Chapter 1

Introduction

Conducting polymer, from its development in the 6th decade of the last century, has significant importance in the field of material research and technology up-gradation in modern times. Scientific and technological importance of conducting polymer (CP)'s is due to their interesting structural, electrical, electronic and magnetic properties. CP's are extensively used in molecular electronics, sensor technology, bio-fuel cells, functional and electrical textiles and in many other applications increasing day by day. An interesting application of CP's comes out from their efficient charge transferring ability from a biochemical system to an electronic circuit. This leads to its application in electrical simulation, modulation of cellular activities including cell adhesion and migration, DNA synthesis and protein secretion [1]. Polypyrrole (PPy) is an important member of the class of conducting polymer, obtained by polymerization of Pyrrole (Py) and is formed as a ring structure of connected Py molecules. It is one of the most studied conducting polymers attracting considerable interest in recent years because of its numerous applications in various electrical and electronic devices, especially due to its bio-compatible character. Such composites are also found to exhibit certain novel properties useful in bio-engineering and bio-chemical applications, sensor technology, opto-electronic devices etc [2]. An understanding of its electronic and optical-electrical properties with changing morphology and crystal structure of PPy composites on incorporation of different bio-molecules as dopant or surfactant during its polymerization is proved to be useful in increasing its environmental stability and improving its mechanical properties. These key issues provide a route for improvement of its device performance also [3].

Synthesis of PPy was first reported by Weiss and his co-workers in 1963 by pyrolysis of Tetraiodopyrrole [4]. At present, different processes are available for its synthesis. However, in the following sub sections here, we discuss only the chemical synthesis of PPy as this route is adopted for experimental sections described in the present dissertation.

1.1 Chemical polymerization of Pyrrole

In this method, PPy is synthesized by oxidative polymerization of Py using an oxidizing agent e.g. $FeCl_3$, either in aqueous medium or in an organic solvent like methanol. The reaction involved is,

$$n\ C_4H_4NH + 2FeCl_3 \rightarrow (C_4H_2NH)_n + 2FeCl_2 + 2HCl$$

It is assumed that polymerization takes place by means of electrophilic attack of $\pi-$ radical cation $C_4H_4NH_+$ on C-2 atom of an un-oxidized Py molecule producing an intermediate dimeric radical $[(C_4H_4NH)_2]_+$ which is finally transformed into the conductive form of PPy by means of p-doping of the polymer [5].

$$(C_4H_2NH)\ n + xFeCl_3 \rightarrow (C_4H_2NH)_n Cl_x + x\ FeCl_2$$

The p-doping can be brought about by chemical or electrochemical oxidation. Though reduced /un-doped PPy is an insulator, its oxidized derivatives are electrically conducting with conductivity ranging from 2-100 S/cm. The conductivity depends on the polymerization condition, reagent used and the nature of dopant [6]. However, doping makes it more brittle. Thin films of reduced PPy are yellow or greenish in colour but doped films are blue, black or greenish black depending on the degree of polymerization, degree of oxidation and film

thickness. It is insoluble in water and most of the organic solvents which creates a lot of problems in its fabrication [7].

Though it has high electrical conductivity and ambient stability, the main drawback of pure PPy is its low elongation and brittleness. However, this problem can be overcome by synthesizing PPy composite with materials having better mechanical properties. Both electrochemical and oxidative polymerization can be used for such a synthesis, but the chemical oxidative polymerization is the preferred one for mass production, due to its cost effectiveness though it is true that PPy produced by oxidative polymerization is less conductive compared to that produced by electrochemical method.

In chemical oxidative polymerization, formation of PPy takes place via one electron oxidation of Py, which subsequently couples with another radical cation to form 2, $2^{/}$ bipyrrole. The process repeats to form a longer chain of PPy later on.

Fig 1.1 given below shows the polymerization scheme of Py by addition of successive rings. PPy can exist either in aromatic or in quinoid structure, depending on the position of the conjugate bond, as shown in the figure 1.2.

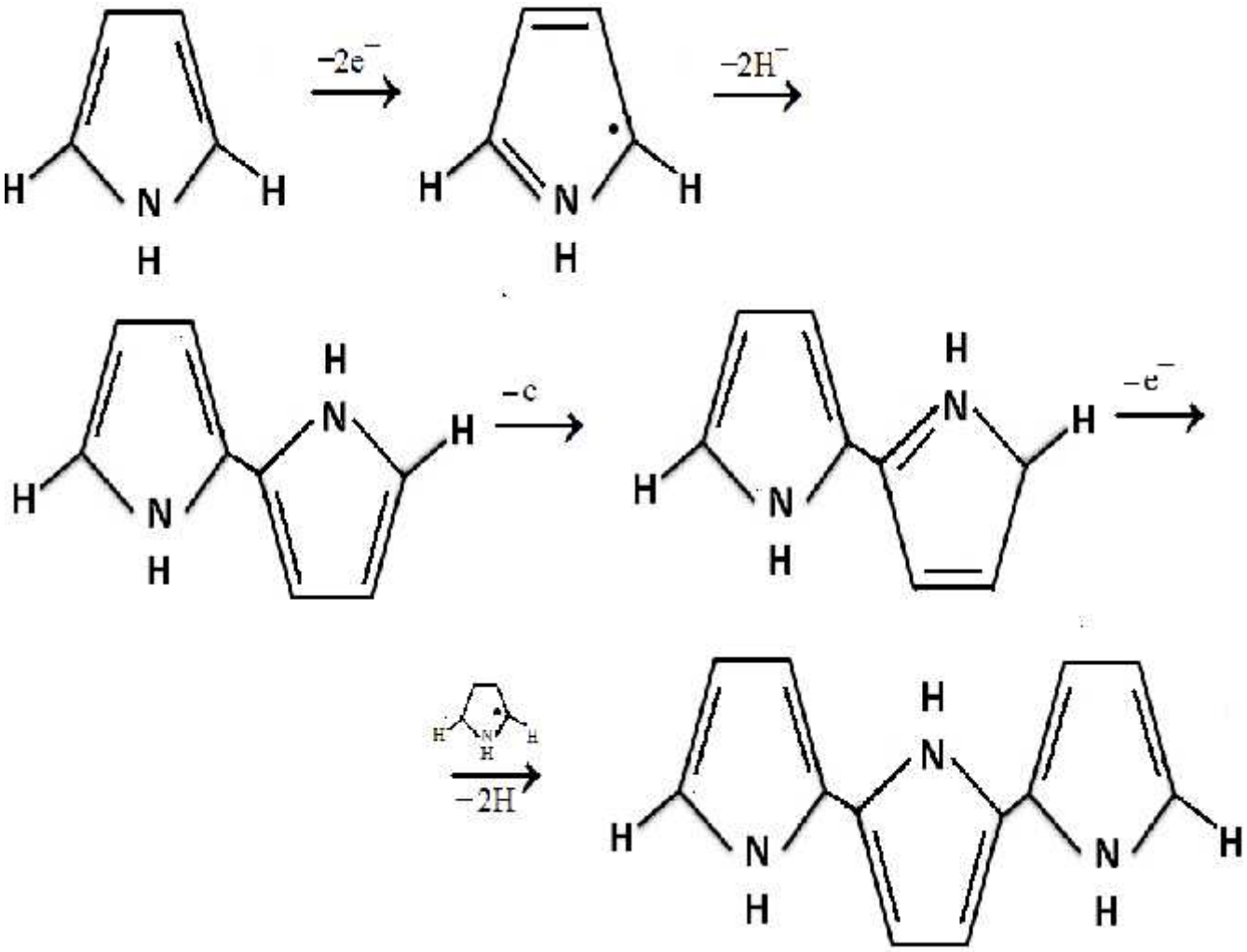
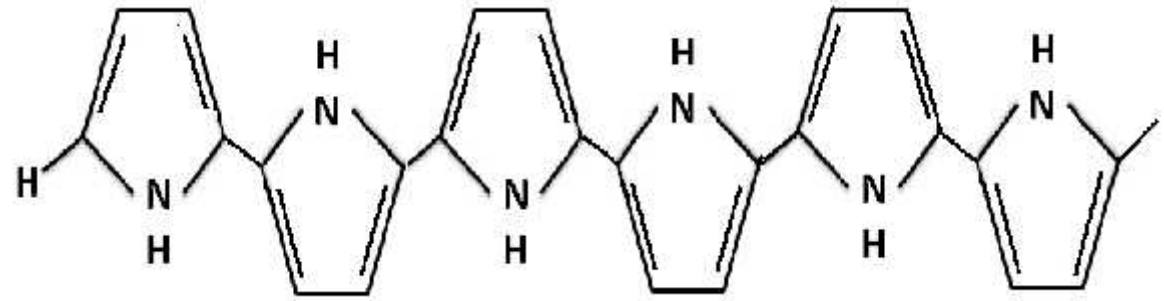
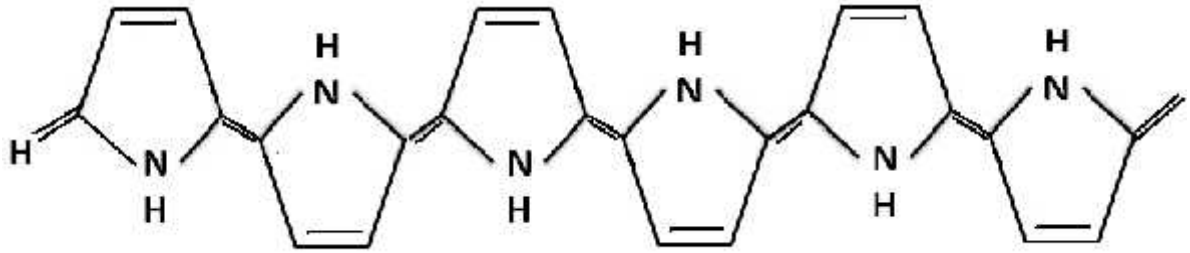


Fig.1.1: Polymerization of PPy



(a)



(b)

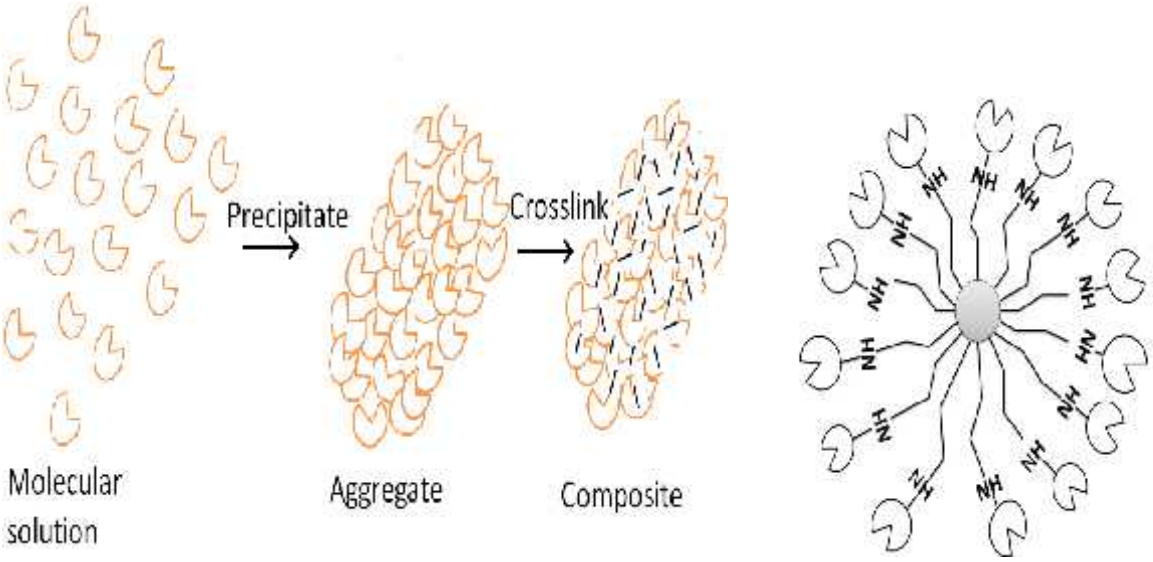
Fig.1.2 Two structures of PPy (a) Aromatic (b) Quinoid

1.2 Incorporation of bio molecule on CP

Remarkable improvement of the mechanical properties of CP can be achieved by incorporation of bio-molecules into its backbone [8-9]. Conducting polymers which are bio-compatible also, have been extensively used as matrix for incorporation of bio-molecule [10-12]. PPy and its derivatives are used as matrix because of their high conductivity, mechanical stability and the tendency to form freestanding films [13-15]. For such incorporations, entrapment is preferred over other methods because of its capacity to entrap

the molecule within the solid matrix of the polymer during the course of *in-situ* polymerization and no chemical treatment, at the risk of decremented molecular activity, is required.

Schematic of bio-molecule incorporation in CP's by physical method is shown in Fig 1.3 given below, representing: (a) encapsulation, (b) cross linking and (c) entrapment



(a) (b) (c)

Fig. 1.3: Aggregation, cross-linking and entrapment of a bio-molecule with the polymer matrix to form the composite

Bio-molecules in some cases act as a catalyst also. Enzymes are the example of catalysts which take part in biological processes and are known for their versatile nature, especially in their bio-active and regulating behavior in the living matter. Some other bio-molecules can also act as good additive for making a polymer bio-active and bio-compatible if it is helpful as a doping material in the polymerization reaction. Incorporation of such a bio-molecule

during the polymerization process can be an effective and enabling route also, for re-cycling of the catalyst [16-18].

If a catalyst, whether it is an enzyme or any other type of bio-molecule, is dissolved in the reaction medium, it is often difficult to retain or reuse it. Immobilizing a catalyst can be a straightforward route to enable recycling of a catalyst [19-20]. It is a process which entails the interaction of two materials: the bio-molecule and the carrier, wherein the surface property of each of the material is very important. However, there is no general method of immobilization or incorporation of a bio-molecule, and generally an approach of trial and error is followed until a satisfactory result is obtained. The most important point in this process is to oversee the stability of the molecule during the process. Other factors to be noticed are activity, ease of handling and cost-effectiveness. The method of incorporation can be broadly classified into two classes, viz. Physical and chemical [21]. Physical methods involve non-covalent localization of the enzyme and are reversible in nature, while the chemical methods involve at least one covalent bond between the enzyme and the support i.e. the substrate. It is irreversible in nature and the original enzyme cannot be regenerated or recovered.

The scheme of chemical immobilization for amino acid linking to a polymer is shown in Fig. 1.4.

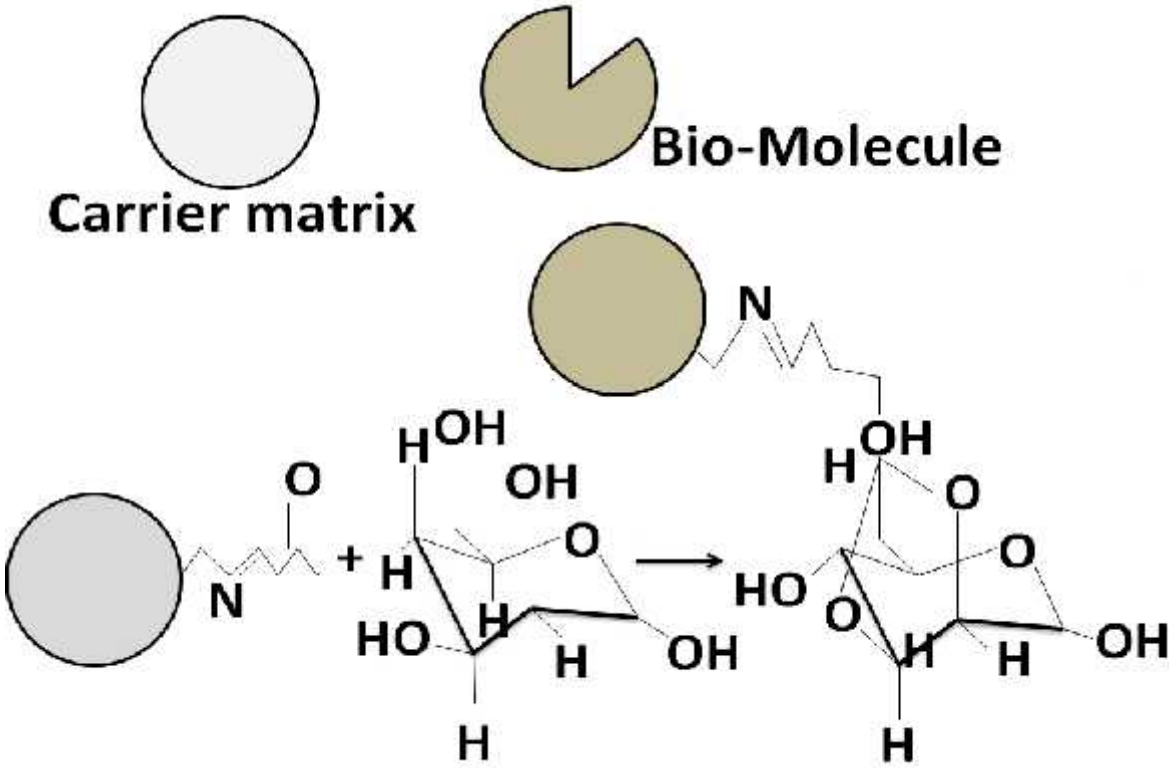


Fig. 1.4: Scheme of amino acid linking to polymer via covalent bonding

1.3 Physical vis-a-vis chemical method of incorporation:

The techniques for incorporation of bio-molecule on CP surface are based on physical or chemical adsorption. The most frequent techniques are: [21, 22]

1. Non covalent adsorption and deposition.
2. Ionic interaction.
3. Entrapment or encapsulation in a polymeric gel**.**
4. Covalent bonding.
5. Cross-linking.

Of which the first three are physical (Physisorption) while the last two are chemical (chemisorptions) in nature.

The characteristic features of each of these techniques are briefly described below.

1.3.1 Non covalent adsorption and deposition

Bio-molecules with large hydrophilic surface area interact well with hydrophilic carrier. When a bio-molecule is used in aqueous phase for adsorption on the carrier surface, one disadvantage is that it tends to percolate from the carrier because it is deposited from its aqueous solution which can be recovered afterwards by precipitation or by evaporation of the aqueous media, without involving any entropic or hydrophobic driving force [23].

1.3.2 Van-der-Waals interaction

For efficient immobilization by means of Van-der-Waals (VDW) interaction or entropy changes, both the substances - the carrier and the material need to have large lipophylic surface area. However, many molecules exhibit surface residue with both hydrophilic and well defined hydrophobic regions. For example lipase used for breaking down fats in production of bile acid and bile salt shows interfacial activation with hydrophobic superficial regions in its surface. This nature is also exhibited by different bile acids and bile salts also, and hence their incorporation on hydrophobic carrier (PPy) is considered to mimic this interfacial activation by means of VDW interaction. The VDW forces are weak but can act as the actual driving force in very short range of molecular interaction [24-25].

1.3.3 Ionic Interaction

The surface of a bio molecule may contain surface charges depending on the pH of the solvent and the charge distribution may be found out using well known modeling systems. Thus an ion exchanging carrier can act as a good substrate for enzyme immobilization by ionic and polar interactions [29]. Depending on the charge distribution pattern on the molecular surface, the ion exchanger should be charged negatively (e.g. in caboxylate) or positively (e.g. in amino groups) [30].The enzyme activity and enantio-selectivity of the enzyme varies with pH and temperature of the medium due to difference in ionic state of the molecular surface during the process of incorporation [31-32].

1.3.4 Covalent bonding

The covalent bonding method for immobilization is based on covalent attachment of enzymes to water insoluble matrices. The advantage of this method is that the enzyme is tightly fixed to the carrier preventing the leaching to the aqueous medium of the carrier, and contamination. This method is preferred when the medium is aqueous and denaturing factors exist in the incorporation process. However, covalent bonding can take place in any medium, with any organic solvent or pure hydrophobic reactant which may be used to avoid leaching [33].

1.3.5 Cross-linking

Cross-linking is the extreme case of covalent bonding generally followed in enzyme immobilization, which involves attachment of the molecule with the formation of covalent bonds by means of bi-functional or multi functional agent (such as glutareldehyde) [34]. This results in cross linked crystal or aggregates insoluble in water. Cross-linked enzyme

aggregate (CLEA) may involve formation of multiple covalent bonds and is prepared by aggregating the enzyme with the carrier when intermolecular as well as intra-molecular cross linking may be established [35].

1.3.6 Encapsulation

This involves entrapping an enzyme within the interstitial spaces of a water insoluble polymer. Polymer such as polyacrilamide, polyvinyl alcohol and CP's such as PPy, PAni etc are used for enzyme immobilization in this way [35]. It is the best method for enzyme immobilization as any structural defect due to incorporation of the enzyme over the substrate surface can be avoided. Out of different methods for encapsulation, sol gel technique is used most widely [36].

1.4 The advantage of bio-molecule incorporation on polymer matrix [37, 38, 39]:

- Low downstream processing cost
- Better stability (e.g. sensitivity of certain bio-molecules towards heat may be reduced by formation of polymer composite)
- Lesser wastage of the bio-molecule due its co-factor binding with the polymer
- Continuous and cost effective production
- Adaptability to multifarious configurations and processes

1.5 The main disadvantages are:

- Possible loss of absolute activity of the bio-molecules due to immobilization
- Additional cost of carrier or surfactant needed to complete the polymerization and mass transfer

1.6 Incorporation of bio-molecule in CP

The most important electrochemical property of CP is its ability to act as an electronic conductor. This property may be further controlled by redox switching at specific potentials. Chemically synthesized CP's are initially formed in their neutral state through oxidation (p-doping) or reduction (n-doping) and mobile charge carriers are generated which make them conductive. The polymer backbone of PPy, for example, is neutral in the reduced state and positively charged in the oxidized state. So, some counter ions are needed to maintain electro-neutrality in the oxidized state, which can diffuse into the polymer matrix during charging and comes out during neutralization. Sometimes, the oxidation process may be accompanied by significant changes in volume of the polymer due to ingression of the mobile charge - a property which can be exploited in sensing and actuator applications [40]. Thus, biosensor and biomedical applications of PPy have been emerging as a potential field of research due to its excellent bio compatibility [41].The possibility of dopant substitution with biological macromolecules such as proteins, polysaccharides and even whole living cells in the polymerization process of PPy have been explored in recent times and is still considered as a grey area in many aspects [42]

1.7 Incorporation of biological moieties on PPy: Recent trend

Garner et al (1999) studied human umbilical vein endothelial cells on PPy-heparin films choosing heparin as counter ion, which is a component of the extracellular matrix of blood vessels having anticoagulant properties [44]. They established that the conditions for synthesis as well as polymer redox state led to variations in the level of the exposed surface of heparin and showed that the PPy-heparin composite supported the growth of endothelial cells with a reduction in the normal amount of heparin required as a medium supplement.

Since cells did not grow on PPy-nitrate, this was attributed to the presence of heparin. Collier et al (2000) studied composites of PPy with glycosaminoglycan and hyaluronic acid (HA) [45]. They showed that HA retained its affinity towards the surface of the polymer. In vitro compatibility studies using PC-12 cells (cell line derived from a transplantable rat phaechromocytoma which acted as a model for primary neuronal cells) confirmed that the PPy-HA composites supported cell attachment and viability. Optimal responses for PPy-coated polyester fabrics of intermediate conductivity has been studied by Jiang et al (2002) [46] who speculated that local release of cations from PPy, which was presumed to be varying between their compositions affects cell behavior due to modification of ionic transport across the neighboring cell membrane. Polyester fabrics so coated have been shown to elicit less or comparable cellular reaction like inflammation and response to acidic/alkaline phosphatase levels when implanted in rats over 3–90 days, compared with their uncoated counterparts. Inspired from this finding, we have experimented with incorporating a PPy coating over bamboo cellulose fibrils which shows characteristic AC conductivity of PPy in cellulose. The details have been described in chapter 6. Neural recording microelectrodes coated with PPy doped fibronectin and laminin fragments [47-48] showed that behavior of the same keratinocyte cell line was modulated by redox state and the morphology of PPy in sylate films is highly modified. We have studied here incorporation of bile acid and bile salt in PPy and their ethanol sensing property (Ch. 3 and 4). Considerable modification of surface morphology of as synthesized PPy in the bile acid composite was observed by us. The same phenomenon was also observed when PPy was incorporated in cellulose fibrils. The inter chain hopping and low frequency dispersion observed in the later experiment was attributed to this surface modification which has been described (ch.5 and 6). The effect of incorporation in presence of an additional dopant (Dodecylbenzenesulfonic acid) was also studied and described in chapters 4 and 5.

1.8 Interaction of bio material with PPy

Oxidation and reduction of Py results in reversible intercalation of anions from the dopant, which maintain electro neutrality [50]. With the incorporation of the bio-molecule in the polymerization process, there is a change in the interaction pattern which is related with the oxidant/dopant type. The process is shown in Fig.1.5 given below where (a) represents PPy oxidized with (Cl^-) ion, and (b) represents the redox process for DBSA doped PPy when the bio-molecule is incorporated. The Oxidation-reduction process of PPy is shown in that figure. It is shown for Cl^- ion which is a small mobile dopant incorporated in oxidation in Fig.1.5a (left side), which is released when the bio-molecule is incorporated into the polymer surface (right side).

The process is shown in Fig.1.5 for DBS^- which is a large immobile dopant and remained entrapped in oxidation (in the left side of the Fig. 1.5) but switchesd back their orientation after incorporation (in the right side of Fig.1.5).

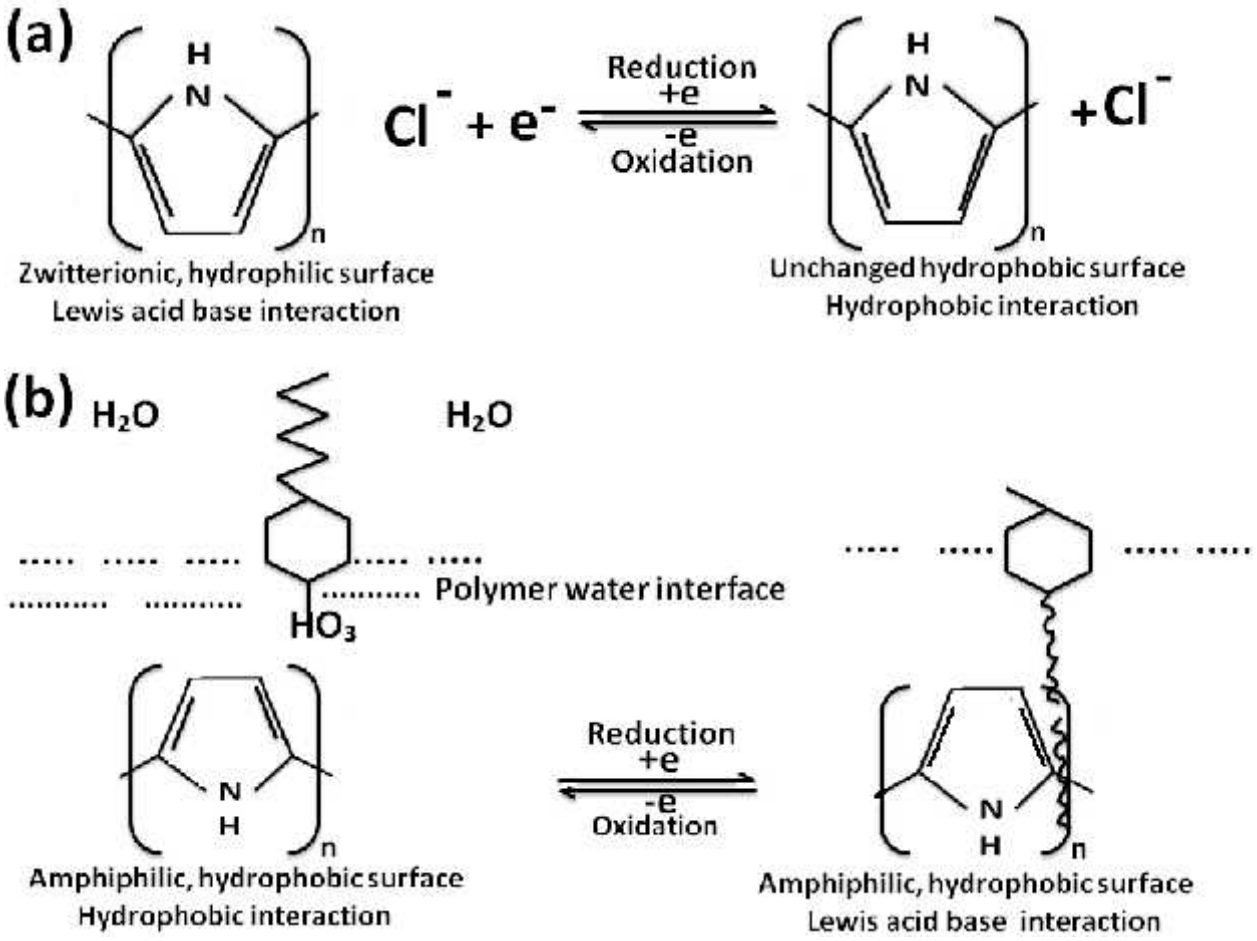


Fig. 1.5 Oxidation – reduction process of PPy in presence of (a) Cl^- and (b) DBS^-

The charge generated from oxidation is confined (localized) over a few repeating units, close to the Cl^- ion. The Cl^- ions progressively loss their hydration shell when brought into aqueous phase and the PPy/ Cl^- polymer forms a hydrophilic, zwitterionic surface in the polymer water interface, which can be reduced to a neutral surface of lower surface energy using counterions which may be, an organic acid with a sulphate group (Dodecylbenzenesulfonic acid) or a biological moiety or both [51]. The orientation of the molecule acting as counterion in the polymer solution may change in order to maintain a favored configuration for lowering

the surface energy of the polymer surface and is generally coordinated through hydrophobic interaction with the neutral polymer backbone. This was observed in our study when hydrophilic cellulose and amphiphilic bile acid and bile salt are incorporated in PPy. Surface properties such as charge, energy etc are determined by the oxidation state of the polymer and the physiochemical interactions of the dopants [52].

1.8.1 Surface energy and interaction of bio-molecule

Surface energy determines the wetting and adhesion properties of a material surface. The two forces taking part in the surface interaction of PPy with bio-molecule are Liftshitz Van-der - Waals (dispersive) and Lewis acid-base (Polar) forces [53-54]. PPy is generally seen to posses medium to high surface energy which is a function of synthesization parameters like dopant type and concentration. The surface energy increase in the doped state is a consequence of increased conductivity due to radical cations/ions. However, in presence of strong hydrophobic groups like hydroxyl and carboxylic groups which can form intermolecular hydrogen bond, the wetting properties of the polymer may vary. The high surface energy of PPy has pointed towards the contribution of Lewis acid base interaction enabling it to bind both acidic and basic species [55]. PPy has predominant Lewis acidity and the acidic sites are probably the most energetic ones because the N-H bonds in Py act as electron (pair) acceptor and the polymer backbone behave as a positively charged one. We successfully synthesized PPy composite with bile salt because of its basic nature and with bile acid due to its amphiphilic nature and in both the cases the Lewis acid-base interaction significantly contributes to enhanced surface energy of the PPy composite. The high surface energy polymers easily absorb molecules in vapor state and this property of polymer–bile

salt/acid composites have been successfully applied in ethanol vapor sensing in our experimental study, described later in Chapters 3 and 4.

1.8.2 Surface potential for bio-interaction of PPy

A special feature of CP's is their ability to switch its surface energy in a dynamic and reversible way using electrical stimulation. Any change in surface energy is related to reorganization of the ion distribution in the polymer-liquid interface [56-57]. Thus the electrical switching of surface energy is a result of the interplay between the dopant type, dopant concentration, doping potential, polymerization time as well as the combined effect of topography and surface chemistry [58]. The effect of electrical stimulation on the polymer surface becomes clearer when we consider the polymer surface as consisting of two layers. The nearest layer is made up of fixed or bound charges, known as Stern layer and the other layer is made up of free charges, forming a diffuse double layer. The double layer is made up of the shear plane and the Gouy Chapmen plane. The potential of the shear plane is termed as zeta potential and is responsible for electrostatic forces between the charged surfaces and for controlling the stability of colloidal dispersions and is dependent on the type of dopant and pH of the medium [59-62]. Isoelectric points (iep) are found in the polymer surface generally in the very high or low pH which means that zeta potential remains as a constant parameter in CP surface and then abruptly changes at the iep. The changes in the zeta potential as a function of pH of the solution occur due to deprotonation and protonation of the polymer. It has been observed that the iep of the PPy particles are typically found in the low and high pH range and they often carry a significant charge under neutral condition which is favorable to bio molecular interaction and has the propensity to electrostatic binding of the bio-molecule with the polymer chain [63].

1.8.3 Intermolecular and biological forces for bio-interaction of PPy

The interactions that control the biological events *in vitro* and *in vivo* can be explained as interaction among the molecules, particles and surfaces. The force of interaction between two interacting particles at a distance D can be expressed as a negative gradient of energy, expressed as,

$$F(D) = - dE(D)/dD \quad \text{..... (1.1)}$$

The force inherently existing in the molecular levels are the Van-der Waals (VDW) force prominent in covalent interaction and the electrostatic force responsible for ionic interaction between the molecules. The VDW force is always attractive between similar molecules and follows power law with distance (D), given by,

$$F(D) = -\frac{1}{6} A D^{-2} \left(\frac{R_1 R_2}{R_1 + R_2} \right) \quad \text{... (1.2)}$$

Where the constant 'A' – known as Haymaker constant reflects the strength of interaction between the particles. A is related to the dispersive component of the surface energy and is dependent on geometry of the interaction.

The non-covalent interaction which is guided by long ranging electrostatic forces with range longer than the VDW force is repulsive for similarly charged and attractive for oppositely charged surfaces. It roughly decays exponentially as a function of distance and is given by

$$F(D) = © \frac{R_1 R_2}{R_1 + R_2} Z e^{-©D} \quad \text{..... (1.3)}$$

The constant Z (Jm^{-1}) is analogous with A in equation (2) and depends on the interacting geometry while κ, the Debye length represents the characteristic decay of the interaction which depends on the condition of the solution medium e.g. type and concentration of the ion, temperature and dielectric constant etc. Covalent bonds with energies ≈ 1eV generally occur in short distances, within 0.1nm which implies that the minimum force required to break this bond is 1eV/0.1nm = 1.6 $x10^{-19}$ J /0.1nm ≈ 1.6 nN which is stronger than non-covalent bond of similar energy as the later extends over long distance (≈ 1nm), and hence the force required to break such bonds are ≈ $1.6x10^{-19}$J /0.1nm ≈ 160 pN [64].

In very short range this force is significantly less than the VDW forces and hence attractive forces dominate the very short range interaction of PPy particles. In most of the bio-molecular interactions of PPy strong electrostatic attraction was typically exhibited between the positively charged polymer and the negatively charged biological moieties when subjected to electrostatic forces, resulting in higher order adhesive forces between the polymer surface and the bio-molecule.

1.8.4 Selective adsorption of bio-molecule on PPy surface

PPy has been observed to follow a selective adsorption pattern with human and bovine serum albumin (SA) which was attributed to variation of iep and effect of zeta potential in different pH for both the protein and PPy. Selective adsorption of protein in CP is generally driven by long ranging electrostatic interactions and may be influenced by the factors like intermolecular forces (e.g. hydrophobic force) and other competing interactions [65]. Decrease in interfacial interaction energy (E_{int}) is proportionate with hydrohobicity and it plays a vital role in the interaction of bio-molecule with the PPy surface [64, 65]. Thus selective adsorption is observed in case of bio-molecule incorporation on PPy. It is proposed

that such interactions induces an alteration in the double helix of the polymer structure and subsequently allows formation of hydrogen bonds and intercalation of the polymer along with the formation of specific N-H-O hydrogen bonds, N-H-S bonds, π- π stacking and N-H- π interaction [66-68]. N-H-O hydrogen bonds are found in abundance in such interactions which has relatively larger lifetime also.

Thus the bio-molecule incorporation in PPy is an emerging and challenging domain in the field of conducting polymer. We synthesize here PPy by chemical oxidative polymerization using Ferric Chloride as oxidant and DBSA as a dopant and surfactant in certain experiments. Our study was mainly confined to synthesis of PPy with bile salt and bile acid *in vitro* so as to study its sensing behavior when exposed to ethanol vapor. We also studied incorporation of cellulose in PPy and find out the effect of topography, polymerization time and DBSA dopant on AC and DC conductivity of the synthesized PPy. The effect of PPy nano-layer on bamboo fibres was studied to observe the effect of charge reorientation with any possible structural changes of the fibre. A brief account of our study as described in the chapters of this dissertation is given below.

1.9 Preview of this dissertation: Chapter-wise description

Chapter 1: Introduction, the present chapter gives the general introduction to the topic where we describe the physico-chemical process of synthesis of PPy relevant to this study and give a brief sequential overview of bio-molecule incorporation on PPy, along with the theoretical description of the effect of such incorporation as well as the related problems and prospects. We tried to link our study with the general trend in this area in matters of its synthesis and application.

Chapter 2: Materials and method, this chapter deals with the materials and techniques used for synthesis and characterization of the polymer composite. Brief description of the apparatus and their working principles of characterizations have been covered in this chapter.

Chapter 3: Synthesis and characterization of PPy/cholic acid bio-material.

Cholic acid, which is one of the components of bile acid obtained from sheep bile, is irreversibly incorporated in PPy and a composite biomaterial is produced which is electro-active and bio compatible for potential use in the field of bio engineering. The composite is produced in powder form and studied for its morphological and electrical properties.

Chapter4: Synthesis and characterization of bile salt incorporated thin film of PPy

Bile salt obtained from fish bile can be incorporated into the polymer matrix of PPy by *in situ* polymerization of the latter which is used for ethanol vapour sensing. Thin film of the composite is prepared and any effect of alcohol vapor on the film is studied, considering protracted difference in structural and opto-electric properties for different exposure time.

Chapter 5: Synthesis and characterization of cellulose/PPy blend with cellulose extract from cotton linter.

In situ polymerization Py with cellulose extract from cotton linter is carried out to result in cellulose/PPy polymer blend which could be useful in industrial applications. With variation of polymerization time, we find that the surface morphology and particle size varied. AC conductivity of the composite shows low frequency dispersion which can have potential use for microelectronic applications.

Chapter 6: Incorporation of PPy nano-particles over the bamboo fibre surface.

PPy nano particles obtained by oxidative polymerization have been incorporated as surface layer over the fibre surface of bamboo cellulose which results in changes in the physical properties of the fibre. Incorporation of PPy nano particles produced by means of *ex-situ* polymerization over the fibre surface was achieved and subsequent characterization exhibited

interesting changes in surface morphology and conductivity opening its potential use as conducting fibre in textile industry and in micro-electronics.

Chapter 7: Overall summary, describes the summary of results and discussion of the studies covered in chapters 3-6.

Finally we add an appendix at the end containing the list of publications of the results of this work in referred journals (with the first page of the reprint) and seminars/conference/workshops attended.

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