such as that of
polyurethane, polystyrene or polymethacrylate. After that the template is dissolved in proper solvents which leave the impression of the molecule (the molecular signature) in the polymer. This impression could act as the specific binding site for the analyte during the sensing that mimics the lock and key model for enzymatic reactions. A schematic representation of the working of imprinted sensor is represented in scheme 1.6. Because of its robustness to both physical and chemical stress, this straightforward synthesis technology can be a good alternative for conventional bioreceptors [302-305].

**Scheme 1.6.** Fabrication of molecularly imprinted polymers for sensing.

Electropolymerized 2-mercaptobenzimidazole MIP on gold electrode was reported as capacitive sensor for cholesterol, which had shown detection up to 30 μM and good storage stability [306]. Tong et al. developed a ceramic carbon electrode modified with MIP-CNT composite matrix for the sensing of cholesterol [307]. Fluorescence based detection of cholesterol using dansyl fluorophore modified cyclodextrin MIP was reported recently [308]. Self assembled hexadecyl mercaptan MIP modified on gold substrate was reported to have good selectivity towards
cholesterol [309]. Methyl methacrylate-co-acrylic acid copolymer based specific cholesterol recognition sites were reported by Ciaardelli et al. [310]. Para-aminothiophenol polymer with gold nanoparticles for cholesterol recognition [311] and alkanethiol SAM on gold electrode were used for the sensing of food cholesterol levels [312]. Chou and Liu prepared a MIP sensor for cholesterol with self assembled hexadecanethiol on gold electrode that detects up to 700 nM [313].

1.5.3 Nonenzymatic cholesterol sensors

There are only a few reports available on the nonenzymatic sensing of cholesterol. Two different approaches the direct and the indirect electrooxidation of cholesterol on electrode surface are adopted for this. The direct electrooxidation and the fabrication of amperometric sensor for cholesterol on platinum nanoparticles decorated on macroporous gold structures were reported [314]. The sensor detects cholesterol in phosphate buffer saline medium. Porous tubular silver nanostructures were reported to detect cholesterol in basic medium with good linear range [315]. Layer by layer deposited platinum nanoparticles on multi walled carbon nanotubes sense cholesterol in neutral pH with good sensitivity and selectivity [316]. Nonenzymatic determination of cholesterol on β-cyclodextrin functionalized graphene is developed by Agnihothri et al. [317]. Carbon nanotubes synthesized from coconut oil and its application on nonenzymatic cholesterol determination was reported by Saha and Das [318]. The application of Cu$_2$S nanoplates for the sensing of cholesterol is explored by Ji et al. [319]. Nanographitic electrode is used for the nonenzymatic cholesterol detection in basic medium by Bhavana et al. with wide detection range [320].

Since the current work is oriented towards nonenzymatic electrochemical detection of cholesterol that involves electrochemical oxidation, a brief overview of the electrooxidation reaction of cholesterol and the products formed is given here. Chemically cholesterol is a homoallylic alcohol with a relatively high hydrophobic moiety. The oxidation sites on cholesterol are dependent on the reaction conditions. The electrooxidation of cholesterol in nonaqueous media was well studied by Kusu et al. [321]. On glassy carbon electrode in acetonitrile/2-propanol medium electrooxidation was carried out and found that the oxidation proceeds via four electrons and four protons to form cholesta-4,6-diene-3-one which indicates that the
oxidation site is the 3β-hydroxyl group [322,323]. On platinum electrode in acetonitrile medium cholesterol oxidizes to cholestenone in the presence of KBr and the reaction is mediated by bromide ions. The oxidation mechanism involves the electrochemical oxidation of Br− to neutral Br by one electron process which further oxidizes to cationic Br+ upon the elimination of another electron. The Br+ acting as the electrocatalyst and the cholesterol oxidizes liberating neutral Br which can further oxidize to Br+ species [324,325]. A schematic representation of the process is given below.

**Scheme 1.7.** Indirect electrooxidation of cholesterol in presence of bromide ions in acetonitrile electrolyte.

In glacial acetic acid the oxidation of cholesterol occurs at the allylic position yielding 7α- and 7β-acetoxy derivatives of cholesterol [326]. Cholesterol oxidation in dichloromethane produces chlorinated compounds followed by one electron oxidation of oxygen atom in C-3 position produces a carbocation. This carbocation reacts with the medium to generate different products [327].

Electrochemical oxidation of cholesterol is also mediated by dioxygen compounds. The most common compounds used are Mn(III/IV) porphyrin-O₂ [328] and Ti(II/III) hematoporphyrin-O₂. The oxidation occurs at C-25 side chain to form tertiary alcohols [329]. Oxidation using Br−/BrO− leads to the cleavage of C5-C6 double bond and also at the allylic C₇ position [330]. Cholesterol acetate oxidation with dioxygen and iron picolinate leads to 7-hydroxy products [331]. Cholesterol oxidation on carbon electrode in acetonitrile produces cholesta-4,6-dien-3-one [323,332]. Figure 1.4 shows the different oxidation sites on cholesterol as the 3β, 5-6 double bond, 7-allyl and side chain C₂₅ [333]. The advances in electrochemical
oxidation of cholesterol by different means and the product formed were reviewed [334,335].

![Figure 1.4: Different oxidation sites on cholesterol (3, 5, 6, 7 and 25 carbon atoms).](image)

1.6 Screen printed carbon electrodes

Screen printing (thick film printing) is a low cost microfabrication technology used for the large scale production of disposable sensors with reproducible results. These sensors showed numerous real life applications, especially in amperometric sensors which are commercialized for glucose and metal ions. Screen printing involves spreading a thixotropic fluid (ink) evenly across a mesh screen which defines the shape and size of the desired electrode via a squeegee. Carbon inks are mainly used since they provide a wide potential window, low cost and low background currents. The electrode fabrication is done by serially printing different inks such as carbon (contains graphite particles, polymer binder and other additives), metal inks of silver or platinum. They are printed on hydrophilic plastic materials such as polyethylene terephthalate. Silver based ink is used as reference electrode [336,337]. This manufacturing process is already employed for the manufacture of capacitors, resistors and conductors used in hybrid electronic circuits, multilayer patterns deposited are repeatable in thickness and geometry [338].

A typical SPE for electrochemical sensors consists of a chemically inert substrate on which three electrodes, namely the working electrode (WE), reference
electrode (RE) and counter electrode (CE), are printed. The working electrode is the main electrode which provides a platform on which the analyte reacts. This is surrounded by the counter and reference electrode to complete the cell. SPEs are comparable with glassy carbon electrode in its performance, are less expensive and also do not require pretreatment.

1.6.1 Biosensors with screen printed electrodes

Large number of electrochemical sensors is reported in literature using screen printed electrodes. Biosensors for glucose and cholesterol are already available in the market for point of care diagnosis. Enzyme incorporation on SPE surface using different methods and their biosensor application was well reviewed [339]. Development of immunosensors using SPE is well reviewed recently by Kalyan et al., and also explains the advantages of the sensors over traditional counterparts [340]. Nanomolar concentrations of progesterone detection using screen printed carbon electrode is performed by Hart et al. [341]. Simultaneous detection of hydroquinone and catechol on prussian blue modified SPE was reported by Buliendra et al. [342]. Enzymatic glucose sensor with prussian blue modified SPE was developed by Chandra Sekar et al. [343]. Lactate determination in saliva was reported with disposable screen printed electrodes by Claver et al. [344]. CuO and graphene modified SPE was developed for nonenzymatic sensing of glucose with high sensitivity [142]. Carbon nanotubes and Cytochrome 450 scc modified SPE were used as cholesterol sensor with good detection range [345]. The application of SPE for environmental analysis was well reviewed [346]. Shuriken like Cu$_2$O on SPE was used as a nonenzymatic glucose sensor [108]. Enzymatic determination of lactate in wine was reported with modified SPE [347]. Our group has reported nonenzymatic glucose sensor using Pt-CuO-graphene nanocomposite modified SPE for highly sensitive and selective detection [348]. In all these cases SPE could act as good platform for the incorporation of various electrocatalysts and is very well suited for commercialization.

1.7 Objectives and scope of the work

The main objective of the present work is to develop highly sensitive, selective and wide detection range nonenzymatic electrochemical sensors for the
determination of glucose and cholesterol. Highly sensitive electrocatalysts such as NiO or CuO were used for the fabrication of glucose sensor. Since the surface area is directly related to sensitivity, nanotubular and nanoporous materials were used as the electrode material. In this study TiO$_2$ nanotube arrays and Ta$_2$O$_5$ nanoporous structures were used to enhance the electrode area. The scope of the work includes,

(i) Development of glucose sensors using,
   - Nickel oxide nanoparticles modified TiO$_2$ nanotube arrays
   - Cobalt-copper alloy nanoparticles modified TiO$_2$ nanotube arrays.
   - Copper oxide and platinum nanoparticles modified tantalum oxide honeycomb nanostructures.

(ii) Fabrication of disposable cholesterol sensors using platinum mesoparticles modified screen printed carbon electrodes.

(iii) Morphological characterization of all the modified electrodes by SEM, AFM and XRD.

(iv) Electrochemical characterization of the sensor electrodes using cyclic voltammetry and electrochemical impedance spectroscopy.

(v) Testing of the analytical performance of the sensors with glucose, cholesterol and interfering molecules.

(vi) Analysis of real samples.