1 Sensors

A sensor is a device which converts the interaction of analyte/change of environment into a readable signal. Sensors can be classified broadly into two groups. One responds to the chemical signal and another group responds to physical changes. For example, hearing is a physical change as it involves recognition of sound waves and smell involves some chemical change of organic molecules. Apart from this, sensors can also be classified as various types based on two basic components receptor and transducer. Figure 1.1 shows schematic representation of a typical sensor [Bandodkar *et al.*, 2014].

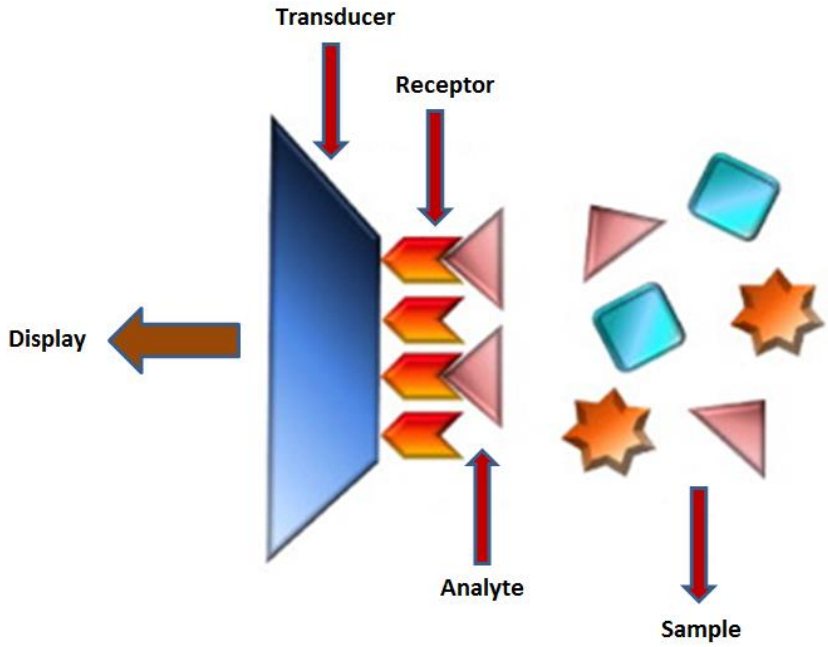


Figure 1.1 Schematic representation showing typical components of a sensor [Bandodkar *et al.*, 2014].

A receptor is an active or recognition part that has ability to interact with a particular type of analyte (makes sensor selective). There may be various types of analytes present but to make sensor selective the receptor should recognize target analyte molecules.

There may be a chemical reaction between analyte and receptor when receptor binds with analyte (typical example of enzyme-substrate reaction) or a bonding/coordination (chelating agents or ligands with analyte) or by its dimension, called steric recognition (antibody-antigen) etc. The changes occurring at the interface of receptor and analyte are perceived by transducers. Transducers are nothing but an energy converter which transforms one form of energy into another form. The changes occurring are converted by transducer into some readable signal such as voltage, current and resistance or light, stress etc. Therefore, based on types of transducers and recognition layer classification of sensors are more important as discussed under headings 1.1 and 1.2. Figure 1.2 showing that chemical information can be originated as a result of a chemical reaction occurring by the binding of receptor with analyte. Further, this information can be converted into electrical signal with the help of transducer [Catterall *et al.*, 1997].

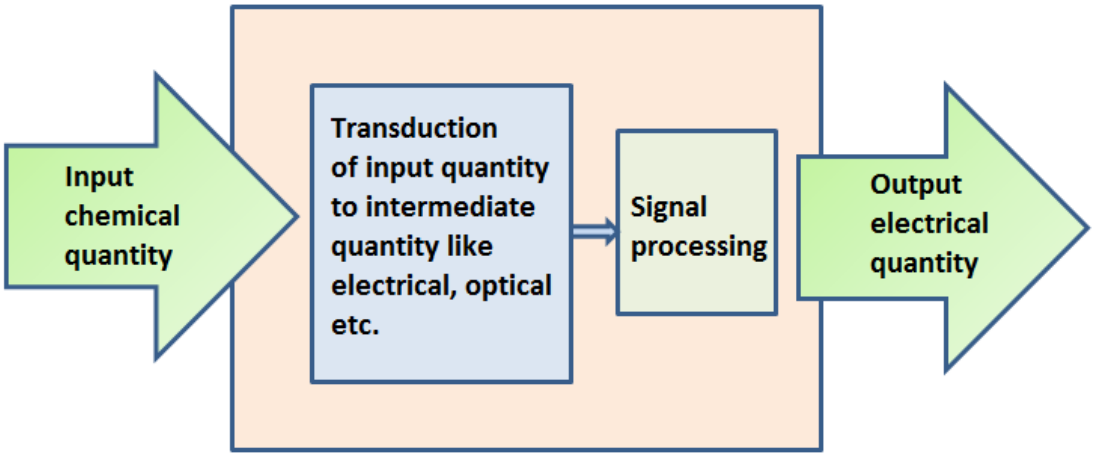


Figure 1.2 Schematic representation of a sensor showing electrical output in response to the presence of an input quantity [Catterall *et al.*, 1997].

Figure 1.3 shows classification of chemical sensors on the basis of various sensing objects like ion sensors, gas sensors, humidity sensors and biosensors. This classification is based on interaction of sensing object/ analyte with the modified

electrode.

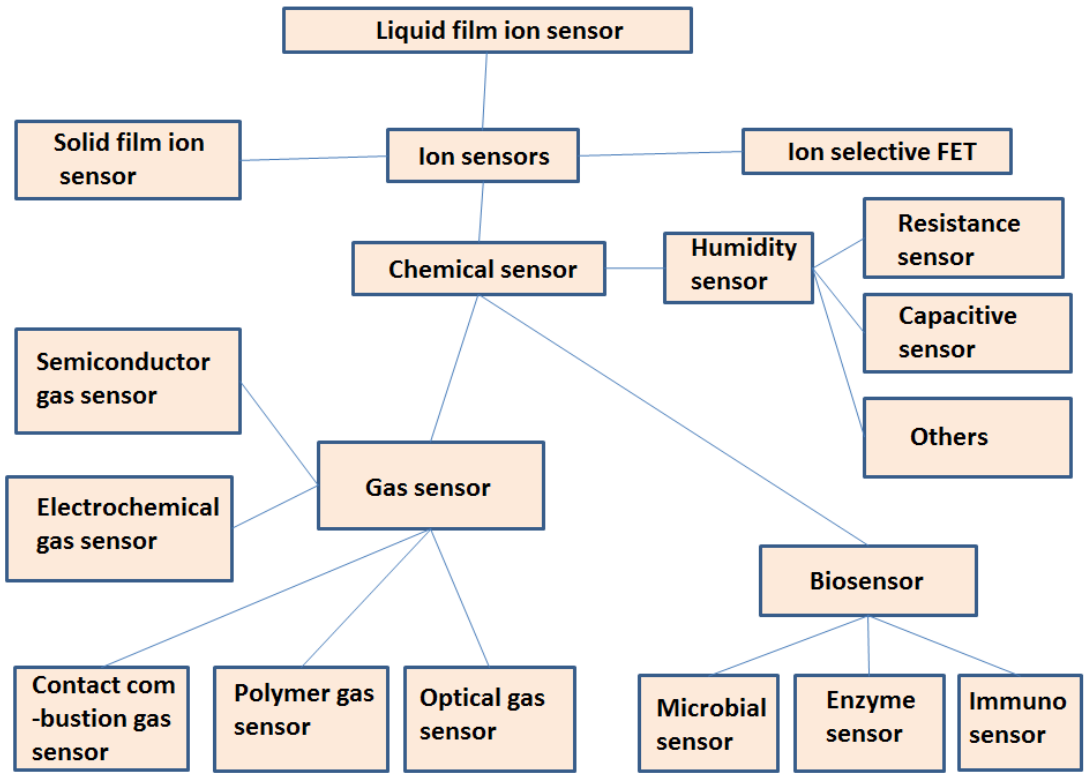


Figure 1.3 Classification of chemical sensors on the basis of sensing objects [Wen, 2016].

1.1 Types of sensors based on transducers: Sensors can be classified into various types on the basis of transducer and operational mode.

1.1.1 On the basis of operational mode: There are four types of sensors.

1.1.1.1 Optical sensors: Such type of sensors exhibits the interaction of light. Light consists of various components like wavelength, phase, polarization, intensity when these components interact with matter. There is a change in various properties of light like absorbance, reflectance and luminescence.

1.1.1.2 Mass sensitive sensors: A mass sensitive sensor measures the analyte conc.

by changing its mass. For example: Quartz crystal microbalance, cantilever sensor etc. Quartz crystal microbalance is ultra- sensitive mass sensors (Figure 1.4). It consists of quartz crystal that resonates at its frequency due to piezoelectric effect. When there is addition or removal of small molecules of analyte from the surface the resonance frequency of the crystal is interrupted which leads to change in frequency observed. This change in frequency further transformed into change in mass through a physical relationship.

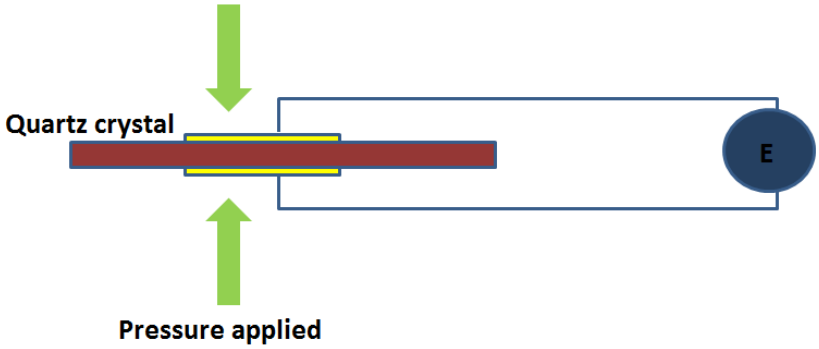


Figure 1.4 Piezoelectric effect: a pressure exercised on a quartz crystal results in an electric field.

1.1.1.3 Electrical/Electrochemical sensor: Some chemical information like concentration of a sample or nature of analyte is converted into an analytically important signal like a change of current, resistance or voltage.

It represents the largest group of chemical sensors. Figure 1.5 depicts typical electrochemical sensors based on three electrode assembly, i.e., auxiliary electrode, working electrode (sensing electrode) and reference electrode. All are connected to an electrochemical analyser and are separated from each other with a thin layer of electrolyte [Rathee *et al.*, 2016].

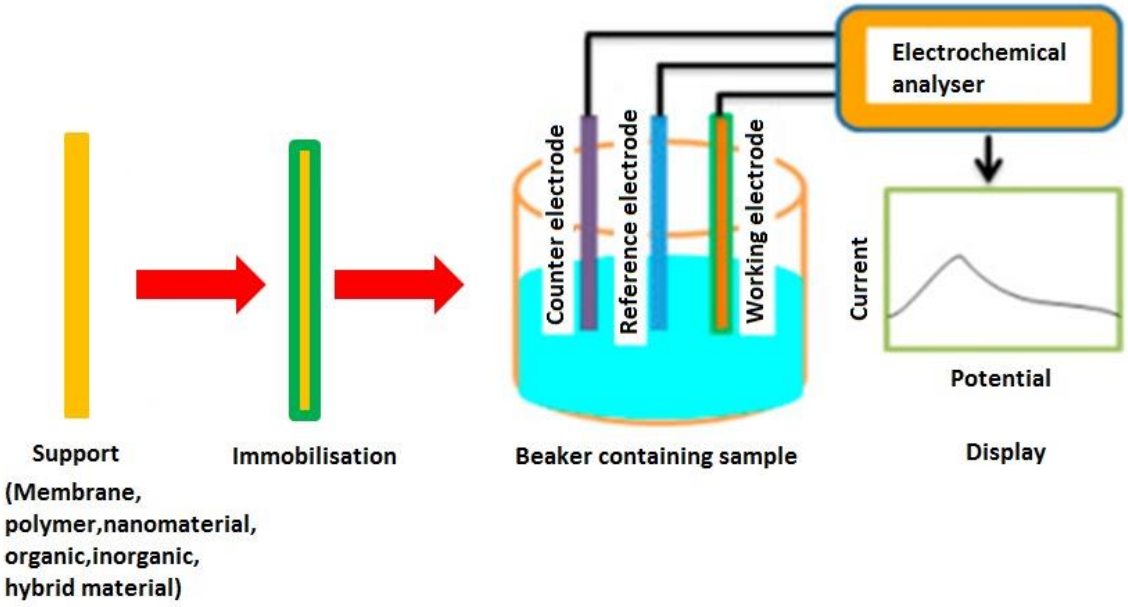


Figure 1.5 Schematic representation of electrochemical cell showing different components [Rathee *et al.*, 2016].

The nature of electrolyte depends on the chemical nature of electro-active species present in the solution for detection, i.e., analyte. There are three types of electrochemical sensors, i.e., potentiometric, amperometric and conductometric sensors. Such type of sensors offer high sensitivity, low cost, portability, low detection limit, selectivity, etc. Due to above reasons, in present time, electrochemical sensors got much attention over other types of sensors.

1.2 Classification based on recognition layer: There are various types of recognition process which follows the equation:

$$\textbf{(Sample) A + (Sensing element) R} \rightleftharpoons \textbf{P (Product)}$$

Double sided arrow confirms the reversibility of the reaction process at equilibrium. Reversibility arises due to non-covalent interactions in product such as hydrogen bonding, ionic bonds and Vander Waals interactions.

$$K_A = \frac{C_P}{C_A C_R}$$

Here, C is the concentration of different species indicated by its subscript. Equilibrium constant shows the affinity interaction between the recognition elements with analyte. Higher the value of equilibrium constant greater will be the interaction between recognition layer and analyte. When sensor response depends on the concentration of products then, response will be defined by the initial concentration. Recognition process is highly selective, i.e., it is based on the interaction between recognition layer and analyte not with any other third interfering species [Banica *et al*., 2012].

There are various types of recognition process which are as follows:

1.2.1 Ion recognition: These are the first chemical sensors developed on large scale. pH glass electrodes were first ion sensors developed based on famous work of F. Haber and Z. Klemensiewicz. Electric charge is the basis of ion recognition. Various types of material having electric charge opposite to analyte are suitable for ion recognition. Selectivity in ion recognition can be developed by some other factors like size of the receptor and partial covalent nature of analyte-receptor bond.

1.2.2 Recognition by affinity interactions: It involves the reversible binding of analyte and receptor through non covalent bonds, hydrogen bonding, and Vander Waals force of attractions. The product formed is denoted by molecular association complex. Both the species must be in complementary with respect to shape, size and their reactivity. The strength of the complex formed depends on its stability constant. Such type of interactions is mostly common in biosensors. For example: antigen antibody interactions. Antibodies are glycoprotein's that are produced by our immune system to

protect our bodies from microorganisms like bacteria and viruses. The specific part of microorganism which interacts with antibodies is called antigen. This interaction is also named as immunochemical reactions. Such reactions are the basis of diagnostic tests in clinical laboratories.

1.2.3 Recognition by nucleic acids: Nucleic acids (DNA and RNA) are the basis of storage and transfer of genetic information within the cells. In DNA there are four nucleoside bases are present: Adenine, Guanine, Thymine and Cytosine. In RNA, Thymine is replaced by Uracil. There is hydrogen bonding type interactions between complementary nucleobases. So, complementary base pairs formed double stranded association complex through the process of hybridization which is the basis of DNA sensors.

1.2.4 Recognition by enzymes: Enzymes are biocatalysts i.e. functions as catalysts in various biological processes. Recognition process through enzyme is a dynamic process involving three main steps: the first step is the binding of enzyme and substrate species leading to the formation of enzyme substrate complex. The second step is the chemical interaction and formation of product-enzyme complex. The final step is the release of products and regeneration of enzymes. Enzyme sensors can perform various functions like determination of substrate, determination of reaction inhibitors and acts as transduction labels which are based on affinity interactions.

1.2.5 Recognition by cells and tissues of biological origin: They form a new class of recognition elements. It has been realized that tissues and cells perform better as compared to the products which are derived from them like enzymes because they are present in its native form. They can react with chemical species by changing their

metabolic processes. Change in metabolic process leads to various phenomenon like evolution of oxygen or excretion of some chemical species.

1.2.6 Gas and vapour sorption: Determination of hazardous gases is very important for controlling of air quality parameter. The recognition process of gas and vapours is based on sorption which can be possible either on surface of electrodes or within the material used for recognition. Polymeric materials, organic and inorganic materials can be used for this purpose.

1.3 Parameters of a sensor:

There are various parameters which are used for validation of a good sensor. Some important parameters are described below:

1.3.1 Accuracy: It shows the closeness of a measured value to actual value. Precision shows the reproducibility of a measurement. For a sensor to be precise as well as accurate the measurement value should be around the average value and also be clumped together.

1.3.2 Sensitivity: It is defined as the extent of change of an output signal by varying the amount of measured quantity. The sensitivity of a sensor is calculated by the slope of the calibration curve, i.e., analyte conc. *vs.* Current.

1.3.3 Limit of detection: The limit of detection of a sensor is defined as the minimum concentration of analyte that can be measured at a fixed signal to noise value. Generally, it is obtained when signal to noise value is 3. The limit of detection is equal to the three times of standard deviation of blank without adding analyte.

$$LOD = 3\,SD/S$$

1.3.4 Selectivity: This refers that a sensor can interact with a specific analyte or a group of analytes present in the reaction mixture. An ideal sensor shows interaction only with the analyte under observation and discards the effect of interfering molecules.

1.3.4 Linearity: Linearity of a sensor is defined as the relative deviation of a measured parameter from a perfect straight line. Ideal sensors always show linearity either in terms of output signal or in terms of some mathematical function like logarithm.

1.3.5 Dynamic range: It is the measurable maximum and minimum linear range of an output signal. Every sensor has a fixed minimum and maximum dynamic linear ranges over this range it can show destruction of sensing behaviour.

1.3.6 Response time: Response time of a sensor is defined as the time period taken to approach its real output value when there is step change in input value. In general, it is the total time taken for chemical interaction and conversion of chemical interaction or change into some signal by transducer. For an ideal sensor response time should be very low so that it can be easily applied for real sample analysis.

1.3.7 Resolution: Resolution of a sensor is defined as the smallest observable change in the input parameter which is clearly detectable in the output parameter.

1.3.8 Shelf life: It is the time period within which sensor remains active or shows good sensing behaviour when subjected to storage.

1.4 Advantages of electrochemical sensor: Among different types of sensors there are various advantages of electrochemical sensors. Some are as follows:

1.4.1 Electrochemical sensors are highly stable, offer low cost and show high reproducibility.

1.4.2 It exhibits low limit of detection. We can detect pico micro molar concentration using electrochemical sensors.

1.4.3 They provide very fast response as compared to other types of sensors. So, there is quick detection of analyte using electrochemical sensing devices and such types of sensors can also be used for the development of portable sensing devices.

1.4.4 Such type of sensors is highly sensitive and selective.

1.4.5 They are specific to the analyte chosen.

1.4.6 They have very large linear dynamic range as compared to other types of sensors. They can also be used for the detection of analyte in real samples also.

1.5 Generation of electrochemical sensors:

Sensors can be categorised on the basis of mode of transfer of electrons, intimacy between recognition layer and transducer, Type of the recognition event etc. into three generations which are described below:

1.5.1 First generation electrochemical sensors:

First generation electrochemical sensor consists of a recognition layer and transducer which are well separated from each other e.g. Determination of glucose using Glucose oxidase. Figure 1.6 describes the first generation electrochemical sensor/biosensor.

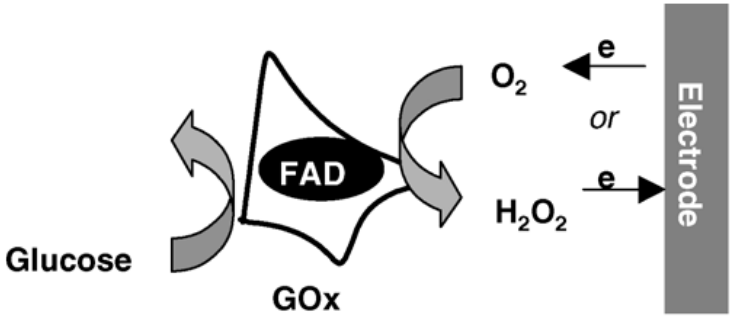


Figure 1.6 Schematic representation showing first generation glucose sensors [Clark *et al.*, 1962]

Oxygen is an electron mediator between glucose oxidase and the surface of electrode. In the presence of glucose GOx reduces O_2 into H_2O_2. First generation glucose sensors has drawback that it suffers from interference of oxygen or other species which are electro-active.

1.5.2 Second generation electrochemical sensors: In second generation electrochemical sensor transducer and recognition layer are in direct contact with the help of some mediator. Figure 1.7 describes a typical second generation electrochemical sensor which uses an artificial mediator molecule for facilitating the process of electron transfer [Cass *et al.*, 1984; Carr *et al.*, 1984] e.g., Glucometer used in home for checking the blood glucose level every day. It utilises FAD as an artificial mediator and functions by the following equations:

$$C_6H_{12}O_6 + (FAD) + H_2O \rightarrow C_6H_{12}O_7 + GOx\ (FADH_2) \quad 1.1$$

$$GOx\ (FADH_2) + 2\ Mox \rightarrow GOx\ (FAD) + 2\ M_{red} + 2\ H^+ \quad 1.2$$

$$2\ M_{red} \rightarrow 2\ M_{Ox} + 2\ e^- \quad 1.3$$

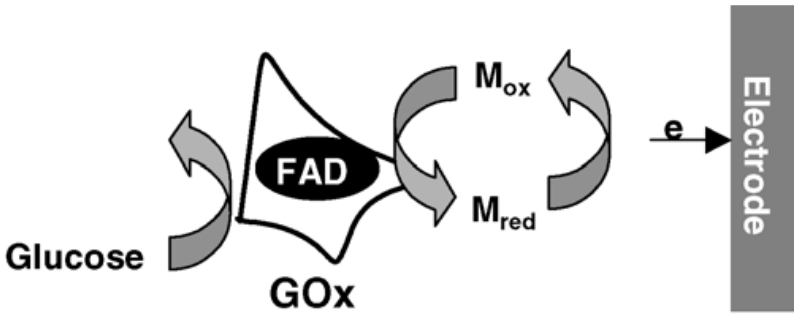


Figure 1.7 Schematic representation of glucose sensor from second generation [Carr *et al.*, 1980; Cass *et al.*, 1984].

The function of artificial mediator is to facilitate fast electron transfer by shuttling electrons in between electrode surface and enzyme. There are various types of artificial mediator which are frequently used like hydroquinone, ferrocene, feeri/ferro cyanide and several organic dyes.

1.5.3 Third generation electrochemical sensors: Such type of sensor doesn't utilise any mediator, there is a direct transfer of electrons between the recognition layer and transducer. The advantages of such type of sensors are the removal of interfering species like oxygen or other electro-active species. Degani and Heller recognized the transfer of electrons directly from GOx to the electrode by covalently tethering a number of electron-relaying centers to the enzyme [Dagini *et al.,* 1987, Heller, 1990]. Willner and co-workers, as illustrated in Figure 1.8 describes a third generation electrochemical sensor in which attached flavin adenine dinucleotide (FAD) to the self-assembled monolayer of electron relays (i.e., pyrroloquinoline quinone, PQQ) on the electrode, and then reconstituted the GOx [Willner *et al.,* 1996]. The third generation electrochemical sensor also suffers from various drawbacks which can be improved [Park *et al.,* 2006]. Mostly work investigated belongs to first and second generation

sensors. In the present time, nanomaterials based platform is being utilised for improving various sensing parameters like high sensitivity, selectivity, low limit of detection and wide linear response range etc.

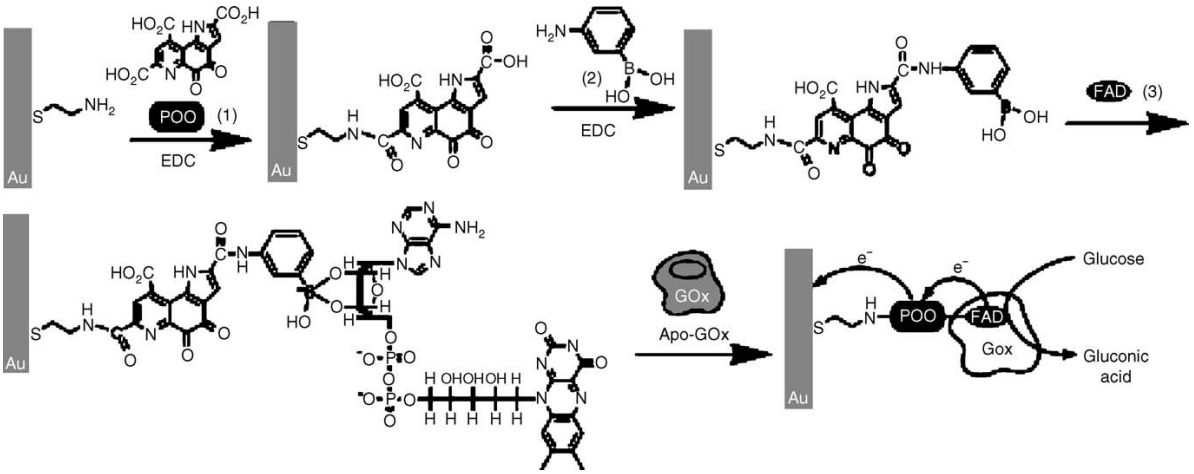


Figure 1.8 Schematic representations of third generation glucose sensors [Willner *et al.*, 1996].

1.6 Need of nanomaterials for electrochemical sensors: When nano word is coupled with the unit "meter" originates the word nanometer, it indicates a spatial unit that is one billionth of a meter or in the range of 1 nm – 100 nm. Nanomaterials includes a long list of nanostructured platforms like metal nanoparticles, nanowire, nanotubes, nanofibers, nanorods, nanocomposite, nanostructured polymers and various allotropes of carbon like carbon nanotubes, graphene, fullerenes etc. as shown in Figure 1.9 [Faraji *et al.*, 2009; Sambyal *et al.*, 2018]. The immense interest in nanomaterials for sensing applications is arising due to its various significant unique properties which are listed as follows:

1.6.1 Nanomaterials have large surface area to volume ratio that is highly useful for the absorption of analytes or in catalytic reactions as large area can be provided in less volume.

1.6.2 The ease of the modification of recognition layer with cost-effective manner.

1.6.3 Low noise level in sensors due to ability to tune the nanoparticles size and morphology

1.6.4 Ease of processing as they are generally soluble in organic solvents or can be dispersed easily so that it can be applied on electrode surface for modification.

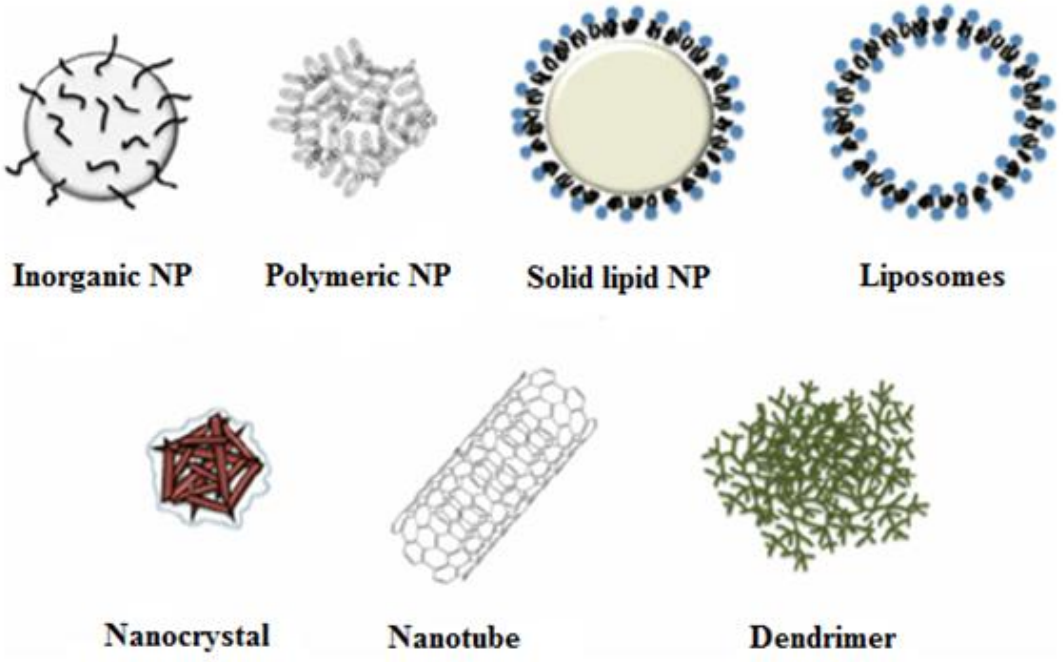


Figure 1.9 Different types of nanoparticles used for different applications [Faraji *et al*., 2009].

1.6.5 Increase air stability, long shelf life as well as long operational life.

1.6.6 Nanomaterials with capping agents and without capping agents are having different electronic and optical properties that are useful for sensing applications.

1.7 Functions of nanomaterials/ nanoparticles: The uniqueness of chemical, physical and optical properties of nanoparticles establish them highly suitable for designing new devices related to chemical sensing and biological sensing. There are various types of nanoparticles available with different sizes and compositions and are highly useful for electrochemical analysis. Different types of nanoparticles perform different functions and sometimes same nanoparticles perform different type of functions like DNA sensor, immunosensors and enzyme sensors [Katz *et al.*, 2004; Wang *et al.*, 2003]. The different functions of nanoparticles can be categorised into five groups like Immobilisation of biological molecules, catalysis of electrochemical reactions, enhancement of electron transfer, labelling molecules and acting as reactant.

1.7.1 Immobilisation of biological molecules: Due to large surface area to volume ratio and highly energetic surface of nanoparticles, biological molecules can easily adsorbed on its surface strongly and plays a vital role in the construction of biosensors. When biological molecules adhere on the surfaces of bulk materials, there is possibility of denaturation and loss of biological activity of biological molecules, but when they get adsorbed on the nanomaterials surface due to their biocompatibility they retain their biological activity. Nanoparticles bear surface charge, they can electro statically interact with nanoparticles and also can interact by some other interactions. For this purpose, metal nanoparticles are generally used. For example**:** Iron nanoparticles are used for the immobilisation of protein molecule [Xu *et al.*, 2014] as shown in Figure 1.10. Generally, for this purpose gold nanoparticles are used [Liu *et al*., 2003].

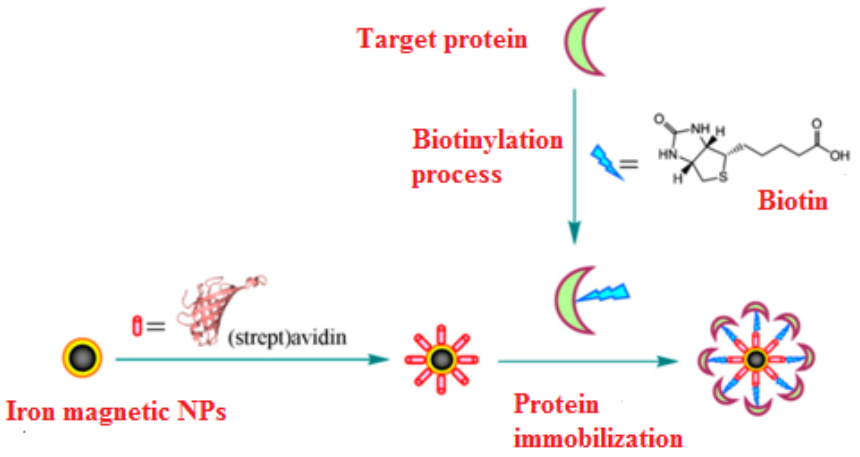


Figure 1.10 Schematic representation showing protein immobilisation onto Fe NPs utilising (strept) avidin-biotin technology [Xu *et al.*, 2014].

1.7.2 Catalysis of electrochemical reactions: Metal nanoparticles exhibit excellent catalytic properties. The introduction of metal nanoparticles into electrochemical sensors and biosensors decreases the over potential of various analytically important reactions. They can also change the irreversibility of some common chemical reactions which are irreversible at the surface of unmodified electrode. Ohsaka and coworkers developed a highly selective electrochemical sensor based on selective determination of dopamine in presence of ascorbic acid, and it is based on the catalytic activity of gold nanoparticles reducing the overpotential of ascorbic acid [Raj *et al.*, 2003]. Q. Geng and co-workers reported the selective reduction of 4-nitrophenol which was catalysed by silver nanoparticles supported on polymeric micelles and vesicles [Geng *et al.*, 2014] as shown in Figure 1.11. Such type of nanoparticles are showing higher dispersibility, higher stability and excellent catalytic effect at a very low concentration of 4-nitrophenol, i.e., 1 ppm. Such types of sensors are highly selective as well as sensitive.

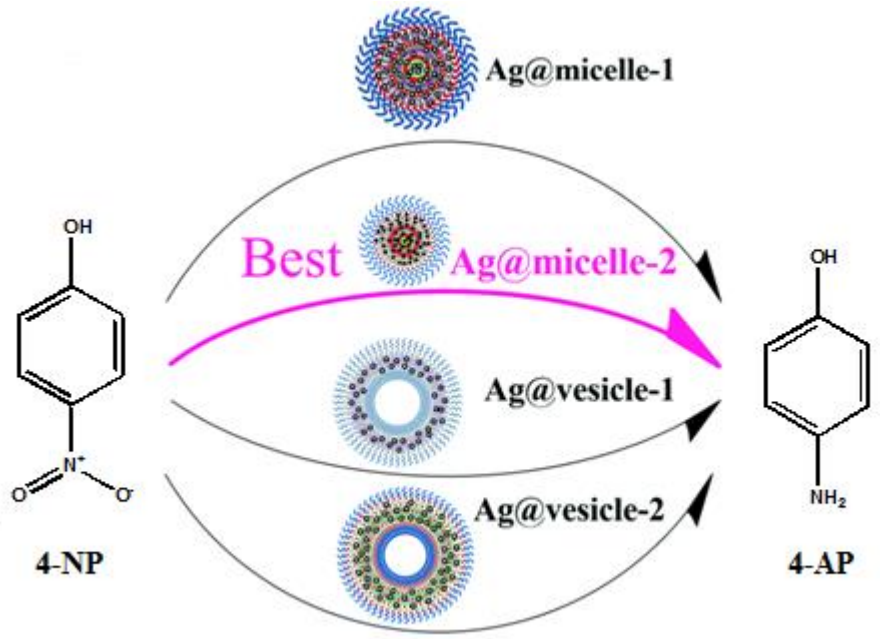


Figure 1.11 Reduction of 4-nitrophenol to 4-aminophenol catalyzed by Ag NPs supported on polymeric micelles and vesicles matrix [Geng *et al.*, 2014].

1.7.3 Enhancement of electron transfer reactions: Highly conducting nature with tiny dimensions of metal nanoparticles made them suitable candidate for increasing the electron transfer process between the active sites of enzymes and electrode surfaces acting as electron transfer mediators. H. Razzaq and co-workers reported that binding of gold nanoclusters incorporating anthraquinone (AQ) redox molecule with dithiol self assembled monolayer (Figure 1.12). The incorporation of nanoclusters of gold showed fast electrical communication behaviour between the redox molecule and electrode resulting into the fast electron transfer behaviour between AQ and Au electrode surface [Razzaq *et al.*, 2014].

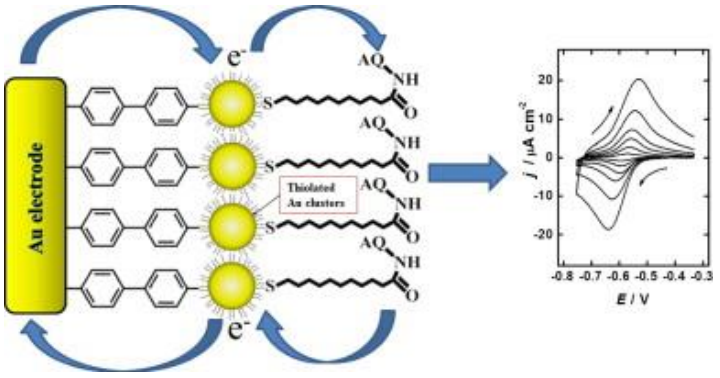


Figure 1.12 Schematic representation showing binding of gold nanoclusters incorporating anthraquinone redox molecule with dithiol self assembled monolayer [Razzaq *et al.,* 2014].

1.7.4 Acting as labelling molecules: For the development of sensitive electrochemical sensors and biosensors labelling of certain biomolecules like antigen, antibodies, and DNA by using metal nanoparticles plays a significant role. Biological molecules remain intact with metal nanoparticles can preserve their bioactivity and has ability to interact with their counterparts. On the basis of concentration of metal nanoparticles we can determine the concentration of biomolecules by using some suitable electro-analytical techniques like stripping voltammetry. For this purpose, we generally choose semiconductor nanoparticles (CdS, PbS), Ag and Au nanoparticles. So, there is indirect detection of biomolecules with high sensitivity due to very small size and modifiability of nanoparticles. N. Jhu. and co-workers reported enhanced electrochemical DNA sensor using platinum nanoparticles and MWCNTs dispersion in nafion for fabrication of glassy carbon electrode as displayed in Figure 1.13. Use of platinum nanoparticles enhanced the process of amplification by many times. The limit of detection is 1.0×10^{-11} M [Jhu *et al.,* 2014].

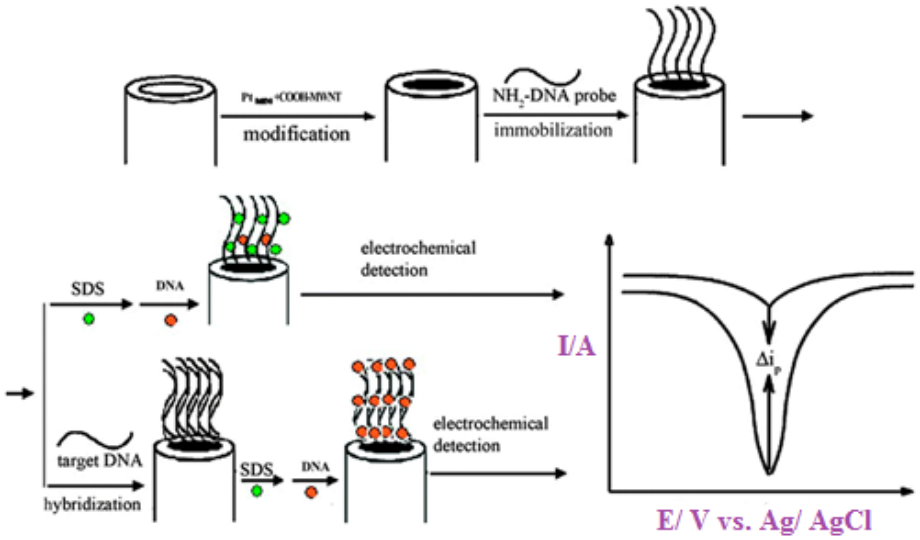


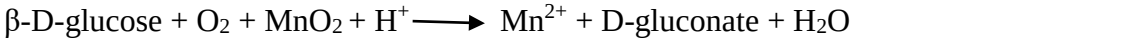
Figure 1.13 Schematic representation of the electrochemical detection of DNA hybridization based on platinum NPs combined with MWNTs [Jhu *et al.*, 2014]..

1.7.5 As a reactant: Nanomaterials are highly reactive due to their large surface energy as compared to its bulk counterpart. Such type of nanoparticles can provide new response mechanism for the detection of biomolecules. For example: MnO_2 nanoparticles. Based on highly reactive nature of MnO_2 nanoparticles Chen and co-workers has developed various electro-analytical systems [Xu *et al.*, 2005, Luo *et al.*, 2004]. Glucose oxidase (GOD) and MnO_2 NPs were immobilised on the gate of an ion field effect transistor (ISFET) and developed glucose biosensor is highly sensitive towards pH change with increasing concentration of glucose at the sensitive membrane. Here the driving force for pH change is based on the chemical reaction between MnO_2 NPs and H_2O_2 [Luo *et al.*, 2004]. It is not based on the pH changes occurring within the cells as a result of gluconic acid formation.

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{GOD} \text{D-gluconate} + H_2O_2 + H^+$$

$$MnO_2 + H_2O_2 + 2H^+ \longrightarrow Mn^{2+} + 2H_2O + O_2$$

Total reaction in the proposed glucose is



$$\beta\text{-D-glucose} + O_2 + MnO_2 + H^+ \longrightarrow Mn^{2+} + \text{D-gluconate} + H_2O$$

1.8 Convenient methods applied for chemical modification of electrode surface:

Nanomaterials have various unique properties useful for various purposes due to this reason they are used for modifying the electrode surface. When the surface of an electrode is dipped into a solution, the chemical species present in the solution get absorbed and form a layer over the electrode surface. Controlled and intentionally modified electrode surface can develop electrodes with new and desirable properties that can be the basis of various new applications in electrochemistry and novel devices. There are various techniques utilized for the modification of electrode surface which are as follows:

1.9.1 Dip coating: This technique consists of immersing bare electrode surface into the electro-active nanomaterials with desired functionalities for sufficient time that allows the formation of spontaneous film at electrode surface via chemisorptions. Further, chemically modified electrode surface is washed with desired pH for removing impurities.

1.9.2 Solvent evaporation: Fine droplets of electro-active hybrid nanomaterials are drop cast over the electrode surface and solvent is allowed to evaporate slowly. It is the most commonly used method to fabricate the electrode surface and maintain the desired thickness of nanomaterials over the electrode surface.

1.9.3 Spin coating: Spin coating or spin casting is a technique which allows depositing uniform film formation on bare electrode surface. A small amount of dilute solution of desired nanomaterials is applied at the centre of substrate which is kept on rotation so that excess solvent spin off from the edges and the remaining nanomaterials spread uniformly and allowed to dry. By this technique, multiple layers of nanomaterials film can be uniformly deposited until we get the desired thickness. By this technique, pinhole free thin films can be easily produced which is highly stable or long lasting.

After chemically modifying the surface of electrodes it can be characterized by various ways including spectroscopic measurements and electrochemical measurements to know the thickness of nanomaterials deposited, conductivity, electron transfer process occurring at the electrode surface, morphology and porosity of layers deposited [Chaki *et al*., 2002; Raj *et al*., 2007].

1.9 Literature survey on nanomaterials based sensors:

Nanomaterials play vital role in the development of sensing devices as they perform various types of functions as described above due to their extremely small size, peculiar structure, and large surface area to volume ratio. Electrodes utilized in an electrochemical measurement can be chemically modified with appropriate nanomaterials so that the target compounds will selectively adsorb onto and enrich the chemically modified surface, making ultra-trace detection possible. Electrochemical detection has several advantages over other methods in that the instrument is simple and suitable for constructing inexpensive and portable detectors [Zacco *et al*, 2006].

H. X. Zhang and co-workers reported the ultra-trace electrochemical detection of nitroaromatic (NACs) compounds using mesoporous MCM-41- and SiO_2 nanosphere-modified glassy carbon electrodes in 0.5 N NaCl solutions using cathodic voltammetry [Zhang *et al.*, 2006]. The voltammograms are recorded in 0.5 M NaCl solution containing different concentrations of NACs. TNT and TNB are showing three well defined reduction peak corresponding to reduction of three nitro groups into amine whereas two reduction peak are obtained in case of DNB and DNT showing stepwise reduction of two nitro groups to the corresponding amine.

A. Salimi *et al.* reported electrochemical determination of As^{3+} using cobalt oxide nanoparticles modified GCE by CV. Arsenic exists in various oxidation states; As^{3+} is the most toxic form of arsenic [Salimi *et al.*, 2008]. It can cause bladder, lung, liver and kidney cancer and several skin diseases like hyperkeratosis, and pigmentation alterations [Chatterjee *et al.*, 1995]. Due to low cost, easy operation, electro-catalysis through various redox mediators electrochemical detection is a powerful technique for the detection of As^{3+} at ultra-trace level. In this case, cobalt oxide nanoparticles are electrodeposited at the surface of glassy carbon electrode and used for the sensing of As^{3+} by CV in wide concentration range (from 10 µM to 0.3 mM). The limit of detection is found to be 0.6 µM. The modified electrode surface is highly electro-catalytic towards detection of As^{3+} in real samples in presence of different reducing compounds without any interference.

H. Zhang *et al.* reported electrochemical detection of hydrazine using electrospun palladium nanoparticles/carbon nanofibers modified electrode. Hydrazine is one of the most hazardous environmental pollutants due to its high toxicity and irritation. It is a carcinogenic and mutagenic substance and showing adverse health especially on the

central nervous system [Garrod *et al*., 2005]. So, sensitive detection of hydrazine is highly important. For this purpose, palladium nanoparticles/ carbon nanofibers (Pd/ CNFs) are synthesized by electro spinning technique and thermal treatments. The electro-catalytic effect of Pd/ CNFs modified GCE was investigated by CV and further employed for the detection of hydrazine. A well defined oxidation peak potential obtained at 0.32 V (*vs.* Ag/AgCl). The developed sensor showed wide linear range 10 µM to 4000 µM, low LOD (2.9 µM) and better reproducibility (RSD = 1.58%) [Zhang *et al.,* 2009].

M. M. Adarkani *et al.* reported simultaneous and selective estimation of norepinephrine (NE), acetaminophen (AM) and folic acid (FA) using ZrO_2 modified carbon paste electrode through DPV technique. Due to high catalytic effect and highly selective nature of ZrO_2 nanoparticles, modified electrode detects an ultra trace level of NE, AM and FA independently in the mixture solution [Ardakani *et al.,* 2010].

N. F. Atta reported electro-sensing of morphine using gold nanoparticles (Au NPs) modified graphite paste electrode by CV and DPV techniques. Morphine is a potent analgesic generally given to the patients who are suffering from acute or severe pain. Morphine gives an anodic peak response at pH 2 in BR buffer corresponding to the oxidation of tertiary amine group into pseudo morphine as the main product. When oxidation behaviour of morphine is checked using electrodeposited Au NPs modified electrode, there is shifting of oxidation potential towards less positive potential side due to enhancement of electron transfer processes, larger surface area and high electro-catalytic effect of Au NPs. Further, modified electrode is used for the electrochemical sensing of morphine by DPV in BR buffer at pH 7.4. Real sample analysis is performed

by taking human urine sample and spiked with different concentration of buffer [Atta *el al.*, 2011].

M. H. Mashhadizadeh and co-workers developed a potential sensor for the determination of Thioridazine (TR) and Olanzapine (OLZ) using carbon paste electrode modified with ZnS nanoparticles. These drugs are used for the treatment of schizophrenia and the control of mania and agitation. It is also practised for the short-term medication of adults with major depression who have varying degrees of associated anxiety. It may be employed in the direction of anxiety conditions, children who have behaviour problems. Olanzapine (OLZ) is a neuroleptic drug for curing schizophrenia, effective against both positive and negative symptoms of schizophrenia. For the treatment of severe forms of schizophrenia often two or more antipsychotic agents are given in combination to achieve sufficient control of psychotic symptoms. Such types of drug are generally associated with sudden death investigation. So, simultaneous detection of these drugs is very important. Electrochemical sensors in which electrodes are fabricated with metal nanoparticles has got much attention due to its high sensitivity, fast enhanced current response and improved reproducibility. M. H. Mashhadizadeh and coworkers used ZnS nanoparticles for modifying the surface of carbon paste electrode and detected both the drugs simultaneously in PBS at pH 7 by DPV. The results obtained showed that both the drugs are simultaneously detected at potentials of 0.2 V and 0.625 V respectively corresponding to the oxidation of OLZ and TR respectively. The modified electrode surface is highly catalytic as well as regenerating and exhibited high sensitivity towards the detection of both the drugs in pharmaceutical tablets and urine samples [Mashhadizadeh *et al*., 2012].

F. Zhang *et al.* studied electrochemical determination of anti-HIV drug Nevirapine (NVP) using Uracil modified carbon paste electrode. NVP is a non-nucleoside reverse transcriptase inhibitor licensed for the treatment of AIDS [Clercq *et al.,* 2009, Alcaro *et al.* 2011]. The function of Nevirapine is to interact with the reverse transcriptase inhibitor and bind with an allosteric site which is located at a short distance from the actual catalytic site of enzyme. In this manner, NVP prevents the enzyme from converting RNA into DNA strands [Parreira *et al.*, 2001]. But these drugs are also having various side effects. So, determination of this drug was very important and most of the detection methods are based on hanging mercury drop electrodes (HMDE).

Zhang and co-workers constructed an electrode by electro-deposition of Uracil on carbon paste electrode (CPE). The mechanism of Uracil grafting is shown in Figure 1.14. Filled CPE was immersed into 0.1 M PBS (pH 7) consists of 1 mM Uracil. Electrochemical oxidation is performed at 1.8 V just 30 minutes for modifying the surface of CPE. The modified electrode is further utilised for the electro-sensing of NVP by CV and DPV techniques. DPV response of NVP from 0.1 µM to 70 µM concentration is obtained in 0.1 M NaOH solution in a linear fashion. The limit of detection and sensitivity were found to be 0.05 µM and 2.073 $\mu AMm^{-1}cm^{-2}$ respectively. The modified electrode showed fast electron transfer kinetics, enhanced oxidation peak current and high electro-catalytic effect, high sensitivity and better reproducibility [Zhang *et al.*, 2013].

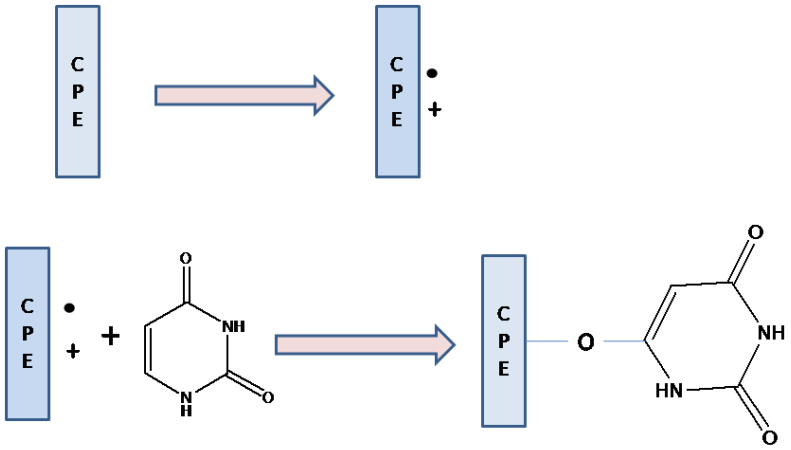


Figure 1.14 Schematic showing the electro-deposition of Uracil on CPE [Zhang *et al.*, 2013].

S. Gupta *et al.* reported electrochemical detection of a third generation antibiotics cefotaxime using GCE modified with photo-chemically synthesized silver nanoparticles which are capped by dithizone (DTZH) and its oxidation products. Figure 1.15(a) showed the reaction scheme for photochemically assisted synthesis of silver nanoparticles which are capped by DTZH and its oxidation products [Gupta *et al.*, 2014]. Figure 1.15 (b) illustrated how silver nanoparticles are stabilized in DTZH matrix and how the nucleation, growth phenomena facilitates the formation of NPs within the matrix. Further, this nanostructured platform is used for the fabrication of GCE and sensing of third generation antibiotics cefotaxime by CV and amperometry. CV response of cefotaxime drug is showing linear behaviour using modified electrode in 0.1 M Tris buffer at pH 7.2 from 30 µM to 240 µM concentration. Silver nanoparticles raise the electrode conductivity and promote fast electron transfer kinetics leading to selectivity and sensitivity towards the detection of drug.

S. Radhakrishnan *et al.* reported a sensitive electrochemical sensor for determination of nitrite using Fe_2O_3 nanoparticles decorated over reduced graphene oxide nanosheets (rGO). Nitrite detection is very crucial as it has detrimental effect on the environment as

well as on human health. The contamination of nitrite in drinking water can cause several diseases such as methemoglobinemia or blue baby syndrome and cancerous growth in stomach due to the formation of N-nitrosoamines when there is an interaction between nitrite ions and amines [Lijinsky *et al.* 1970, Nakamura *et al.* 2006, Mirvish *et al.* 1995]. They synthesized graphene oxide by using modified hammers method, and Fe_2O_3/rGO composite is synthesized by a green route, i.e., through hydrothermal method as shown in Figure 1.16.

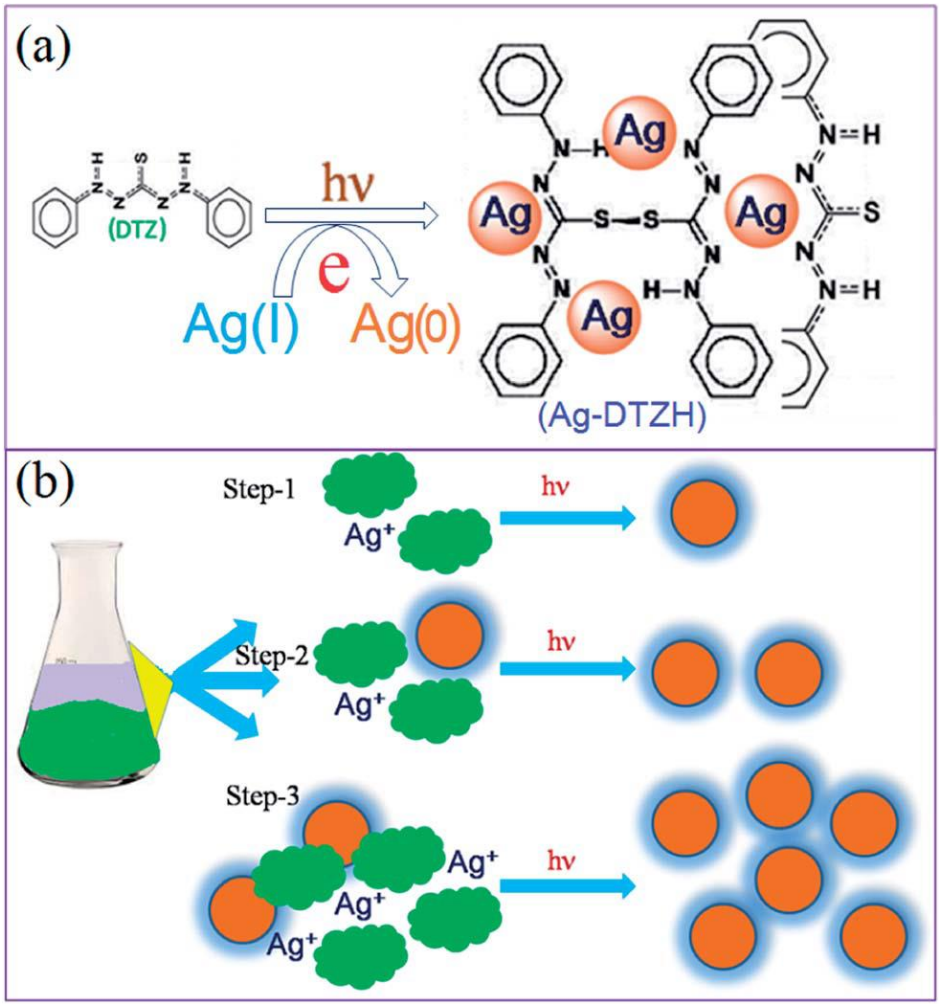


Figure 1.15 (a) Schematic representation showing photochemically assisted synthesis of silver NPs stabilized by DTZH. **(b)**Schematic showing various phenomenon occurring during photochemically assisted synthesis of Ag NPs like nucleation and growth phenomenon [Gupta *et al.*, 2014].

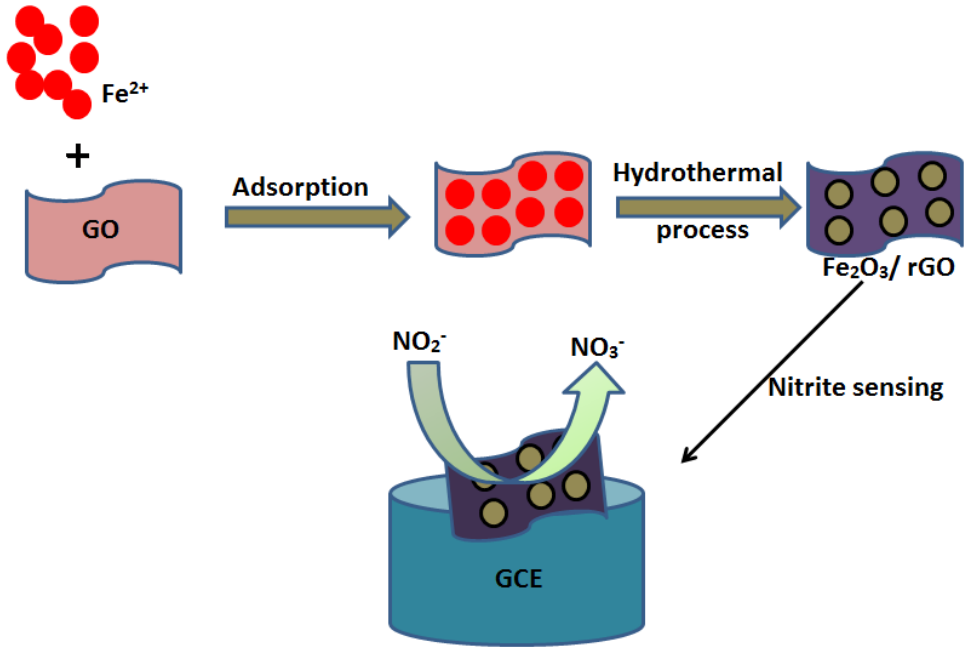


Figure 1.16 Schematic representation showing detection of nitrite using Fe_2O_3/ rGO composite [Radhakrishnan *et al.*, 2014].

Microstructure and morphology of different components involved are investigated through FESEM. GO and rGO are folded into a wrinkle shape, Fe_2O_3 nanoparticles are well decorated into the rGO matrix and Fe_2O_3 NPs are spherical in shape having dimension in between 10-100 nm. The metal nanoparticles present over conducting rGO sheet may provide better interaction between the modified electrode and analyte. Further, Fe_2O_3/ rGO composite is utilised for electrochemical determination of nitrite by DPV. DPV response showed a well defined oxidation peak at 0.05 V at scan rate of 10 mVs^{-1} for nitrite due to high catalytic effect of Fe_2O_3/rGO modified electrode. DPV current response enhanced linearly with increasing concentration of nitrite from 0.05 µM to 780 µM and 0.015 µM LOD [Radhakrishnan *et al.* 2014]. The developed sensor showed good sensitivity, selectivity, stability and reproducibility that it can be utilised for environmental analysis and food industry.

Li *et al.* reported sensitive electrochemical sensor for glucose based on anodized CuO nanowires (NW) on 3-dimensional porous copper foam (CF). CF provided high surface area due to its 3-dimensional structure of foam leading to high sensitivity and excellent electro-catalytic activity towards non-enzymatic glucose detection. CuO NWs/CF electrode shows high selectivity, good repeatability, reproducibility and stability towards detection of glucose. Prepared glucose sensor also applied for practical applications and its concentration in human serum collected from hospitals was measured and results obtained were in good agreement with the commercial available glucose sensor [Li *et al.* 2015].

S. D. Bukkitgar *et al.* presented nanostructured platform modified with 5% barium doped zinc oxide nanoparticles for determination of mefenamic acid (MFA). MFA is an anti-inflammatory, non-steroidal drug employed for the treatment of various diseased conditions like osteoarthritis, musculoskeletal illness, sports injuries and menstrual pain [Robertson *et al.*, 1980]. Consumption of MFA leads to hepatotoxicity and nephrotoxicity due to the formation of metabolite which can covalently bind and interact with tissue proteins causing immunity dysfunction. 5% Ba doped ZnO NPs were synthesized using a co-precipitation method. Further, this synthesized material is utilized for the modification of GCE and CV studies exhibited modified electrode possess high conductivity for the oxidation of MFA. Electrochemical determination of MFA is performed by DPV technique from 0.001 µM to 0.6 µM concentration with 20 nM LOD. The applicability of suggested sensor for determination of MFA was analysed in spiked human urine [Bukkitgar *et al.* 2015].

H. K. Maleh reported simultaneous determination of three anti-cancerous drugs like 6-mercaptopruine (6-MP), 6-thioguanine (6-TG) and dasatinib (DA) using Pt/MWCNTs

and 1-butyl-3-methylimidazolium hexafluorophosphate modified electrode. 6-MP is an important thiopurine drug for treatment of childhood acute lymphoblastic leukaemia [Montgomery *et al.* 1970] and inflammatory bowel disease [Shen *et al.,* 2006]. Its high concentration in human serum causes various side effects. 6-TG is another thiopurine anti-cancerous drug used for the treatment of various types of leukemia [Albertini *et al.*, 1982]. 6- MP and 6-TG are two important chemotherapeutic drugs used for the treatment of childhood acute lymphoblastic leukemia [Kuśmierek *et al.* 2009]. DA is an anti-cancerous drug employed for the treatment of prostate cancer in the trade name of sprycel [Jesus *et al.* 2015]. A major dogma of cancer therapeutics is that it utilises a combination of drugs that are used with dissimilar mechanisms of action and dissimilar toxicities [Kaur *et al.,* 2013]. Simultaneous determination of these three drugs is very significant for effective chemotherapy for cancer. Simultaneouse electrochemical detection of these drugs is performed using electrode modified with Pt/ MWCNTs and 1-butyl-3- methylimidazoleium hexafluorophosphate [Maleh *et al.,* 2016].

The main advantage of the developed sensor is that it shows well separated peak potential for the three drugs which is clear in SWV response. There is 200 mV peak potential difference between 6-MP and 6-TG and 290 mV difference between 6-TG and DA. The sensitivities calculated for different drugs in presence and absence of other compounds is almost same using Pt/MWCNTs-BMIHFP-CPE modified electrode. This investigation showed that the oxidation process on modified electrode surface is independent and can be performed simultaneously without any interference. This sensor also exhibited its potentiality in real sample analysis due to high catalytic effect of the modified electrode surface.

L. A. Arribas *et al.* described electrochemical oxidation behaviour of an anti-retroviral drug Nelfinavir (NFV) on screen printed electrode modified with the carbon nanotube. NFV is a protease inhibitor (PI). The main function of viral protease is to prevent the cleavage of the gag-pol poly protein leading to the formation of mature non-infectious virus. It is like other anti-retroviral drugs given in combinations of two or three drugs. It is the first PI utilised in paediatric formulations. Its high dose intake can cause various side effects, so its electrochemical detection was very important. For this purpose, screen printed electrodes are used due to its disposability, low cost and fast mass production [Arribus *et al.*, 2014]. The screen printed electrodes (SPE) are made up of conducting inks. The working electrode surface is modified by the dispersion of multi-walled carbon nanotubes in DMF and solvent is allowed to evaporate at room temperature overnight. Electrochemical measurement of NFV is performed using modified SPE by Square wave voltammograms. The performance of various types of SPEs is checked towards NFV determination by SWV in supporting electrolyte solution at pH 2. Different calibration curves are obtained from 10 µM to 150 µM concentration. But such electrodes are able to produce only a single calibration curve due to contamination of working electrode surface by deposition of NFV and its oxidised products [Arribus *et al.*, 2016].

Y. Wang *et al.* reported electrochemical determination of an anti-cancerous gastric drug using modified screen printed electrode in lobelia chinensis. Helicobacter pylori, which is found in stomach cause of peptic ulcer disease and gastric cancer. It is carcinogenic in nature. Apigenin is an important drug used against the treatment of Helicobacter pylori-induced gastric epithelial cell inflammation [Fox *et al.*, 2007]. This drug can be derived from a medicinal plant Lobelia *chinensis* and has great potential for inhibiting the effect

of Helicobacter pylori [Miean *et al,* 2001]. Activated bare screen printed electrodes are doped with Ni nanoparticles by electro deposition method and used for the detection of this drug in herbal plants by CV. Electrochemical determination of this drug is performed in 0.1 M BR buffer at pH 3 from 0.9 µM to 200 µM with good linearity and 5 nM LOD. The developed sensing platform is highly reliable and reproducible and can be utilised for detection of Apogenin drug clinically [Wang *et al*., 2017].

M. G. Mahmoud *et al.* reported simultaneous voltammetric determination of Acetaminophen and Isoniazid employing Bismuth oxide nanorod modified SPE sensing platforms. Hepatotoxicity means liver damage caused by the excess use of chemical substances. Acetaaminophen is a major drug utilised for relieving pain related with headache, backache, arthritis and postoperative pain [Fan et al., 2011]. It is also effective for the treatment of cough, cold and fever symptoms. Isoniazid (INH) is an antibacterial drug widely used for the prevention and treatment of tuberculosis drug due to restriction of mycobacterium stains and it is highly associated with hepatotoxicity and neuropathy [Byrd *et al.,* 1972, Kaufmann *et al*., 1978]. Some antipyretic drugs can be combined with INH to check fever during the course of medication. So, simultaneous determination of both these drug is necessary to check the chances of liver failure. For this purpose, bismuth oxide modified screen printed electrodes are used. BiO nanorods are synthesized via one pot synthesis approach through hydrothermal method. BiO modified SPE offered highly electro-catalytic surface for the significant decrease in overvoltage and improved electrochemical response for determination of APAP and INH. LOD for APAP and INH determination is calculated to be 30 $nmolL^{-1}$ and 1.85 $\mu molL^{-1}$ respectively. Further, this modified SPE nanostructured platform is used for the detection of drugs in human serum [Mahmoud *et al*., 2017].

N. G. Mphuthi *et al.* investigated electro-catalytic properties of metal oxides (MO = Fe_3O_4 and ZnO) NPs doped phthalocyanine (PC) and functionalized MWCNTs (MWCNT/ ZnO /29H, 31H-Pc), decorated on GCE towards ultra-trace detection of dopamine (DA). DA is a neurotransmitter in human brain tissue belongs to the family catecholamine. It plays a significant physiological role in proper functioning of central nervous, hormonal and cardiovascular system as a chemical messenger and in drug addiction. Abnormal concentration of DA may cause various diseases like Parkinson's disease, Huntington's disease and Alzheimer's disease where DA level in the body is found lower than in a healthy individuals. In case of schizophrenia, DA concentration is increased [Hua *et al.*, 2012, Wang *et al.*, 2012]. Therefore, it is necessary to measure DA concentration in biological fluids for clinical diagnosing. Electrochemical oxidation of DA was successfully performed on MWCNT/ZnO/29H, 31H-Pc modified GCE. The developed sensor showed good sensitivity, very low detection limit, easy electrode fabrication process, resistance to electrode fouling and excellent ability to determine DA without interference from other signals. The increased current response was obtained due to high electrical conductivity of MWCNTs, ZnO, and phthalocyanine and excellent strong super magnetic properties of Fe_3O_4 [Mphuti *et al*., 2017].

Shukla *et al.* reported the effect of aspect ratio and surface defect density on ZnO nanorods array which are hydrothermally grown over Platinum coated glass substrate and further this substrate is used for development of biosensors of glucose using CV and amperometry. Glucose biosensor with least surface defects and highest aspect ratio showing highest performance with the highest sensitivity and large linear range [Shukla *et al.*, 2017].

Mahato *et al.* investigated use of nanomaterials for bio sensing applications. Nanomaterials based biosensors are used for enhancing the performance of biosensing mechanism and helpful in the development of user friendly, robust and sensitive prototype module by providing different sensing mechanism [Mahato *et al.*, 2018]. Kumar *et al.* reported the fabrication of Pd decorated SiC nanocauliflowers (NCs) thin films for development of H_2 gas sensors. The prepared gas sensor is promising, easy and cost effective making it robust sensor for the detection of H_2 gas in harsh conditions [Kumar *et al.*, 2018].

1.10 Need of chemically modified electrode: There are various types of biologically and environmentally hazardous compounds present in nature that show no response within defined potential window at solid state electrode or required high over-potential value. Direct electrochemical detection of such compounds offers very high potential and produce very large background current leading to inferior detection of compounds. There is also high possibility of electrode surface contamination due to adsorption of large molecules and interferences due to the presence of other coexisting species. These factors directly affect the stability of the electrode. Sometimes complex sample pre-treatment is applied to eliminate such type of issues. To overcome above issues, there is need to modify the chemical nature of electrode surface or tailoring of the electrode surface. Chemically modified electrodes (CMEs) have attracted much attention over the past decades. The electrode surface can be modified by utilising nanomaterials due to its specific properties as above mentioned [Baldwin *et al.*, 1991].

1.10.1 Mechanism of electro-catalysis over CME: A significant aspect of CME is electro-catalysis of the reactions occurring at the surface of electrode of an analytically useful substrate.

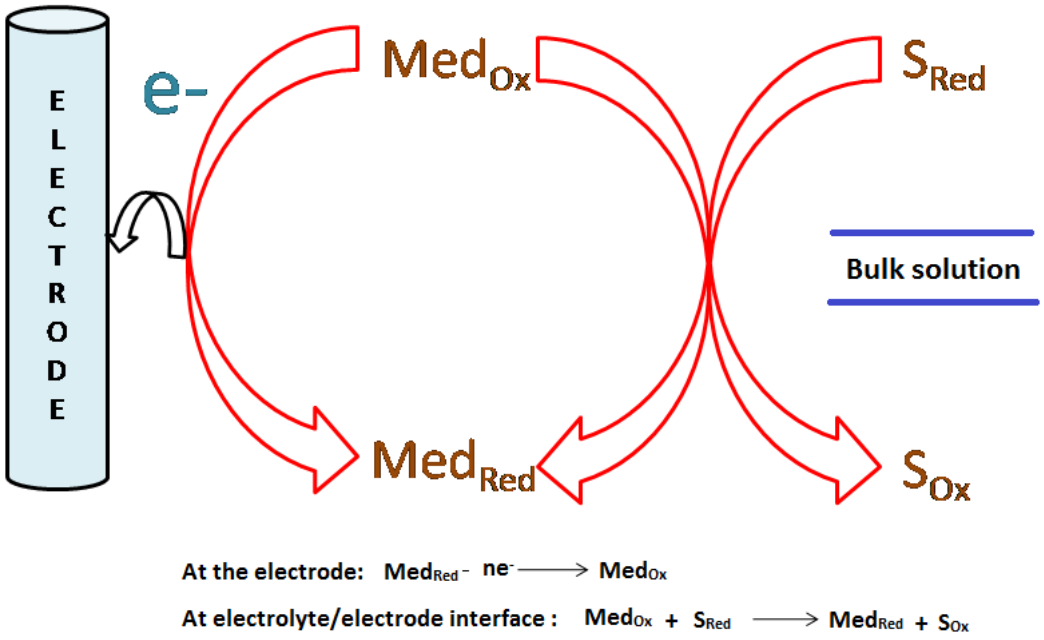


Figure 1.17 Model showing electro-catalytic reaction on CME [Ciucu *et al.*, 2014].

The various processes involved in CMEs electro-catalysis by a surface confined electron transfer mediator are clearly explained in Figure 1.17 for a generalized chemical reaction. The detailed explanations of the various electrochemical reactions occurring at nanomaterials modified electrode surfaces are as follows:

1.10.1.1 The nanomaterials modified electrodes surfaces are highly electro-catalytic causes either oxidation or reduction of analyte of interest at the redox potential of the mediator catalyst couple unless there is formation of catalyst substrate adduct.

1.10.1.2 The potential of substrate and catalyst mediator should be almost similar.

1.10.1.3 At the electro-catalytic surface of CME successful catalysed reaction of substrate S occur either at lower positive potential or negative potential for oxidation or reduction process respectively in comparison of unmodified electrode surface [Ciucu *et al.*, 2014].

1. 11 Motivation for the thesis work: In view of recent literature survey it was observed that there is urgent need for the development of sensitive, selective and low cost sensors for the hazardous chemicals especially for drugs given to patients during the treatment of critical diseases. There are various types of drugs which are employed for the treatment of life threatening diseases like cancer, AIDS or HIV infection and tuberculosis. Drugs specific to these diseases offers several adverse side effects which are not manageable. When the concentration level of such drugs increases in human serum above a certain level they can harm or affect proper functioning of certain body organs. So, there is a strong need to maintain the minimum concentration of these drugs in human serum. There are various types of techniques which are used for the detection of these drugs like high performance liquid chromatography, high performance thin layer chromatography, calorimetric, radioimmunoassay and electrochemical techniques. Electrochemical techniques are very fast, offers high sensitivity as well as portability to the developed sensing platform. There are several types of electrochemical sensors developed for the detection of such types of drugs but they involved either less sensitive and selective carbon paste electrodes or complex electrode fabrication processes. So, there is a very strong demand to develop sensitive sensors for the detection of such types of drugs which involves easy electrode fabrication procedures and also provides portability to developed sensors. Portable sensors are highly useful for continuous monitoring of concentration of drugs in human serum during medication. For this purpose Anti-HIV drugs are selected because HIV infection is still fast growing deadly diseases and it is not fully eradicated. The drugs used for the treatment of HIV infections causing adverse side effects and high doses of these drugs are given to the patients suffering from HIV infections which is causing damage of organs.

1.12 Objective of the thesis: In view of above the main objective of the present thesis is the development of novel functionalized nanomaterials for sensing electrode modification for sensitive, selective and low cost sensing of toxic drugs. Due to toxicity of the drugs used in critical diseases like HIV-1 a highly sensitive and selective sensor development is aimed based on novel functionalized nanomaterials. Further in order to achieve stability and low cost of the sensor probe the focus is made on the synthesis of noble metallic nanomaterials or composites stabilised by suitable capping agents in order to enhance the conductivity and catalytic properties of modified electrode surface. The modified nanomaterials are aimed to use to fabricate the screen printed graphite electrode (SPGE) based sensor probe in order to provide the portability to the developed sensors for practical applications.