CHAPTER I

INTRODUCTION

This chapter deals with the basics of conducting polymers with their historical development, salient features and structures. Interesting features of conducting polymers such as band formation and generation of charge carriers upon doping are described. The general principles of biosensors and their classification are highlighted. The mechanisms of different bioreceptors and transducers based biosensors have been discussed. The chapter also describes different synthesis methods and applications of conducting polymer in diverse fields including biomedical applications. The chapter ends with the outlines of the scope of the thesis and statement of the thesis problem.

1.1 Conducting polymers

Most materials fall into the category of conductors ($\sigma \gtrsim 10^2$ S/cm), semiconductors ($10^2 \gtrsim \sigma \gtrsim 10^{-7}$ S/cm) and insulators ($\sigma \lesssim 10^{-7}$ S/cm) based on their electrical conductivity (σ), besides superconductors. Superconductors are zero electrical resistance materials which also expel magnetic flux when cooled below a characteristic critical temperature [1], whereas in a conductor, the valence electrons move freely throughout the material and show high conductivity [2]. A group of materials, called semiconductors e.g. germanium, silicon etc. posses conduction ability between that of a conductor and an insulator [3]. Lastly, insulators have very low conductivity and wide energy bands that are either completely filled or completely empty [4]. Polymers (insulators) have long been used as insulating materials in form of plastics bags, insulating plastic coating in metal cables, packaging etc. However, there are at least four major classes of semiconducting polymers that have since been developed. They include conjugated conducting polymers, charge transfer polymers, ionically conducting polymers and conductively filled polymers. A traditional concept which considered organic polymer as an excellent insulator has been discarded after the discovery of conducting polymer in the year 1977 [5]. The imitation of organic polymer like that of metal was first discovered by Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa. They

have been awarded with Nobel Prize in chemistry for this breakthrough in the year 2000 [6]. Their landmark discovery was the nine fold increase in conductivity in polyacetylene after treating with halogen [5, 7]. The possibility to tune the conductivity of polymers makes them a significant material from both technological applications and industrial point of view. Gradually, the development of other conducting polymers and their derivatives such as polythiophene (PTh), polyaniline (PAni), polypyrrole (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT) took place in 1980s [8]. Some of the most significant conducting polymers are shown in Figure 1.1.

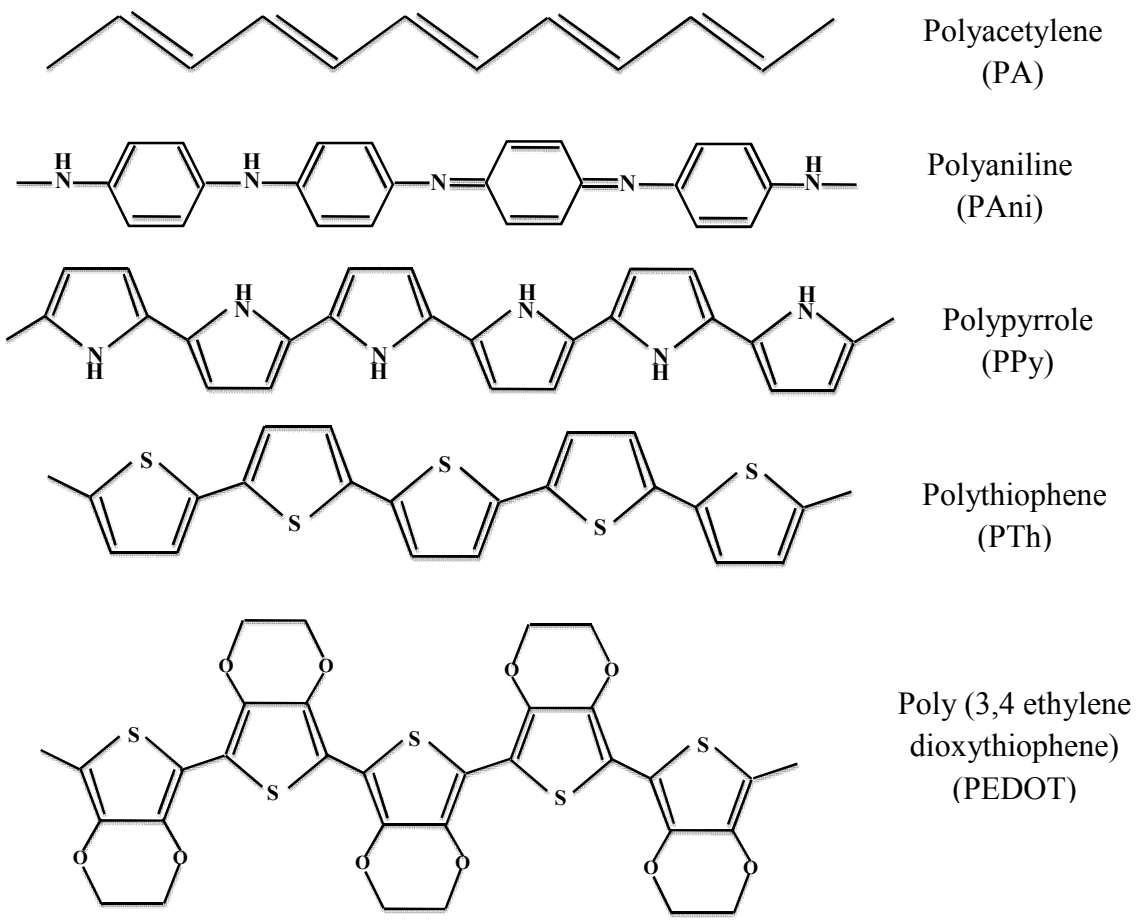

Figure 1.1: Structural representation of most significant conducting polymers.

1.1.1 Historical Development:

Polymers are large macromolecules with molecular mass ranging from 10^3 to 10^7 gm/mole. They are composed of multiple repetition of chemical units ('mers') connected together resembling beads on a string. Polymers are mostly organic made up of hydrocarbon molecules and each polymeric chain may contain hundreds to thousands of monomer in each chain [9]. The term polymer was coined by a Swedish chemist Jons Jacob Berzelius who is also considered as one of founder of modern chemistry [10]. Naturally existing polymers such as DNA, proteins, RNA and polysaccharides play essential roles in plant and animal life. From prehistoric times, the polymeric materials have been exploited by mankind for the purpose of decorations, clothing, shelters, tools, fibers, skins and other requirements [11].

The plastic industry was begun in the year 1868 by the synthesis of cellulose nitrate. The origin of today's macromolecules polymer industry was accepted after the development of concept of modification of certain natural polymers by Hermann Staudinger during 1920s [12]. For this discovery in the field of extremely high molecular weight polymers, he was awarded with the Nobel Prize in chemistry in the year 1953 [13]. This was followed by development of synthetic fiber by Wallace Carothers who gave confirmation of existence of polymeric macromolecules [14]. The field of modern polymer science was more highlighted by contribution of Noble laureates Giulio Natta and Karl Ziegler and Pierre-Gilles de Gennes [15, 16]. However the discovered polymers were electrically insulating in nature, therefore it failed to be a potential material from the point of view of electronic materials.

The early discovery of conductive polymers was triggered in the year 1960s when Pohl, Katon and their co-workers first synthesized partially conducting polymers [17-19]. The interesting electrical properties of poly(sulfurnitride) were the steps towards research on conducting polymer. Later in 1977, a Chem Comm paper was published by Shirakawa, Mac Diarmid and Heeger where they have observed the conductivity of polyacetylene (PA) upon the oxidation using halogen vapour [20]. The 10^9 fold increase in conductivity of PA after incorporation of halogen introduces the concept of doping of conducting polymers. The sudden break through not only had emerged the traditional concept of polymers as insulators, but also begun a new field of conducting polymers, which also called as "Synthetic Metals" [21].

The enhancement of electrical properties and switching to conducting nature from insulating was subsequently ascribed as doping. Electrochemical and chemical methods for the synthesis of π-conjugated polymers such as polypyrrole, polyaniline, polythiophene etc were reported in the nineteenth century and later on the concept of doping was applied [22-24]. In the beginning the research in conducting polymers was bounded to the synthesis procedure of new conducting polymers. The study of conduction mechanism in undoped or doped conducting polymers was discussed gradually along with their optical and physical properties [25, 26]. The unique chemical structure of conducting polymers which can be tailored to alter their physical properties makes it a vital material for diverse applications such as field affect transistor [27, 28], photovoltaic devices [29, 30], super capacitor [31, 32], electro chromic windows [33, 34], actuators [35, 36], chemical sensors [37, 38] and biosensors [39, 40, 41] etc.

1.1.2 Band Theory:

The electronic structure of a material is responsible for their electrical conductivity and the electrons move within distinct energy states called bands. The electronic structure of a material can be discussed using band theory, according to which materials can be categorized into conductors, semiconductors and insulators. The physical chemistry introduced a concept of the band theory by relating it to the quantum theory of atomic structures.

According to Quantum mechanics a single isolated atom has well defined discrete energy and the energy states are very sharp [42]. The spectral emission lines when an electron jumps from one allowed energy state to another giving rise to corresponding narrow line widths. In solids, atoms are in close proximity and cannot be viewed as separate entities with independent existence. The atoms having different electronic energy level are chemically bonded to each other and the orbital of one atom overlaps with the orbital of the neighboring atom giving rise to molecular orbital [43]. As a result of which the electrons in same orbit exhibits different energy levels and these molecular orbital space together in a certain energy range to form a continuum and broadening of sharp atomic energy states takes place which is known as energy band [43]. At absolute zero temperature, the electrons are present in highest range of electron energy called the valence band, while the lowest

vacant state is the conduction band. The energy difference between the highest and lowest state is called band gap (E_g) of a material. The energy band theory can be employed to differentiate among conductors, insulators, and semiconductors. The overlapping of valence band and conduction band allows the electrons to move freely and propagate in the conduction band. This is an intrinsic characteristic of conductors. The band diagrams of insulator, semiconductor and metal are depicted in Figure 1.2.

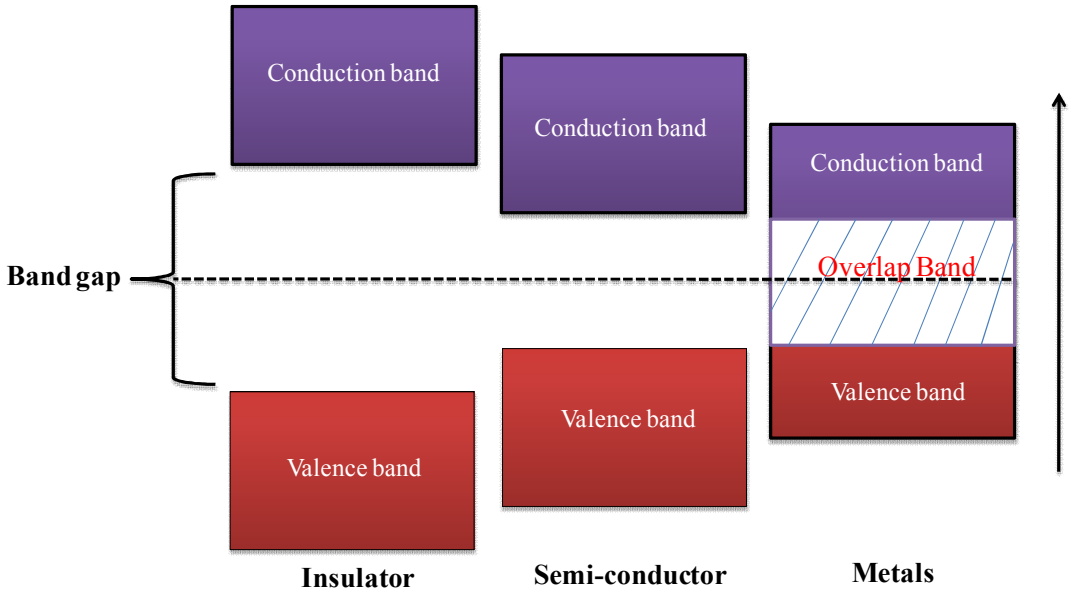

Figure 1.2: Diagrammatic representation of band gap in insulator, semiconductor and metal.

The transport properties of CPs have been discussed in molecular level [44, 45]. The conjugated conducting polymers have alternate single and double bonding along the polymeric chain and π- orbital system allows the electron to move freely from one end of the polymer to the other. The structure of organic polymers is based on aromatic or linear chain of carbon atoms or carbon atoms with some heterogeneous atoms such as oxygen, nitrogen, sulphur etc. The alternate single and double bond along the polymer skeleton results in SP^2 hybridization of carbon atom consisting of one s-orbital and two p-orbitals [46]. In the hybridization process of carbon with ground state configuration of $1s^2\ 2s^2\ 2p_x^1\ 2p_y^1$, overlapping of the 2s orbital with two of the available 2p orbitals produces three sp^2 hybridized orbitals. These hybridized

orbitals are lying in the same directing towards the corner of an equilateral triangle making an angle of 120^0 with each other. The fourth unhybrid P_z-orbital remains unaffected and lies perpendicular to the plane of Sp^2 hybridized orbitals.

The head on overlapping of orbitals resulting from a strong chemical covalent bonding of two carbon atom along the polymer chain axis produces strong σ (sigma) bonds which accounts for the formulation of polymer chains (Figure 1.3). On the other hand, p_z orbitals of two carbon atoms, perpendicular to the plane of nuclei, forms (π) pi bonds by side to side overlapping with each other. The continuous overlapping of p_z orbitals cause the π-bond between the first and second carbon atoms to move to the position between the second and third carbon atoms. This attraction of π-electron between a carbon atom with the nuclei of the neighboring carbon atoms results in tendency of delocalization of π- electron along the polymeric chain [47, 48]. The unusual properties of conducting polymer are due to presence of delocalized electrons moving along the backbone of polymer chain [49]. Therefore the conjugated polymers can be doped to become conducting and even metallic. The unhybridized p_z orbitals of one carbon atom can overlap with other, resulting in the formation of two molecular orbitals which are known as bonding (π) and anti-bonding (π*) molecular orbitals. These molecular orbitals delocalized over both atoms, and the bonding molecular orbital possess lower energy than atomic orbital of both the carbon atoms, while the anti-bonding molecular orbital has higher energy.

The width of individual bands resulting from bonding and antibonding orbitals across the range of energy levels is called band width. The valence band (VB) corresponds to the highest occupied molecular orbital (HOMO) and the edge of the conduction band corresponds to the lowest unoccupied molecular orbital (LUMO). The gap between the HOMO and LUMO is called band gap (Eg) and it is generally in the range of 1.5 - 4 eV in the case of conducting polymers. The weaker π bond in conducting polymer is highly prone to the doping process, which can be achieved by chemical and electrochemical oxidation and reduction process. The schematic representation of the formation of HOMO and LUMO band in conducting polymer is presented in Figure 1.4. During the doping process, the electrical conduction in conducting polymer can be attributed to the formation of nonlinear local excitations (e.g., solitons, polarons, and bipolarons) as charge carriers [50].

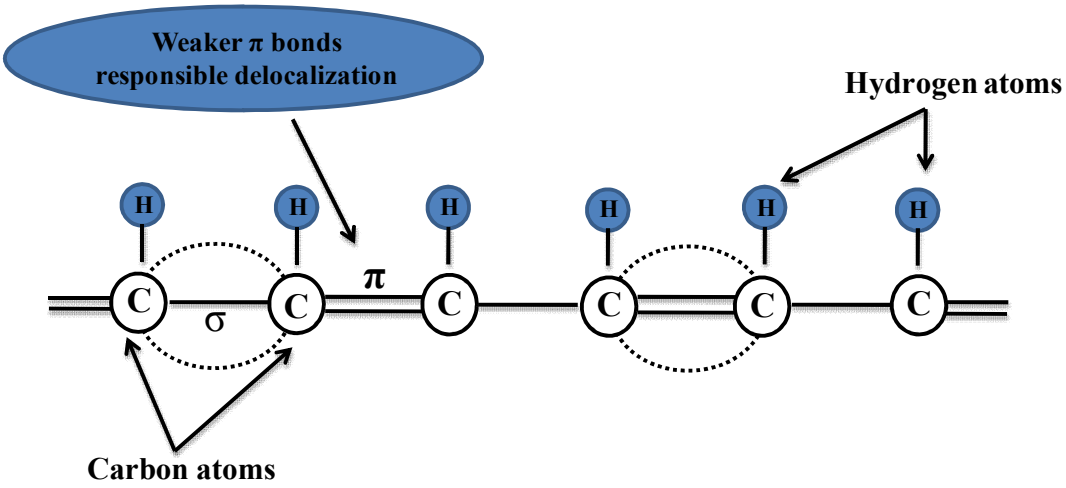

Figure 1.3: A simplified schematic representation of a conjugated backbone: a chain containing alternating single and double bonds.

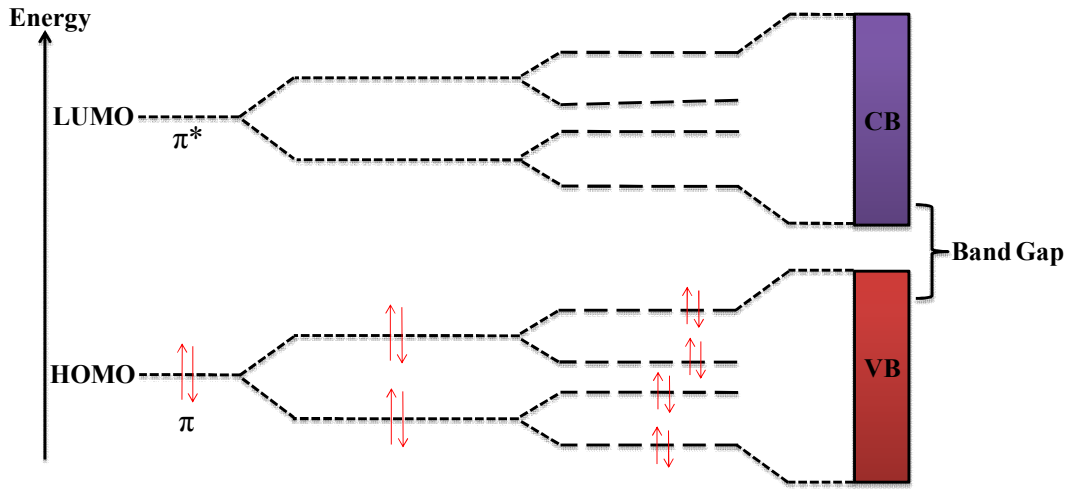

Figure 1.4: Schematic diagram of band formation in conducting polymers

1.1.3 Doping in Conducting Polymers:

Doping is a method by which the property of material like conductivity can be enhanced by generating charge carriers upon addition of different element (foreign atoms) into it. A pure semiconductor (no different elements added) can be categorized into either intrinsic or extrinsic semiconductors based on nature of dopant added. The mechanism of doping in CPs is completely different from that of

semiconductors as dopants in the polymer undergo redox processes and remains in interstitial positions without substituting the host atoms [51]. The oxidation and reduction of conjugated main polymer chain by charge injection should be accompanied with counterions doping for charge neutrality. The term “doping” in conducting polymers suggests (i) the transfer of electron charge i.e. oxidation, p-type or reduction, n-type, (ii) addition of counter ion and (iii) simultaneous control of Fermi level or chemical potential [52]. Doping results in generation of charge carriers like polaron, bipolaron and soliton which can enhance the conductivity of conducting polymer. The concentration of the charge carriers created within the polymer during the process of doping depends on the concentration of the injected dopant. The doping degree is defined as the number of counteranions per monomer unit of the conducting polymer (or the concentration of the charge carrier in the conjugated main chain of the conducting polymer) [53].

The common electronic feature of pristine CPs is the presence of weakly bonded π orbital electrons that are susceptible to doping. Most of the un-doped polymers do not even have intrinsic charge carriers and have been reported as insulators but upon doping their conductivity can be tuned from insulating to metallic. They have been doped using different methods in order to provide required charge carriers by partial oxidation (p-doping) with electron acceptor or by partial reduction with electron donors [54]. The conducting polymers with enhanced conductivity which has been obtained by creating negative or positive charge on polymer chain is called doped conducting polymer. The dopants can be categorized into two groups based on their molecular size viz. small dopants and large dopants [55]. Large dopants like sodium polystyrene sulfonate (PSS) can be introduced during polymerization and the strong incorporation will not allow leaching of the dopant with the change in external stimuli granting better stability of the composite. On other hand the small dopants are comparatively mobile and on application of external electric field they can leave and re-enter the polymer matrix [55]. Therefore conducting polymer can be cycled between doping (oxidized) and de-doping (reduced) state. The integration of dopants can affect both bulk and surface material properties like wettability, surface roughness, porosity of conductive polymers [56-58]. Biologically active proteins, enzymes, antibodies whole cells can be used as dopants forming the basis of biosensing applications of conducting polymers. Doping can be achieved by chemical or electrochemical methods, charge injection or

photo doping simultaneously during the synthesis of conducting polymers [59]. In case of biological dopants different methods like chemically covalent attachment or electrochemical entrapment has to be used [60].

1.1.3.1 Chemical doping:

Chemical doping is categorized into two types based on oxidation and reduction i.e. p- doping and n-doping [61, 62]. The oxidant such as I_2, AsF_5, Br_2, Cl_2, etc. results in the formation of polarons along the conjugated polymer chain and the gain of electron by the dopants forms counter-anion [63]. p-Doping can be explained by considering oxidation of trans-polyacetylene:

$$(CH)_x \rightarrow [CH^{y+}]_x + (xy)e^- \quad 1.1$$

Electrical neutrality has been balanced by formation of counter-anion, A^-;

$$[(CH)^{y+}]_x + (xy)A^- \rightarrow \left[(CH)^{y+}A_y^-\right]_x \quad 1.2$$

n-Type redox doping makes the polymer chain negatively charged to gain high conductivities due to reduction process, for example,

$$(CH)_x + (xy)e^- \rightarrow [(CH)^{y-}]_x \quad 1.3$$

$$[(CH)^{y+}]_x + (xy)M^+ \rightarrow \left[(CH)^{y+}M_y^+\right]_x \quad 1.4$$

where M^+ is the counter cation produced for charge neutrality.

1.1.3.2 Electrochemical doping:

In the electrochemical doping, the p-type and n-type doping is done by applying appropriate oxidation and reduction potential, respectively to a working electrode coated with polymer. The experiment is carried out in an electrochemical cell where the polymer electrode is used as working electrode in an electrolyte solution along with separate counter and reference electrode.

Conducting polymers can be easily electrochemically oxidized or reduced owing to the extensive delocalization of π-electrons. During electrochemical p-doping, the polymeric chain loses an electron accomplished by applying potential is associated with doping of counter-anions from electrolyte. Similarly in electrochemical n-doping, the reduction of polymer is accompanied by doping of counter-cations present in the electrolyte. Since no external oxidizing agent is used the doped polymer film is obtained in as standing film with greater purity as

compared to chemical doping [64]. The stoichiometric equation represents the electrochemical doping process in CPs:

$$CP - e' + A^- \leftrightarrow CP^+(A^-); \qquad CP + e' + M^+ \leftrightarrow CP^-(M^+) \qquad 1.5$$

where A^- and M^+ denotes the solution anion and cation , $CP^+(A^-)$ and $CP^-(M^+)$ represents the conducting polymer with the main chain oxidized and reduced, respectively.

1.1.3.3 Photo-doping:

When a conjugated conducting polymer (e.g. trans-polyacetylene) is exposed to a photon radiation of energy greater than its band gap, electrons are promoted to the higher energy state i.e. conduction band from the valence band. Rapid recombination of electrons and holes do not allow the photo generated charge carriers to sustain after the irradiation is ceased. Photoconductivity can be obtained by applying an appropriate potential during irradiation, which could separate electrons from holes [65].

1.1.3.4 Charge injection doping:

This is another method of doping a conductor where a multilayer structured configuration of metal/insulator/semiconductor (MIS) is applied for charge injection. Using a field effect transistor geometry, the surface charge layers can be generated by applying an appropriate potential across the MIS and electrons and holes are introduced from the metallic side to the π* and π bands of conducting polymers, respectively [66]. During this doping the electron is either added to π* band or removed from π band of conducting polymers and no counter ion is associated.

1.1.4 Charge carriers in conducting polymers:

The electrons and holes are the dominant electronic excitation in inorganic semiconductor where the four fold or six fold coordination of every atom makes them a rigid structure [45]. However the scenario is different in case of conducting polymer and two fold symmetry makes them more susceptible to structural distortion. Thus soliton, polaron and bipolaron are generated by nonlinear excitation and the dominant charge carriers in conducting polymers [67]. Based on the bond structures in ground state, CPs can be grouped into degenerate and non-degenerate systems.

The conducting polymers with degenerate ground state polymers possess two fold geometry where the charged cations can freely move along the polymer chain without any boundaries.

In organic non-degenerate CPs, the ionized states have different equilibrium geometry from that in ground state. During ionization of a molecule, the energy level involved can be schematically described as shown in Figure 1.5 [67] and $E_{IP-\upsilon}$ is the energy require for a vertical Franck-Condon ionization process. If the system in ionized state undergoes geometry relaxation then relaxation energy (E_{rel}) is gained by the ionized state. On the other if a molecule adopts the equilibrium geometry of the ionized state after distortion in the ground state, it will cost energy E_{dis}.

If single electron energy levels of the molecule is taken under consideration then this deformation with energy E_{dis} required to maintain the equilibrium, will cause a modification along the orbital by shifting the highest occupied molecular orbital (HOMO) upwards and a downward shift of the lowest unoccupied molecular orbital (LUMO) by an amount of $\Delta\varepsilon$ as shown in Figure 1.5. Therefore, the distorted molecule requires an amount of energy E_{IP-d} for ionization. The ionization energy is lowered by an amount of $\Delta\varepsilon$ upon the removal of an electron (oxidation) from the conducting polymer chain.

In this process of lattice distortion, polaron, a radical ion comprising of unpaired electrons with spin ½ and a positive charge coupled via resonance, is created [68]. It is the major charge carrier in conducting polymers. Polaron with positive charge P^+ and negative charge P^- (spin 1/2) are formed after oxidation and reduction of the conjugated polymeric chain [69]. The localized electronic states present in the gap represent the polaron states where an electron is associated with molecules and carries its own polarization filed. The polaron bands are created just above HOMO and just beneath the LUMO within the energy band gap. The polaron binding energy is given by the quantity $\Delta\varepsilon - E_{dis}$ (= E_{rel}) [67].

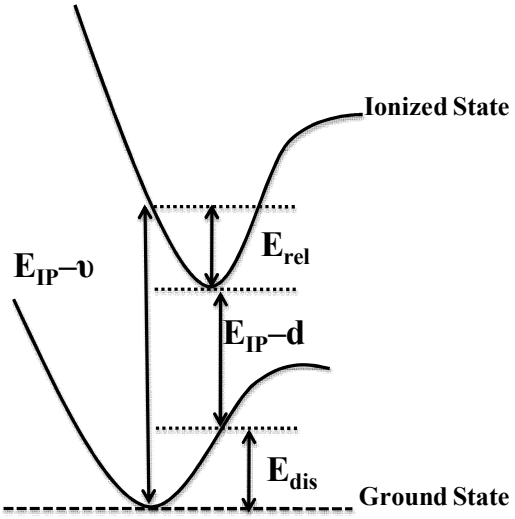

Figure 1.5: Schematic representation of molecular ionization with energies involved in the process.

Further oxidation results in the removal of second electron either from polaron or from the neutral portion of the chain. The former case creates a bipolaron which is defined as pair of like charges coupled through a lattice vibration [70]. The bipolaron is spinless and it is created within the conjugated polymer chain when the concentration of polarons is high. The latter case i.e. the removal of another electron from the neutral chain of the polymer results two polarons.

The creation energy of bipolaron relative to that of two polarons of many conducting polymers have been compared and result indicates that the distortion energy required during the formation of two polarons is almost equal to that one bipolaron (Figure 1.6). Moreover the decrease in ionization energy is much more significant in case of bipolaron ($2\Delta\varepsilon^{bip}$) than that in creation of two polarons ($2\Delta\varepsilon^{pol}$) [67]. These reasons make a bipolaron thermodynamically more stable than two protons. As the degree of doping increases, the bipolaron levels overlap to form continuum known as bipolaron bands.

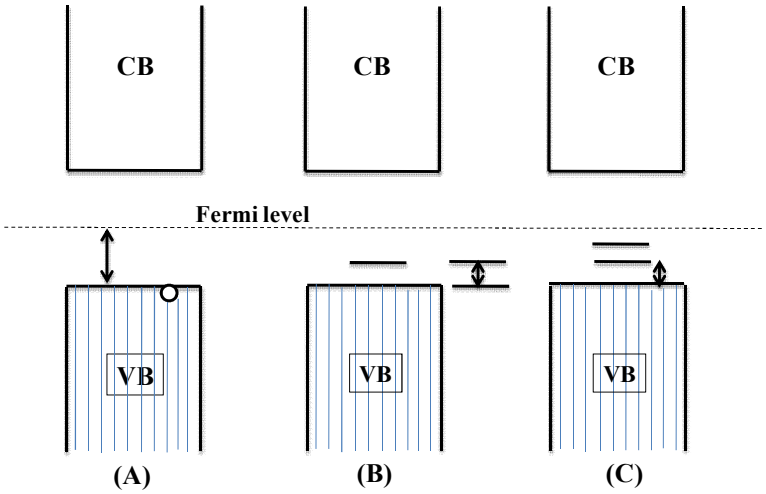

Figure 1.6: Illustration of the band structure of a polymeric chain in the case of (A) a vertical ionization process and (B) the formation of a polaron (C) formation of bipolaron.

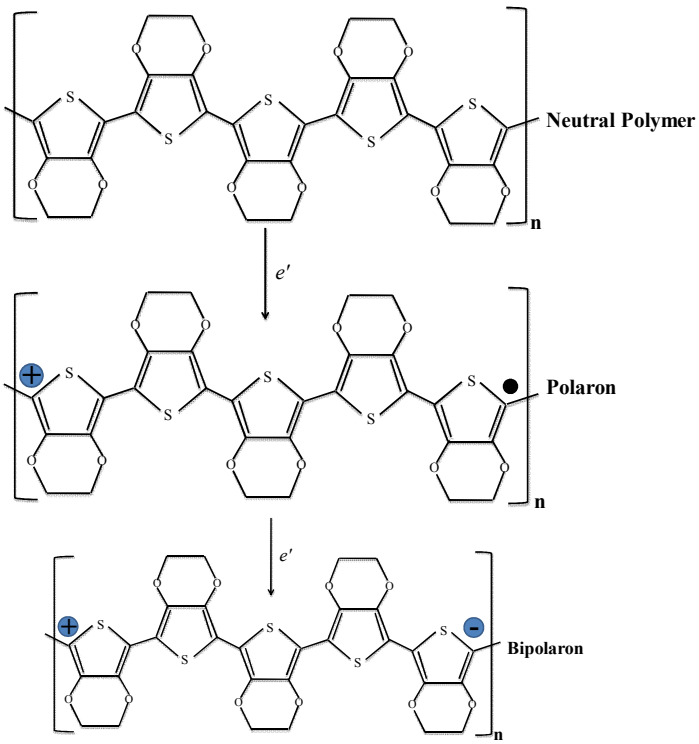

Figure 1.7: Illustration of formation of polarons and bipolaron in PEDOT upon doping.

In case of conducting polymers with degenerate basic state which consists of two separate phases of opposite orientation and indistinguishable energy (eg. trans-polyacetylene). The charged cations can move freely across the polymer matrix. Initially the removal of electron leads to the formation of the polaron, which is same to that of non degenerate conducting polymer. At higher doping level, a different type of charge defects is generated and they are called solitons [71, 72]. The soliton (S) is an unpaired π-electron resembling the charge on free radicals, which can be delocalized on a long conjugated polymer main chain. The creation of polaron and soliton in trans-polyacetylene is displayed in Figure 1.8.

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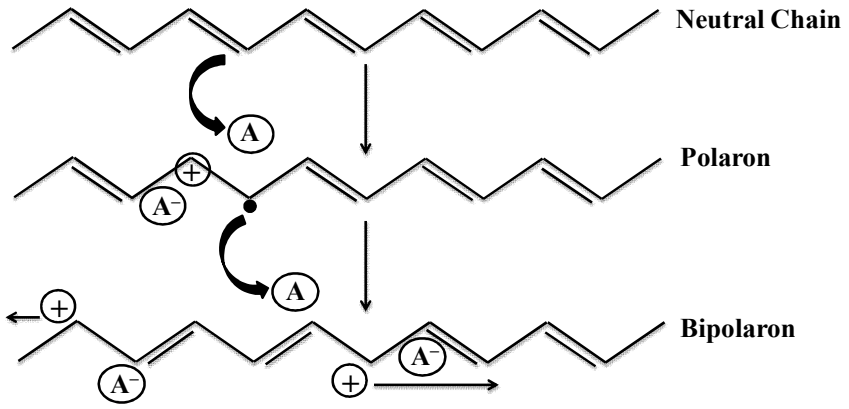

Figure 1.8: Formation of Polaron and solitons in trans-polyacetylene.

1.2 Synthesis of Conducting Polymer:

1.2.1 Chemical Synthesis:

Chemical polymerization or oxidative coupling mechanism was investigated in 1990s and it is a feasible technique to prepare conducting polymer [73]. The chemical route of polymerization is done to get large amounts of polymers. The monomer solution is mixed with oxidizing agent such as ferric chloride, ammonium per sulfate or peroxydisulfate in acid media for the polymerization of monomer [74]. The resultant polymer is found in high conducting state where the ionic species are accompanied by the solution used in the synthesis procedure. Both powdered and thick filmed polymer can be synthesized using this method which makes it a preference for commercial applications. This method can create all types of conducting polymers together with some novel conducting polymers that cannot be prepared with the electrochemical method [8]. The polymerization is considered as

radical mechanism rather than radical cation as shown in Figure 1.9 [75]. During the chemical polymerization in presence of $FeCl_3$, the iron (III) ions with strong lewis acid character take the active part because of the presence of one free orbital. Hypothesized mechanism involves oxidation of monomer producing a radical cation which reacts with another neutral monomer unit. Dimeric radical cation is formed as a result of further oxidation and deprotonation. They combine with neutral monomer to form an oligomer and a chain of monomers is formed [75]. The generation of charge carriers via doping initializes the oxidative polymerization of monomer which is followed by formation of structure and electronic defects. As the doping of pristine or non conducting state proceeds first predominant defect called polarons which results in formation of localized states within the band gap [54].

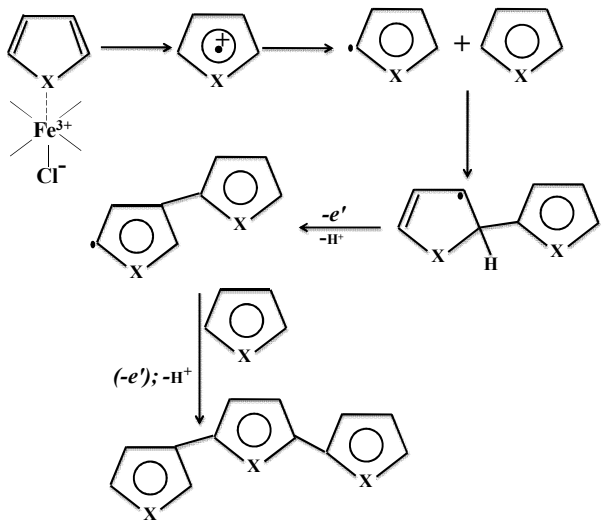

Figure 1.9: Chemical polymerization via radical mechanism.

1.2.2 Electrochemical Synthesis:

This method requires a standard three electrode system comprising a working electrode (platinum, glassy carbon, ITO, gold etc.) where the film has been deposited, a reference electrode (Ag/AgCl, $Hg/HgCl_2$, calomel electrode etc.) with respect to which the potential is applied to the working electrode and the counter electrode (platinum wire) which completes the circuit [76]. The electrode systems are dipped in an appropriate solution containing both supporting electrolyte and colloidal suspension of monomer as shown in Figure 1.10 [77]. The oxidation potential is applied to the working electrode and the current causes monomer to get

oxidized and the charge neutrality is maintain after incorporation of anion into the polymer and at the end an insoluble polymeric film deposit onto the working electrode forming polymeric film [8]. The film thickness can be controlled and a 20 nm thin film can be achieved using this method [8, 78]. However the Polymeric films thickness or geometry can be controlled by optimizing factors like working potential, electrode system, concentration of doping agent and solvent.

Electrochemical polymerization can be carried out using three techniques: galvanostatic, potentiostatic and potentiodynamic [79-83]. During potentiostatic process, a constant potential is applied while the current varies. It maintains the integration of the component to be coated. In galvanostatic polymerization, current is kept constant and steady and controlled deposition of polymers can be achieved. In potentiodynamic method, the applied potential is cycled from an initial lower limit to a higher potential limit at a constant scan rate. During deposition, each layer becomes electro-active which helps in deposition of next layer onto it therefore layer by layer deposition can be achieved. The mechanism of polymerization can be deduced from several observations. Firstly, on application of specific oxidation potential, the monomer gets oxidized and generates a radical cation making an electro-philic attack on a neutral molecule [84]. When the working electrode is perturbed, the diffusion of the monomer from the bulk solution occurs and the reactive radicals are highly assembled near the electrode-electrolyte interface. The second step involves the coupling of one radical with another monomeric radical cation to produce a dihydro dimer dication which may lose proton leads to a dimer and re-aromatization. Since the oxidation potentials of the dimers and oligomers are lower than the monomer therefore applied potential results in more oxidation of dimers than the monomer and generated radical undergoes a further coupling with a monomeric radical. Finally the termination of chain growth results in deposition of polymer film on the anodic electrode [84].

The whole process indicates that electro-polymerization of monomer involves a nucleation step and a chain of successive electrochemical and chemical steps as shown in Figure 1.10 [84]. Electro-polymerization method can synthesize thin and uniform standing films with high degree of geometrical conformity and controllable thickness. This method allows deposition of films on small surface area [85].

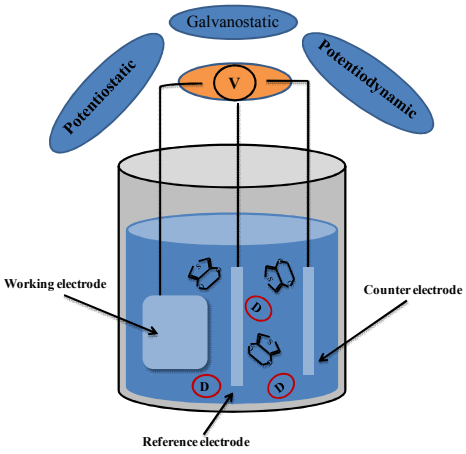

Figure 1.10: Experimental set up for electrochemical polymerization of conducting polymer.

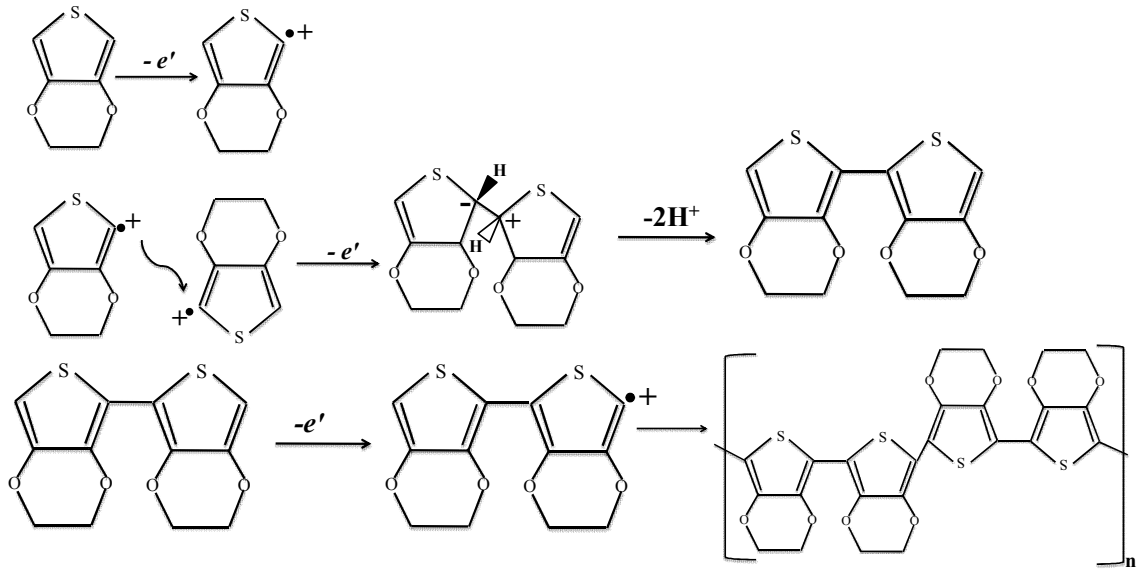

Figure 1.11: Electrochemical polymerization of EDOT into PEDOT.

1.3 Application of conducting polymers:

The tunable optical, electronic and magnetic properties of conducting polymers and their ability to be electrically switched between its conductive and resistive states established conducting polymer for a wide range of applications.

1.3.1 Application in Dye sensitized solar cell:

The dye-sensitized solar cell (DSSC) has been considered as a potential candidate for the next-generation and have attracted broad research interest solar cell due to its

comparatively high energy conversion efficiency, low cost, and environmentally friendly and easy fabrication process. In the development of the dye-sensitized solar cell (DSSC), platinum (Pt) is used as the most common CE due to its high conductivity, stability and efficient catalytic activity towards redox couple (I^-/I^{3-}). However, the replacement of expensive component noble metal Pt is significantly important, and a challenging research to discover new counter electrode (CE) materials [86].

Various conducting polymers PPy [87-89], PAni [90-92] and PEDOT [93-95] have been used as versatile counter electrode material. Conducting polymers are found to have efficient electrocatalytic activity towards the redox reaction of iodide species along with their appropriate features like lower cost, easy fabrication, environment stability; a high degree of processability makes them a suitable material for the fabrication of CE.

1.3.2 Application in energy storage and conversion:

Tailorability in the synthesis procedures and processability of conducting polymer enable them as an ideal material for energy storage applications. Conducting polymers are considered as a versatile material and attracted extensive attention for super capacitor as they exhibit high specific pseudo capacitance, fast Faradaic reaction, high power density and enhanced charge-discharge rates [96-98]. The frequent charging and discharging process causes volume expansion or shrinkage which results in low rate capability or poor cycling of conducting polymer based super capacitor electrode. However this restriction towards the application of CPs as super capacitor can be overcome by fabricating conducting polymer based nanocomposites with graphene, carbon nanotubes, etc [99-101].

1.3.3 Application in chemical sensor:

Conducting polymers and their derivatives have been used as active layer since early 1980 for different chemical sensors for the detection of gases like NH_3, H_2, CH_3OH, NO_2, CO, vapour. Because of their macroscopic and molecular structure, they allow gases to enter their inner structure and chemical modulation of their electronic properties occurs resulting from interacting with gases [37, 38,103].

1.3.4 Application in electrochromic device:

Conducting polymers have also been investigated as an important electro-chromic material because of their color tailorability for varying coloration region under an applied electric field [33, 34, 103]. Moreover, conducting polymers based nanocomposites have been used in microwave absorption and electromagnetic shielding.

1.3.5 Application in tissue engineering:

Electro-active polymers such as polyaniline, polypyrrole, polythiophene, and their derivatives (mainly aniline oligomer and poly (3,4-ethylenedioxythiophene)) exhibits excellent environmental stability and has been shown to have the ability to support cell adhesion and growth of a variety of cell types [104, 105]. The biocompatible and non toxic conducting polymers are considered as suitable material for tissue engineering as they allow cell activities including cell adhesion, migration, proliferation, differentiation, and protein secretion [106, 107]. Apart from facile synthesis, tunable physical and chemical properties, conducting polymers can be functionalized to the specific needs of their applications. Since conducting polymers offer vast variation in fabricating ‘‘smart’’ biomaterials” and therefore they are believed to revolutionize the world of tissue engineering.

1.3.6 Application in Biosensors:

In a biosensor the biological component is integrated within or connected to the transducer, which converts the biochemical signal to an electronic signal. The conducting polymer which possesses many excellent qualities which makes it a very promising efficient biomaterial and it has been used as transducer element [108]. The use of conducting polymer as transducer in biosensors has grown over the past decade due to their ability to efficiently transfer electric charge produced by the biochemical reaction to electronic circuit. Some of the properties of conducting polymers that makes them a suitable matrix for biosensing application are: (a) its unique electrical, electronic, magnetic and optical properties that can be tailored to the specific needs of their application, (b) availability of wide range of monomer and easy fabrication, (c) they can be match up with various range of fabrication techniques such as electrochemical, optical, mass-based, etc, (d) they can be

deposited over defined area of electrode (e) they can be prepared in the range of neutral aqueous solutions allowing entrapment of a wide range of proteins viz. enzymes, antibodies, whole cell, etc within the matrix of CPs, (f) biosensor properties like linearity, sensitivity, detection limits can be enhanced by preparing composites of conducting polymers with carbon compounds, metal nano-particles, (g) functional groups like -COOH, -NH_2 can be introduced to the surface of ramified polymer network which prevents leaching out of biocatalytic layer [109-113].

The key aspect of development of efficient biosensor is the successful immobilization of biomolecules within a solid matrix [114]. In the recent years, organic conducting polymers have been considered as convenient component for the immobilization of protein molecules [115]. The biomolecules can be entrapped into a platform but complete retention of the biological activity of the immobilized protein is a crucial problem [116]. This is a hindrance towards commercial development of biomolecules based miniaturized biosensor [116]. The activity bioreceptors depends on various factors like pH of the operating solution [117], temperature [118], porosity, hydrophillicity [119] and the bonding of the biological moieties with the surface of the matrix [115]. In this regard, conducting polymer is considered as an excellent host for the encapsulation of biomolecules due to its low toxicity, high conductivity and exceptional redox active property [108, 116]. Moreover, the polymers itself can be modified using different agent for efficient binding of protein molecules. Additionally, the conductive polymers can be functionalized with biocompatible molecules, segments and side chains to improve its biocompatibility towards the protein molecule [120]. Different approaches have been made and successful immobilization can be achieved using physical adsorption [121], electrochemical entrapment [122], covalent bonding [123] or cross linking and affinity immobilization [124].

Physical adsorption is a simple process where a solution of functionalizing agent is kept in contact with polymer surface or the platform is bathed in biomolecule solution. The driving force of physical adsorption is the static interactions between the polymer matrix and charged protein. The limitation of this method is that the synthesized bioelectrodes are susceptible to pH and the biomolecule is prone to leaching out of the conducting polymer film [121].

The entrapment of protein molecule can be achieved by performing electrochemical polymerization of monomer in presence of suitable solvent mixed

with functionalizing molecules in electrochemical cell. The enzyme or antibodies are zwitterionic in nature and becomes negatively or positively charged when kept in solution (PBS) of pH below or above their isoelectric point [125]. Therefore during polymerization, the oxidation of the monomer results in positively charged polymer matrix and simultaneous incorporation of negatively charged biomolecule takes place [126]. This technique is mainly applied to bind large molecules (e.g. enzymes, DNA), and after entrapment the molecules are unable to leave the polymer due to their size. However the high operating voltage applied for the oxidation of monomer might result in enzyme activity loss. Another method of immobilization is the pre-modification of the CP layer with functional groups using different agent. During this process the functional groups on the polymer surface can be activated using EDC/NHS and hence a covalent bond is formed [127]. This method results in close intimate bonding of the biological molecules with the transducer surface thereby enhancing the long-term stability of the polymer based bioelectrode. Crosslinking of the biomolecule with the polymer surface can be done by using bifunctional cross-linker like glutaraldehyde, which can form stable inter- and intra-subunit covalent bonds [128].

1.4 Biosensor:

A biosensor is a chemical sensing device that integrates the biologically active components like enzymes, tissues, microorganisms, antibodies, nucleic acids and etc. in intimate contact with an appropriate transduction element for the function of selective detection of the concentration of analyte in interest. The detection principle is based on two steps: recognition step and transducing step. In the recognition step, the confined biological element can recognize the analyte either in the solution or in the atmosphere [129, 130].

Bioreceptors can be classified according to the embedding biorecognition elements; they may be biological molecular species (e.g. antibodies, enzymes, proteins, or nucleic acids) or living biological systems (e.g cells, tissue, or whole organisms) [131]. When a bioreceptor comes in contact with its analyte, it gives a biological response which is then converted into measurable quantitative equivalent electrical signals by the transducer. Transduction of the biological signal can be electrochemical, optical, piezoelectric, calorimetric etc [132-135].

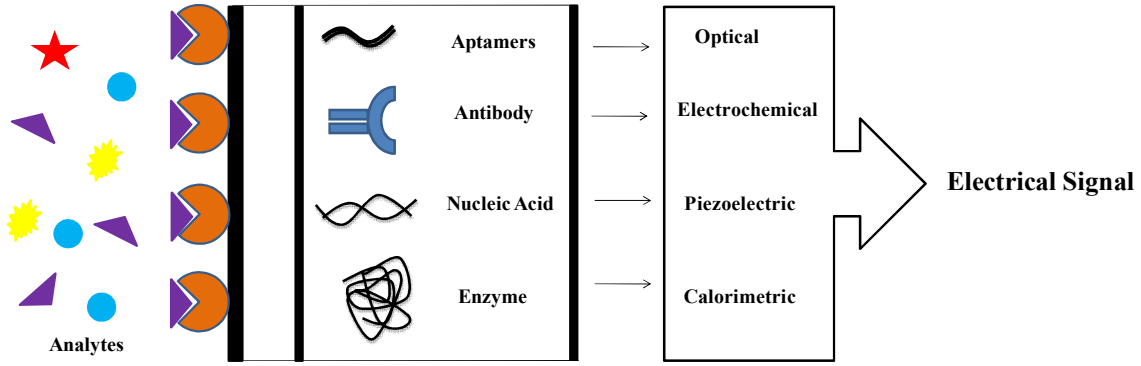

***Figure 1.12**:* Schematic diagram of Biosensor.

Biosensors have been categorized mainly into three generations [136]. The first generation or mediator-less biosensor is based on Clark biosensors where the biologically active molecule is either coupled or encapsulated in a membrane, which is attached to the surface of transducer. Diffusion of reaction product across the interface of membrane and the transducer cause the measurable electrical signal. The second generation biosensors exploit a specific mediator to transfer the signal from the active site of the bioreceptor to the transducer. The motivation of using a mediator is to attain good sensitivity. A suitable mediator should have a lower redox potential compared to that of electro active compounds in the working solution.

The third generation biosensor is also known as direct biosensor is based on bioelectrocatalysis where the bio-component is an integral part of the biosensor and therefore no mediator is required. The bio-active agent is attached to the transducer and the signal to directly measure by the sensor element. Conducting polymer-based biosensors falls under third generation biosensors and the redox polymers helps in signal propagation [136].

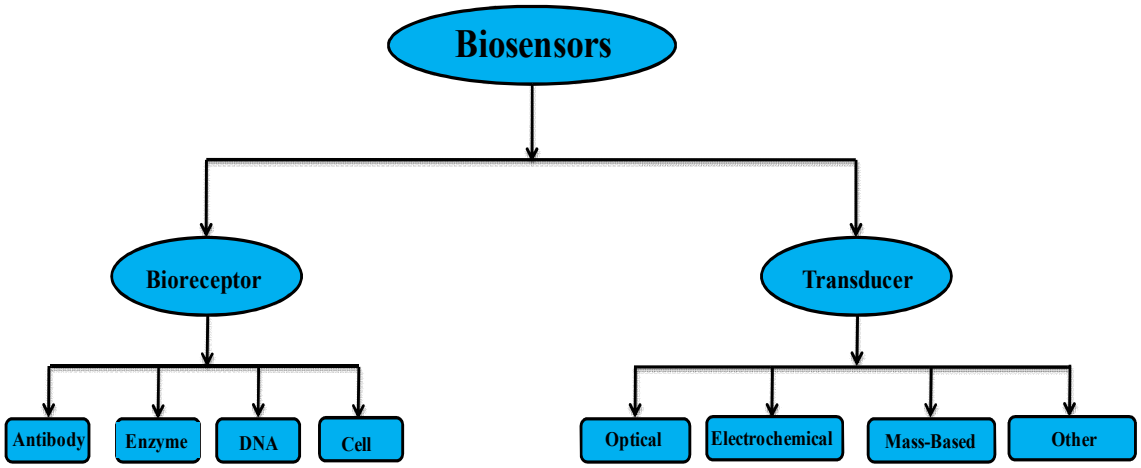

Figure 1.13: The flow chart depicting the classification of Biosensors.

1.4.1 Biocomponent:

The recognition bioreceptor confined within the biosensor possesses an exquisite level of specificity and selectivity but it is susceptible to change in environmental condition like temperature, change in solution properties like pH, ionic strength, toxicity etc [137]. The sensitivity, linearity, detection of specific analytes, shelf life of biosensor depends on the activity of bioreceptor [138]. Therefore the factors like porosity, surface area, surface characteristics (hydrophobic or hydrophillic) of the matrix have to be taken under consideration to retain the activity of the bioreceptor. The methodology for immobilization of the biological component is a crucial factor and it varies with different class of bioreceptor [139].

1.4.2 Types of Biosensors based on different transducers:

1.4.2.1 Electrochemical Biosensor:

Electrochemical biosensor is based on a principle of measuring the electrical properties which generates during interaction between the immobilized biomolecule and target analyte. The electrochemical transducer is used for extracting information from biological systems and the intensity of quantifiable signal is proportional to the concentration of the compound detected. The typical biochemical reaction generates a current signal (*amperometric*), alters the oxidation or reduction potential (*potentiometric*) or changes the conductive properties of the reaction medium

(*conductometric*) between the electrodes in an electrochemical cell. The resistance or reactance between the electrode and the electrolyte (*impedimetric*) during the biochemical reaction can also be used as the electrochemical signal [140-142].

1.4.2.2 Amperometric Biosensor:

The most widespread method of electrochemical detection is based on measuring the current signal generating during a biochemical reaction. The enzyme catalyzed electro-oxidation or electro-reduction or hydrolysis/phosphorylation or antibody based bioaffinity reaction facilitates the electro-oxidation/reduction process. During amperometric sensing the redox active site inside the enzyme layer generates a current signal of nA to μA range and this signal can be correlated to the concentration of the analyte of interest. The value of applied potential is set to a value where the analyte produces oxidation or reduction current and therefore it has been considered as the driving force of electron transfer. The biochemical reaction mediators help in enhancing the electron transfer by participating in the redox reaction [143].

1.4.2.3 Potentiometric Biosensor:

Potentiometric transducer uses the potential at which the biochemical reaction between the analyte and the biocatalyst occur. The potentiometric biosensor is based on the principle of applying voltage to an electrode system in a solution as a result of which current flow occurs. The measured oxidation or reduction potential at which the electrochemical reaction occurs indicates a particular reaction and particular analyte. The potential difference between two electrodes is measured by high impedance voltmeter considering nearly zero passing through the system [143, 144].

1.4.2.4 Conductometric Biosensor:

The overall conductivity of the solution changes with variation of ionic species produced during a bio-recognition event. This transducer measures the change in electrical conductance with the increase of concentration of analyte in interest. The conductometric transducer has relatively low sensitivity. The method relies on a principle of applying AC voltage on a set up consists of two metal electrodes and the measuring the conductance between the metal electrodes [142].

1.4.2.5 Impedimetric Biosensor:

The impedimetric biosensor is based on measuring the resistance to the flow of charge due to the biochemical interaction occuring across the working electrode. In this process, an AC signal is applied over a frequency ranging from higher to lower value which causes a flow of current through the bio-electrode. This technique is widely used in case of bio-affinity interaction and the bound antigen at the surface of electrode results in increase in impedance which may be detected via impedimetric transduction. This hindrance to the flow of current between working bioelectrode and the counter electrode is directly proportional to the amount of analyte assembled at the sensor surface. The EIS method allows label free detection of the analyte [145, 146].

1.4.2.6 Optical Biosensor:

Optical biosensors are based on the detecting the analytes by measuring the light absorbed or emitted in both catalytic and affinity reactions. Optical detection of the analyte can be divided into different classes viz. reflection, absorption, infrared, Raman, fluorescence and resonance depending on the nature of biochemical reaction. The surface plasmon resonance and fluorescence are most commonly used techniques of optical detection due to their efficient sensitivity and selectivity towards the biochemical reaction. A metal (gold) layer is taken as a substrate where the bio-active agents (antibody or enzyme) are covalently bind or adsorbed as a result of which refractive index changes [147]. When the analyte is allowed to flow over the immobilized receptor on the metal electrode and the minute changes in refractive index at and near the electrode surface is measured by SPR transducer. The transduced signal resulting due to the variation of refractive index of the medium is in the form of light [148, 135].

A fluorescence based device monitors the change in the frequency of incident electromagnetic radiation. These biosensors can be applied in vivo detection as they are non-electrical and multiple analytes sensing is possible by using different range of wavelengths [149].

1.4.2.7 Piezoelectric Biosensor:

They are considered as mass-based biosensors and follow the principle of acoustics i.e. sound vibrations. The general idea is based on coupling the surface of piezoelectric component with bioelement. Materials that exhibit piezoelectric effect like quartz, lithium niobate, aluminium nitride, oriented zinc oxide and tourmaline are used to coat the surface of metal electrodes. The piezoelectric crystal can vibrate at a specific frequency which defines the specificity of piezoelectric transducer. The interaction of attached biomolecules with analyte causes mechanical vibrations that can be converted into a measurable electrical signal. The increase in mass due to the formation of complex results in more change of oscillation frequency and the measurable electrical signal is directly proportional to it [150].

1.4.2.8 Calorimetric Biosensor:

Calorimetric transducer employs one of the basic property of biological interaction namely adsorption or expulsion of heat. The exothermic or endothermic interaction between the biocomponent and its analyte changes the temperature of the medium where the reaction occurs.

The experimental set up is designed in such a way that the immobilized enzymes are kept in closed small bed column combined with temperature sensors at the entrance and the exit. In a close air tight system, 80 % heat generated can be attributed to the change in temperature in the sample stream. The adsorbed or produced heat inside the chamber is directly proportional to the number of molecules involved in the reaction and the molar enthalpy. Thermistors are generally used to monitor the change in temperature before and after interaction and the difference in enthalpy is calibrated against the concentration of the analyte. Calorimetric biosensors are generally used to detect pesticides and bacteria [151].

1.4.3 Enzyme based electrochemical Sensors:

The enzyme based bioelectrode probe can be designed by immobilizing a thin layer of enzyme on the surface of a working electrode. The enzyme is the most significant component of biocatalytic sensors as it catalyzes the biochemical reaction to produce biological signal. The increase in reaction product, which is electro-active in nature, can be monitored using amperometry, potentiometry, cyclic voltammetry, pulse voltammetry, impedance spectroscopy etc. The rate of decrease of reactant can also

be considered alternatively as the biosensing signal. The inhibition of enzyme activity is another tool for detecting analytes (Figure 1.14) and pesticides viz. carbamates, organophosphates fall under this category [152, 153]. Glucose oxidase (GOx) coated electrodes for the detection of glucose was first used by Clark and Lyon in 1950s and 1960s [154]. They used platinum electrode where enzyme was immobilized behind a semi permeable membrane. However the direct transfer of electron between the enzyme and the working electrode causes denaturation of protein. Later on mediated electrode immobilized with enzyme showed a better performance. Mediators like quinones, organic conducting salts, dyes, ruthenium complexes, ferrocene, and ferricyanide derivatives have been employed for development of enzyme based sensors [152].

The third generation enzyme based biosensor was fabricated by co-immobilizing of enzyme and mediator onto the surface of electrode. It has been done to achieve better sensitivity and longevity of the enzyme electrodes [153].

1.4.3.1 Enzyme:

Enzymes, a biological catalyst known to speed up a biochemical reaction, are often used as biomaterials for the development of biosensors. Enzyme being very specific towards its analyte and enhances the rate of one particular reaction, without being consumed [155, 156]. The catalytic biochemical reaction of enzyme takes place in specific region within the protein called active site. They can be re-used as long as their specific site remains active but in some case their activity may lose. Some enzymes require assistance in carrying out their function as in case of glucose oxidase, FAD is the co-factor which is the main active site and undergoes the redox reaction involving gain or loss of electrons [157]. The chemical interaction of enzyme with its analyte is mainly based on two mechanisms, lock and key theory and induced fit theory.

The Lock and key mechanism, first postulated in 1894 by Emil Fischer [158], describes the enzymatic action with a single substrate. In this theory, enzyme is considered analogous to the lock and substrate as the key (Figure 1.14). The reaction begins with the binding of the substrate to the active site on the enzyme. This specific binding results in exchange of electrons between the substrate and redox active site of enzyme that results in the formation of product. Perfect sized key

(substrate) fits into the key opening of the lock i.e. the active site of enzyme. The smaller or larger keys which are incorrectly positioned may not fit into the lock (enzyme). Therefore, the unique geometry of the active site that is matching only with similar geometrical substrate makes an enzyme very specific.

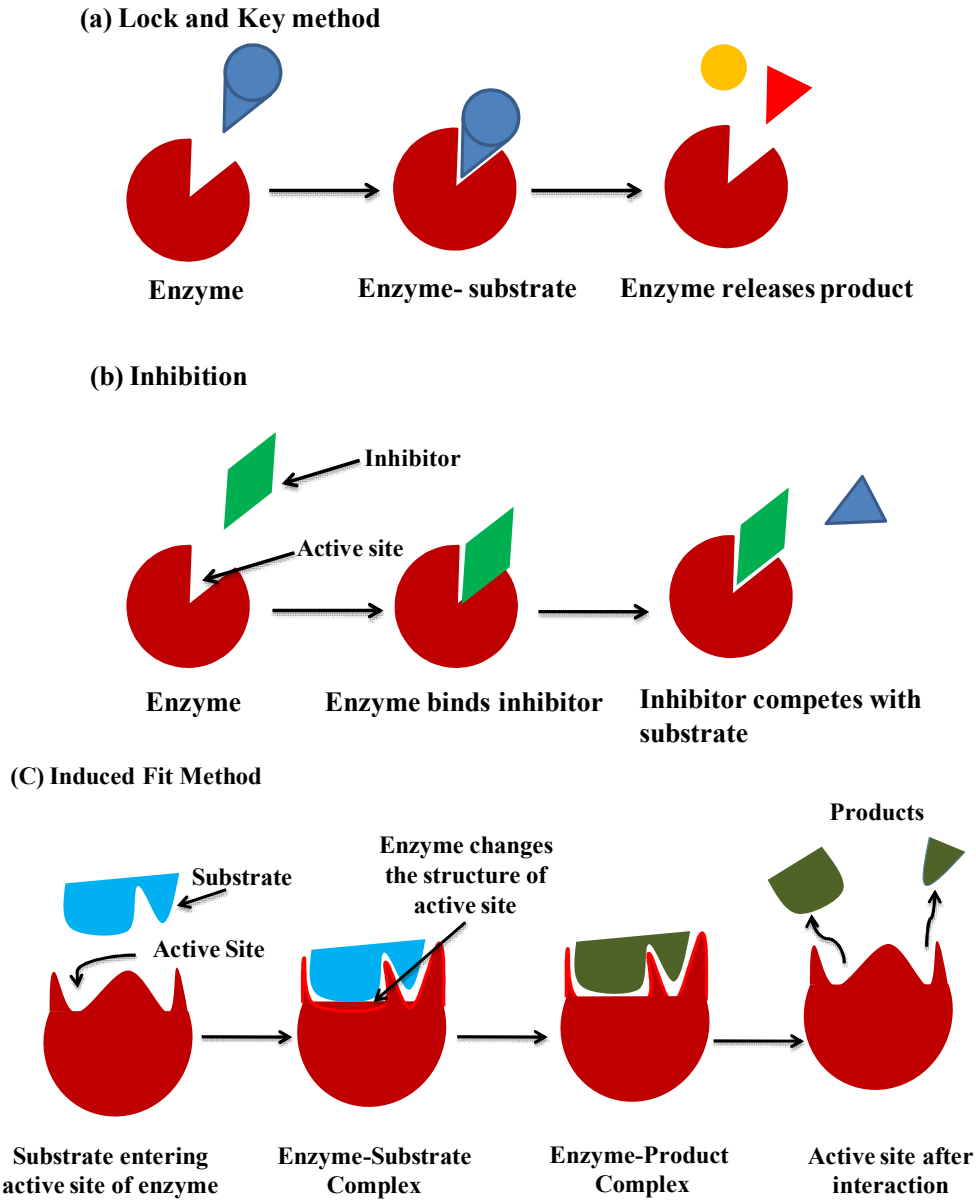

Figure 1.14: Schematic Representation of interaction of enzyme with its substrate.

In 1958, Daniel Koshland [159, 160] proposed another mechanism called induced fit model which is considered the more accurate version. According to this theory initially the active site of an enzyme remains partially flexible and considered as an imperfect match for the substrate binding. The final shape of the enzyme is

determined by the binding of the substrate and the structural rearrangement causing a better accommodation for the substrate [160]. This explains the unsuccessful binding of the enzyme with certain compounds because of distortion of it. Small molecules failed to induce appropriate alignment of the active site.

The activity of enzyme is dependent on the pH value of the operating solution, the temperature condition and the substrate concentration. Being zwitterionic in nature, the interaction of amino acid present in the active site of the enzyme with substrate depends on its electrostatic nature as well as their spatial orientation. The amino acid group is positively charged at acidic medium while at basic pH it becomes negatively charged. Each enzyme has an optimal pH that helps in maintaining the configuration [117]. Temperature is another crucial factor affecting the enzyme kinetics and it has been found that at high temperature more effective collisions between the enzyme and substrate takes place. Beyond a certain point, the thermal energy is enough for the breakage of active forces holding the enzyme in its 3D form and denaturation of enzyme occurs. For the majority of the commercial enzymes, the optimal temperature range is between 40 ºC and 60 ºC [118].

1.4.3.2 Preparation of enzyme electrodes:

The enzyme based electrochemical transducer can be prepared using different physical and chemical methods of immobilization. To minimize the effect of external factors on the activity of the enzyme, the method of entrapment is introduced in the fabrication of biosensor to avoid the leakage of biological component. Free enzyme when mixed with substrate in solution cannot be recovered after its reaction which hinders its practical application [161]. The motivation towards the immobilization of enzyme is to achieve (1) high degree specificity without any structural rearrangement of the moiety, (2) better alignment of enzyme to achieve greater stability towards temperature, ionic strength, pH, redox potential (3) the chance of immobilizing more the two biological components on one substrate matrix [161]. Adsorption is the easiest method of immobilizing enzyme protein on the surface of water insoluble matrix [162]. Different methods have been used to achieve physical adsorption of enzyme molecule (Figure 1.15). The enzyme solution is allowed to come in contact with matrix without stirring which is called Static

process. In dynamic batch process, the carrier matrix is stirred with the enzyme for proper mixing. The weak bonds like ionic, hydrogen, coordinated bonds stabilize the enzymes and no permanent bond is formed between the immobilized enzyme and the carrier [163].

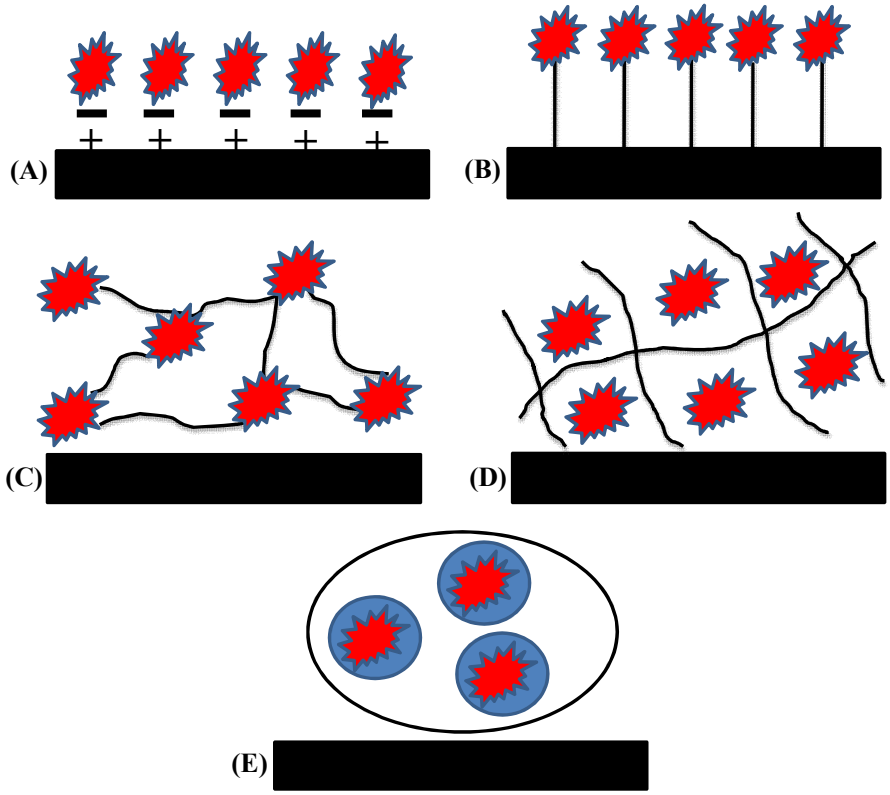

Figure 1.15: The diagrammatic presentation of (A) Adsorption (B) covalent bonding (C) cross linking (D) entrapment and (E) encapsulation methods of immobilization of enzyme.

Another method is the covalent bonding between chemical groups present in enzyme and the supporting matrix forms a stable complex. It is most widely used method of enzyme immobilization. The presence of functional groups like hydroxyl groups, carboxyl groups, thiol groups, amino groups, imidazole groups in the insoluble matrix are responsible for the accomplishment of covalent immobilization **[**164**].** Carriers used for covalent coupling are carbohydrates, synthetic agents, protein carriers and amino group bearing matrices. The main advantage of covalent binding is minimum leakage of enzyme during its practical applications [165]. Another vital method of immobilizing is entrapment where the enzymes are physically trapped

inside a porous matrix. No direct attachment of protein occurs and the enzymes stabilize inside the matrix via covalent or non-covalent bonding. It is carried out by polymerizing the monomer in a solution mixed with the biocatalyst [166]. The biopolymers like chitosan, collagen, cellulose acetate or polyacrylamide etc are used as matrices. Pore size of the polymer matrix can be adjusted in order to minimize the leaching of the immobilized enzyme. The leaching of low molecular weight enzymes from the marix hinders the use of this for immobilization.

In the cross linking method the enzyme got linked with the matrix using a bifunctional or polyfunctional agents like glutaraldehyde and diazonium salt. The cross linker agent can induce an intermolecular bonding between the biocatalyst and the solid support [167].

1.4.4 Affinity Electrochemical Biosensors:

Immunosensors are biological tools used to detect the reaction of an antibody with an antigen. The binding event between antibody and antigen results in formation of a stable complex which is non-conducting in nature [168, 169]. The main motivation of using electrochemical immunosensor is to fulfill several aspects including the sensitivity of the method, the cost of the assay and the opportunity to adopt different procedures of immobilization. The critical part in fabricating immunoelectrode is to maintain the right orientation of antibody after immobilization on the solid surface. The detection of antigen is based on exploring the decrease or increase in current or resistance using voltammetric, potentiometric, conductometric or impedimetric processes [168].

1.4.4.1 Antibody:

Antibodies represent one of the most diverse classes of protein made of millions of different amino acid which comprise 20% of the total plasma proteins and together they are called immunoglobulins (I_g) [170]. Properties of antibodies are based on the structure of I_g which comprises of a constant and a variable region. The tail region of the antibody composed of an identical protein fragment called the F_c region which represents the constant region of antibody and it is responsible for the interaction with cell surface receptors [171]. The simplest antibody can be schematically represented by Y-shaped molecules with two identical binding sites for antigen. The variable end called the F_{ab} region contains variable sections which has a unique

configuration to bind with a specific target. The F_{ab} region, also known as the arms of the antibody, has a sequence of different amino acid for different antibody molecules, making them specific towards one unique epitope [171]. Unlike enzymes, they do not act as reaction catalyst rather they bind reversibly with their specific antigen. Based on the epitope, antibodies are classified as monoclonal and polyclonal. Monoclonal antibodies, originating from one mouse, bind to a single epitope and therefore considered as highly specific [172], diminishing the chance of cross-reactivity. Whereas Polyclonal antibodies shows heterogeneous response as it can interact with multiple epitopes on an antigen.

The chemical structures of antibodies are responsible for its properties like binding specificity, binding versatility and biological activity [173]. The interaction of antibody and antigen are non covalent with rare exceptional case and involves random bumping of both the molecules into each other [174]. The immunoglobulin molecule i.e. paratope binds to a small part of the epitope using non covalent bonds. The force of this binding must be greater than the repulsive force between the two molecules to overcome zeta potential. In the molecular level, long range forces like ionic and hydrophobic bonds are responsible for the binding of epitope, when it is in a distance of several nanometers from the antibody [175]. Eventually the water molecules are expelled and the existing forces overcome the hydration energy of the two molecules as a result of which the epitope and paratope move towards each other more closely. At a very close distance, the forces like vander waals force, hydrogen force and electrostatic force of opposite charges on amino acids have been shown to contribute the biochemical interaction [176].

Antibody affinity is the concept that measures the strength of reversible bio-molecular interaction between a single antigenic determinant and F_{ab} region of the antibody [177]. It is dependent on variety of factors including valency and the accurate fit of the antigen in the antigen binding groove. The sensitivity of an immune assay depends on the affinity of the antibody towards the respective antigen. The affinity can be further categorized into intrinsic affinity and functional affinity. Former is the strength of interaction between monovalent epitope on antigen and the paratope on antibody and it can be determined by equilibrium association constant. The later is the affinity of an intact antibody with multivalent antigen [178].
Different factors effects the activity of antibody and their interaction .The acidity and alkalinity of a medium affects the affinity of an antibody and extreme pH values

results in strong inhibition of antibody-antigen reaction. A conformational change in the structure of antibody molecules occurs at very high or very low pH and therefore destroy the bonding with antigen. Maximum affinity of most of the antibody is found between the optimal pH ranges of 6.5 to 8.2, beyond and below which the results become unreliable [179].

The time required for the reaction of antibody and antigenic determinants is an important factor as they require sufficient time for interaction but prolonged incubation may cause dissociation of antibody-antigen complex. Similarly optimum temperature for efficient antibody-antigen reaction is considered to in the range between 37^0 C and 56^0 C [180]. In presence of low ionic strength saline solution the sensitivity of antigen-antibody reactions can be increased in low ionic [181].

1.4.4.2 Preparation of antibody based electrode:

Like enzymes, antibodies can also be immobilized through hydrophilic or hydrophobic interactions between antibodies and solid matrix. Immobilized antibodies are randomly oriented which may cause denaturation and their binding abilities [182]. The orientation of the antibody on a solid surface can be assumed as "head on", "side on" and "lying on". Random orientation or non specific interaction results in denaturation of the antibody binding sites. The simplest method of immobilizing antibody is physical adsorption but it is associated with poor binding which hinders its use in practical application [183]. Plasma treatment [184], self assembled monolayers [185], molecularly imprinted polymers [186] are different techniques applied for the modification of surface of the interface for specific target binding.

The covalent coupling of the antibody on substrate surface is considered as the most robust method of immobilization. Lysine is the amino acid present in antibodies which contain primary amine groups. The carboxyl groups present across the antibody surface due to the presence of aspirate and glutamate. Theses carboxyl and amine groups can be targeted for covalent bonding (between antibody and solid substrate) and can be attained using carbodiimide chemistry that utilizes EDC combined with succinimidyl esters (such as NHS). Thiols groups present in cysteines can also take part in covalent bonding of antibody with the substrate. Additionally the glycosylation at the Fc region can provide a target for successful immobilization.

Biotin–avidin/streptavidin interaction is another significant method of antibody immobilization [187].

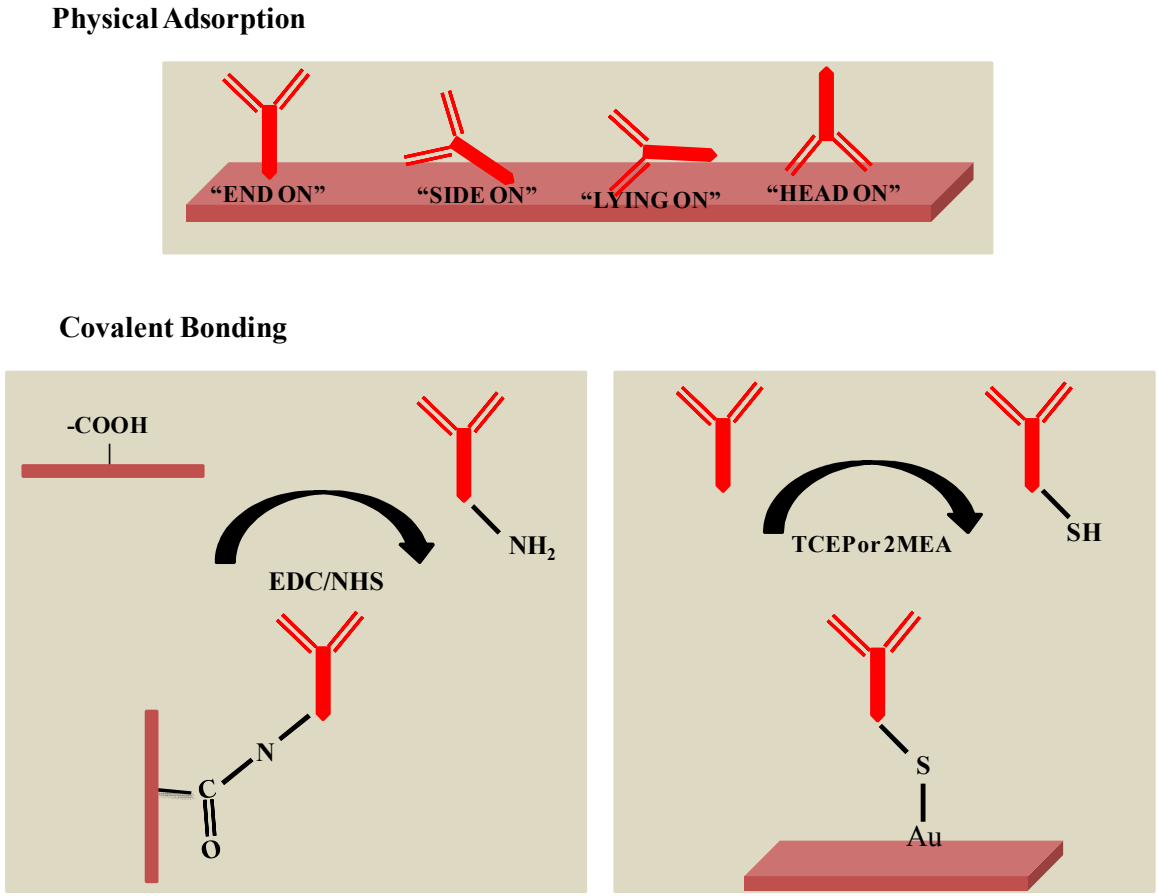

Figure 1.16: Diagrammatic presentation of Immobilization of antibodies.

1.5 Poly (3,4-ethylenedioxythiophene) (PEDOT):

Poly (3,4-ethylenedioxythiophene) is one of the polythiophene derivatives developed during the second half of the 1980s, at the Bayer AG research laboratories in Germany [188]. It is one of the most useful and promising conducting polymer for fundamental research and from application point of view. It is generally abbreviated as PEDOT or PEDT. The main motive towards using PEDOT in different applications is the presence of dioyalkylene bridging across the α-β and β-β coupling of its heterocyclic ring [189]. Among thiophene derivatives, PEDOT is highly conductive and allows both n and p doping. The blockage of undesired 3 and 4 position results in lowering its band gap and therefore enhanced the electrical

conductivity of PEDOT. It has a very high conductivity value of ca. 300 S/cm with higher range of potential of 1.4 V. The PEDOT thin film is almost transparent and highly stable in its oxidized form [190]. The presence of 3,4-disubstituted thiophene ring makes PEDOT a high environmentally stable conducting polymer. In case of other conducting polymers like polypyrrole, polyaniline these positions are susceptible to unwanted oxidation leading to carbonyl formation and consequently lower conductivity. Being a non degenerate conducting polymer with moderate band gap of around 1.5 -1.6 eV, low redox potential -0.6 V, high electrical conductivity and transparency, PEDOT has drawn attention in various applications [190].

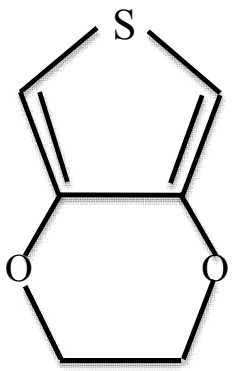

Figure 1.17: Chemical structure of 3, 4-ethylenedioxythiophene

The polymerization of EDOT takes place via 2, 5- coupling since the 3- and 4-position have been occupied by oxygen which increases the electron density of the thiophene ring [191]. Different methods like electrochemical polymerization, chemical polymerization, vapor phase polymerization etc. can be applied for successful polymerization of EDOT. The most common method is chemical oxidative polymerization and electrochemical polymerization. The mechanism of electrochemical polymerization of EDOT monomer is shown in Figure 1.11. Thin film of PEDOT can be deposited on glassy carbon electrode, ITO, platinum electrode and the introduction of a thin PEDOT film increases the effective surface area of the electrode substrate without sacrificing the conductive property of the electrode [191]. PEDOT is considered as an ideal candidate for biological and biomedical applications such as tissue engineering, biosensors and bio-interfaces [192]. The tailorable mechanical strength of PEDOT could endow fine mechanical property for biological applications. Functionalization of PEDOT with different

functional groups makes the polymer an ideal platform for antibody or enzyme immobilization for biosensing applications [193]. Moreover, it can easily be functionalized with negatively charged dopants like GO, functionalized CNTs, PSS and metal nanoparticles as the electro-polymerization of the EDOT results in positively charged polymer backbone.

1.6 Scope of the thesis and statement of the thesis:

Organic conducting polymers have recently emerged as a unique class of electroactive materials and a interesting subject for research and development [194]. In the past decade conducting polymers have attracted intensive interest for technological applications after the discovery that it is possible to control the electrical conductivity of polymers over the range from insulating to metallic [195]. They are the resonance stabilized conjugated organic polymers that have presented a strong fundamental scientific challenge which has been taken up by a diverse community of chemists, physicists, materials scientists, and theoreticians both in academia and in industry. The special chain structure of conducting polymers results in their electrical properties being affected by both structure of polymeric chain (i.e. conjugated length) and dopant nature [196]. The process of doping creates non linear defects, such as solitons, polarons and bipolarons which are responsible for the physical and chemical properties of the organic polymers and the electrical characteristics of metals [197]. Conducting polymers are intelligent material as they are capable of recognizing appropriate environmental stimuli, processing the information arising from stimuli and responding to it in an appropriate manner and time frame. Additionally, they have certain properties like high electrical conductivity, environmental and thermal stability, biocompatibility etc. Hence, they are worldwide used in construction or improvement of intelligent materials systems or structures by many research groups [198, 199]. Due to their ability to undergo molecular interaction with particular species of interest, conducting polymers e.g. Polypyrrole (PPy), Polythiopene (PT), Polyaniline (PAni) and poly (3,4-ehtylenedioxythiophene) (PEDOT) have been investigated for numerous applications such as polymer batteries [200] , micro electrodes [201], supercapacitor [202], biosensors and actuators [203] , drug delivery systems and tissues engineering. Biosensing device has received much interest known as an analytical device that

consists of a confined biological component in conjunction with a transducer device that converts a biochemical signal into an amplified electrical signal. The challenge is to efficiently detect the biochemical signal formed after the interaction of the biological component and the analyte [204].

Aflatoxin B_1 are the most commonly found mycotoxins that are considered most hepatotoxic and hepatocarcinogenic classified into group I by the International Agency for Research on Cancer. The major immunochemical methods used in aflatoxin analysis are radio immunoassay, Enzyme linked immunosorbant assay (ELISA), immunoaffinity column assay and immunosensors [205]. Though these methods are sensitive but more rapid, simple and cost-effective approaches are required by the food industries [206]. In this regard, portable biosensors for toxins detection are very efficient and cost-effective with high sensitivity and selectivity. The present research thrust is towards the development of user-friendly biosensors for Aflatoxin B_1 detection to replace conventional expensive chromatographic and ELISA techniques. On the other hand organophosphates (OPs) have been widely used as pesticides in modern agriculture due to their low persistence and high insecticidal activity particularly in developing countries [207]. The toxins may enter the food chain directly or indirectly and affects the human health adversely by inhibiting the activity of acetylcholinesterase (AChE), which is an enzyme that stabilizes the levels of the neurotransmitter acetylcholine by catalyzing the hydrolysis of acetylcholine to thiocholine [208]. In this regard conducting polymer that contain a π-conjugated system with alternating single and double bonds in the polymer chain which confer upon them the charge transfer property, making it compatible for integration with proteins, allowing electron transfer to the electrode surface. Conducting polymers and their derivatives have been used as active layers of gas sensors since early 1980s [209]. Conducting polymers can be controllably deposited on a substrate surface via the application of a potential sufficient to oxidize and polymerize the specific monomer. The introduction of a thin conducting polymer film increases the effective surface area in addition to incorporating a specific reactive surface on the electrode substrate without sacrificing the conductive property of the electrode. The ingenious concept to combine the recognition properties of macromolecular biological molecules to the sensitivity of electrochemical devices has led to the emergence of biosensors as valuable analytical tools for the monitoring of target analytes in different technological areas. In

comparison with most of the commercially available sensors, based usually on metal oxides and operated at high temperature, the sensors made of conducting polymers have many improved characteristics such as high sensitivity, selectivity and short response time and workability at room temperature.

Among the use of various CP like polythiophene, polyaniline (PANI), polypyrrole (PPy) etc. Poly(3,4-ethylenedioxythiophene) (PEDOT) has recently sparked much interest in the research field due to its evidently superior qualities such as high stability, enhanced light transmission, processibility and easy synthesis over other polymers. Conductivity in PEDOT is a result of conjugated backbone with high degree of π orbital overlap. It has been used in biological and biomedical areas such as biosensors and bio-interface [210, 211] as the deposition of PEDOT on the electrode surface would effectively improved the electrochemical performance of the modified electrodes [212]. Because of the high electronic and ionic conductivity, thermal and chemical stability, PEDOT as compared to other polymers (PANI, PPy etc.) can be used as a mediator in biosensor. PEDOT can be electro polymerized from the monomer EDOT and the oxidative polymerization of monomer results in positive charges on the polymer backbone, this allows for the incorporation of negatively charged doping agents [213]. During the last decade, nanomaterials of various shapes, sizes and compositions have been synthesized, e.g., noble metal nanoparticles like gold nanoparticles (AuNPs) [214], carbon nanomaterials like graphene oxide , graphite [215, 216] and carbon nanotubes [217] and hybrid nanostructures for biosensing application. Compared with bulk materials, nanomaterials exhibit some inherent advantages such as good biocompatibility, high surface-to-volume ratio, and unique physical and chemical properties, which endow nanomaterials as excellent candidates for the fabrication of chemical and biological probes [218]. Gold nanoparticles and carbon nanomaterials based immunological sensors, using electrochemical, optical and piezoelectric detection methods have drawn particular interest in the bioanalytical field due to their superior physical and chemical properties such as easy fabrication, compatibility and high catalytic activity. They modify the electrode substrate by creating nanostructured surfaces with improved electrochemical response and also act as a carrier for the immobilization of biomolecules such as enzymes, antibodies and protein conjugates, for electrochemical signal transduction and amplification.

The present doctoral thesis is a detailed description of synthesis and study of the electro-catalytic properties of the PEDOT based nanocomposites. The effect of the functionalization of the PEDOT with the gold nanoparticles (AuNPs), graphene oxide (GO), polystyrene sulfonate (PSS) and carbon nanotubes (CNTs) has been studied in order to overcome the above mentioned drawbacks. The introduction of these doping agents has the added benefit of incorporating additional reactive functional groups as a result of which covalent bonding of the enzyme/antibody as well enhances the charge transfer properties of the conducting polymer based matrix. Spherical gold nanoparticles have been used in biosensing applications by enhancing the catalytic behavior of the bioelectrode which may be due to their highly conducting nature. AuNPs can provide a microenvironment similar to that of biomolecules in native system and thereby the activity of biomolecules is retained after immobilization [219]. It can not only provide a biocompatible matrix for protein but also helps in the immobilization through ionic interaction and chemisorption covalent bond between the gold nanoparticle and free sulfhydryl (thiol) groups of the Protein [220]. CP along with the carbon materials (GO) are extensively used to combine the properties of the individual components for a synergistic performance in biosensor fabrication. CNTs have been widely used in biosensor designs and nanoscale electronic devices due to their favorable microenvironment around enzyme or other biomolecules. Carbon nanotubes have ability to provide high surface to volume area, biocompatibility, non-toxic and chemically stable matrix for protein immobilization. They can also enhance the transfer of electrons which may result in high sensitivity, low limit of detection of the sensing material. Chemically modified CNTs are mainly merged into a polymeric matrix. Carboxylation of the CNTs with sulphuric and nitric acid makes them more soluble in polar solvents and provides functional groups for the immobilization of biomolecules [220]. PSS have been used to get uniform film of PEDOT with excellent processibilty.

In the view of the foregoing discussion, the present work aspires to develop PEDOT based nanocomposites to fabricate electrochemical biosensors for the detection of Aflatoxin B_1 and organophosphates. Thus the present thesis work has aimed to achieve a new electrochemical strategy for the fabrication of novel cost effective device for the excellent sensing of the Aflatoxin B_1 and organophosphate. The main motivation towards the functionalization of the conducting polymer based

matrix is to enhance the sensing parameters like linearity, sensitivity, and response time, limit of detection and limit of quantification. Besides, gaining an insight into the electron transfer processes and electro-catalytic behavior of the PEDOT based electrodes constitute the prime objectives of the present thesis.

The following PEDOT based systems have been investigated to achieve the objectives set in the present thesis:

1. Synthesis of gold nanoparticles (AuNPs) incorporated PEDOT nanocomposites for efficient detection of Aflatoxin B_1 and organophosphate.
2. Electrochemical biosensor based on gold nanoparticles functionalized PEDOT doped with graphene oxide for the detection of Aflatoxin B_1 and organophosphate with an aim to achieve enhanced sensitivity and linearity.
3. Multi component nanohybrid of PEDOT, PSS and multiwall carbon nanotubes for detection of Aflatoxin B_1 and organophosphate.

The above-mentioned PEDOT based electrode systems have been characterized by Field emission Scanning Electron Microscope (FESEM) (JEOL-JSM-6390LV) to study the morphology of the synthesized nanocomposites. The surface characteristics have been studied using contact angle measurements have been made by Hydrophilicity measurement set-up (Model: DSA 15B). The conformational changes in the materials have been studied using vibrational spectroscopy employing Fourier Transform Infrared (FTIR) spectroscopy. The electrochemical impedance spectroscopy (EIS) has been employed to investigate the resistance enocountered in the charge transfer mechanism at the interface of the electrode and the electrolyte solution. The electro-catalytic properties and the electron transfer mechanism have been studied using cyclic voltammetry (CV). The sensitivity of the synthesized composites towards analytes has been studied and the real sample analysis of spiked and unspiked samples has been carried out using differential pulse voltammetry techniques.