CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE SURVEY

2.1 Theoretical Background

2.1.1 Electrospining Technique

As the name suggests, electrospinning depends on electrostatic forces to form fibers. A high electric field is applied between a reservoir containing a polymer solution and the collector. Under the influence of the electric field, a droplet of the polymer solution at the tip is deformed into a conical shape (Taylor cone) shown in **Fig 2.1**. If the electrostatic repulsive forces overcome the surface tension, a fine charged jet is ejected. The jet moves toward a counter electrode. Because of the extensional viscosity and entanglement of the polymer, the jet remains stable. As the solvent evaporates, the liquid jet is stretched to many times its original length to produce ultra thin fibers. Until now, most by polymers have electrospun via solution-based. Recently, some polymers were also supplied from melt (melt-based electrospinning).

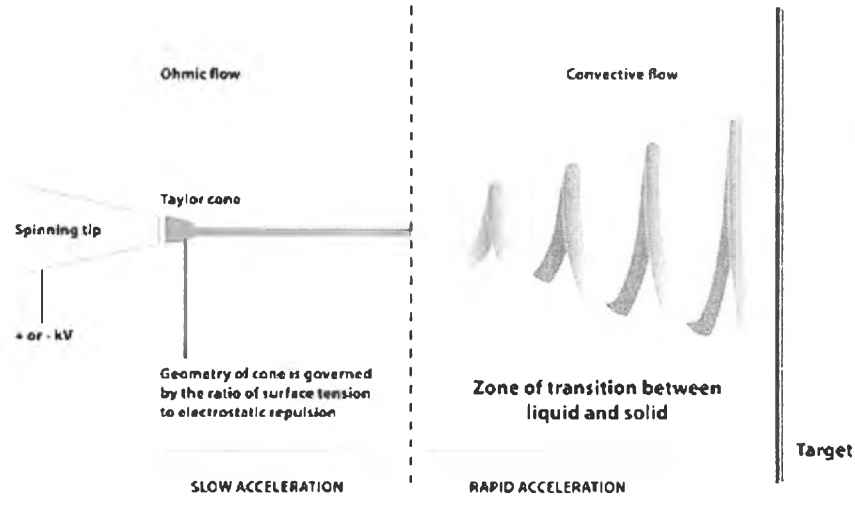


Figure 2.1 Electrospining process

Although electrospinning has received considerable attention for the past few years, the idea originated by Formhals in 1934. He issued several patents

using electrostatic force for fiber fabrication. Since then, electrospinning has been done sporadically. Acrylic microfibers ranging from 0.05 μm to 1 μm were electrospun by Baumgarten. He found that fiber diameter increased with solution viscosity, and was proportional to jet length. He also analyzed the effects of flow rate and humidity. In the mid 1990s, several research groups (including Reneker's at the University of Akron) revived interest in electrospinning by demonstrating the fabrication of various kinds of polymers. Reneker suggested the splaying phenomenon, in which electric repulsive forces splayed the jet into many smaller jets making smaller fibers. After splaying, a number of fine jets moved toward the collector. The fiber diameter was between 0.05 μm and 5 μm, and other morphologies (such as beads, coils, and ribbons) were obtained. Reneker's group published a series of papers about electrospinning, and produced more than 10 kinds of polymer nanofibers. They approached electrospinning theoretically. In fact, the first nanoscale fibers were obtained by Reneker's group. Since then, more than 100 kinds of polymer nanofibers have been produced. When it became possible to make nanofibers by electrospinning, research on electrospinning expanded rapidly.

2.1.2 Conducting Polymers

Conducting polymers (CPs) are the resonance stabilized π −conjugated organic polymers. They are also named as "synthetic metals" since they mimic the electrical, electronic, magnetic and optical properties of metals retaining the ease of chemical and physical modification associated with ordinary polymers.

An intelligent material can be defined as a material capable of recognizing appropriate environmental stimuli, processing the information arising from stimuli and responding to it in an appropriate manner and time frame. The intelligent material systems and structures sense or recognize the stimuli, process the information, convert or store energy, and then actuate or generate response. Conducting polymers are sensitive to numerous stimuli and can be made to respond. Additionally, they can store information and energy and are capable of performing intelligent functions. Hence, they are worldwide used in construction or improvement of intelligent materials systems or structures by many research groups as well as companies. Their unique and practical advantage is the behavior manipulation in situ

using appropriate stimuli **Table 2.1**. Currently, the chemical structures od CPs were widely used for research shown in **Figure 2.2**.

Table 2.1 Property changes typically observed upon electrical stimulation to switch CPs between oxidized and reduced states

Property	Typical change	Potential application
Conductivity	From 10^{-7} to 10^{3} S/cm	Electronic components,sensors
Volume	10%	Electromechanical actuators
Color	300 nm shift in absorbance band	Displays, smart windows
Mechanical	Ductile to brittle transition	-
Ion permeability	From 0 to 10^{-8} mol $cm^{-2}s^{-1}$ in solution	Membranes

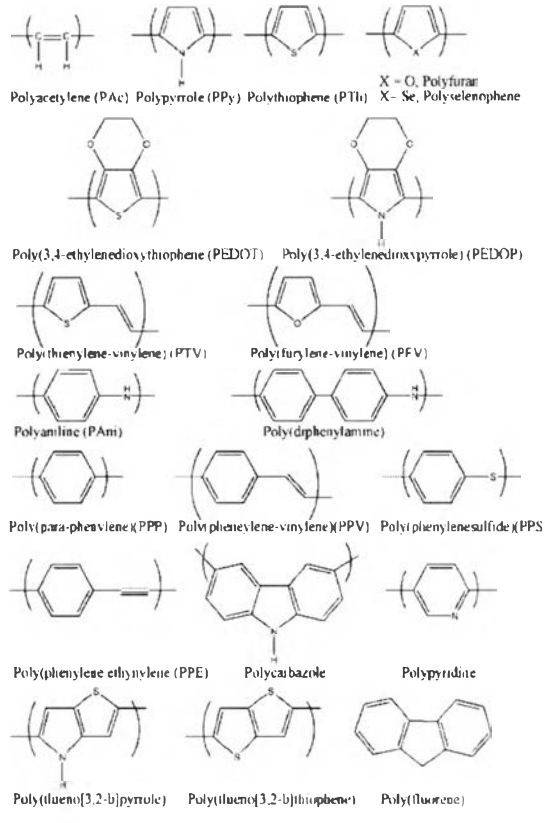


Figure 2.2 Typical conducting polymer structures

2.1.3. Synthesis of Conducting Polymers

Conducting polymers can be synthesized using standard methods of polymerization including conventional as well as specific routes such as Witting, Horner and Grignard reactions, polycondensation processes and metal or enzyme catalyzed reactions. The following polymerization techniques can be applied

1. Chemical polymerization
2. Electrochemical polymerization
3. Photochemical polymerization
4. Metathesis polymerization
5. Concentrated emulsion polymerization
6. Inclusion polymerization
7. Solid-state polymerization

8. Plasma polymerization
9. Pyrolysis
10. Soluble precursor polymer preparation

Chemical polymerization (oxidative coupling) is the most useful technique to prepare large amounts of conducting polymers. The monomers can be polymerized using $FeCl_3$, one of the most common oxidizing agents as indicated below in **Fig 2.3.**

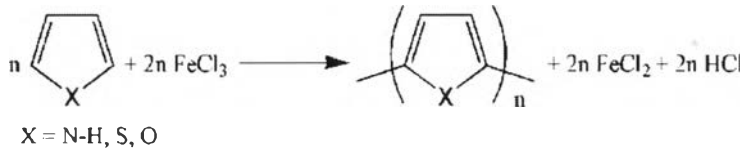


Figure 2.3 Synthesis CPs via chemical polymerization.

Chemical polymerization mechanism was investigated in 1990s and a feasible polymerization mechanism for 3-alkyl thiophenes was developed on the basis of crystal structure of $FeCl_3$ and quantum chemical computations of thiophene derivatives. The polymerization is hypothesized to proceed through a radical mechanism rather than a radical cation mechanism. According to Niemi et.al. in solid $FeCl_3$, the iron (III) ions are mostly hidden within the crystal and chemically inert, additionally each chloride ion is coordinated to two iron(III) ions. On the other hand, at the surface some chloride ions are coordinated to only one iron (III) ion; consequently each iron (III) at the surface of the crystal has one unshared chloride ion and one free orbital. Hence, the active sites in polymerization are the iron (III) ions at the surface of the crystal with strong Lewis acid character because of the one free orbital. The chloride ion that is no longer coordinated to the iron (III) ioncapture a proton from the radical cation forming HCl molecule. The suggested mechanism is shown in **Fig 2.4.** Solution or spin casting as well as vacuum deposition can be used to obtain polymer films after chemical polymerization if conducting polymers synthesized are soluble in common solvents.

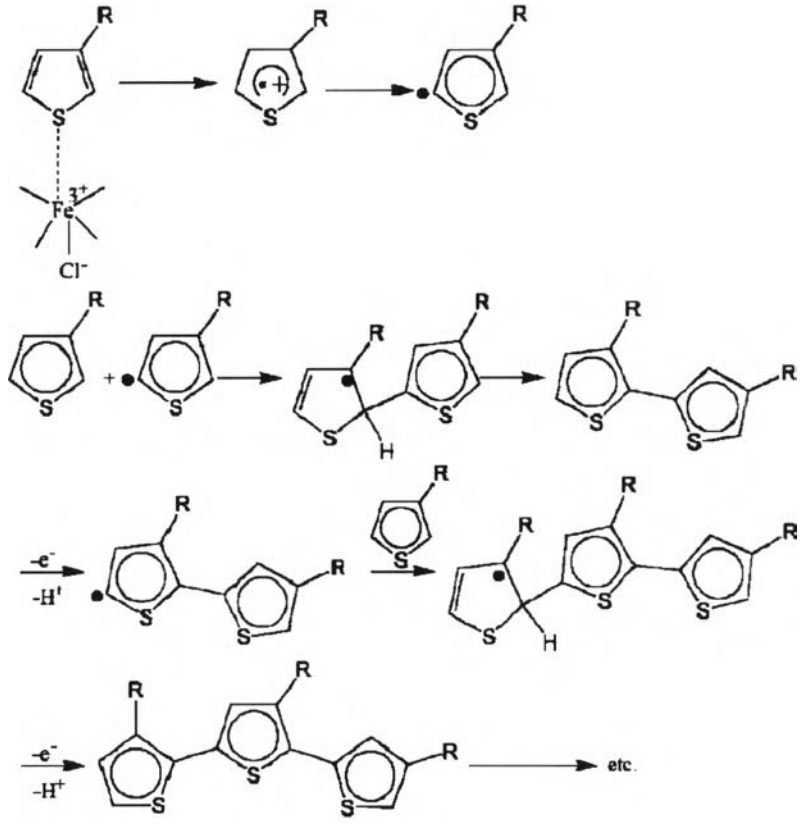


Figure 2.4 Chemical polymerization via radical mechanism

2.1.4 Synthesis of Conducting Polymers by Vapor-phase Polymerization

Alternatively, a conjugated polymer can be deposited by in situ oxidative polymerization directly at the surface. On conducting substrates this can be achieved by electrochemical polymerization, which generally gives coatings of a high quality. This method is however rarely suited for large-scale applications and cannot be used to add electronic functionalities to nonconducting surfaces. Chemical oxidation, on the other hand, is more versatile and less restricted by the substrate. Chemical oxidation can be performed by coating the surface with a mixture of monomer and oxidant where the spontaneous reaction is suppressed in such a way that it will first occur after the mixture is spread onto the surface. Often such mixtures will have a limited pot life, and more freedom in the design of the coating process can be achieved if the monomer and oxidant are applied separately. One way to achieve this

is to apply the oxidant by solvent coating and subsequently expose the coated surface to monomer vapor, a process that has been coined vapor phase polymerization (VPP)

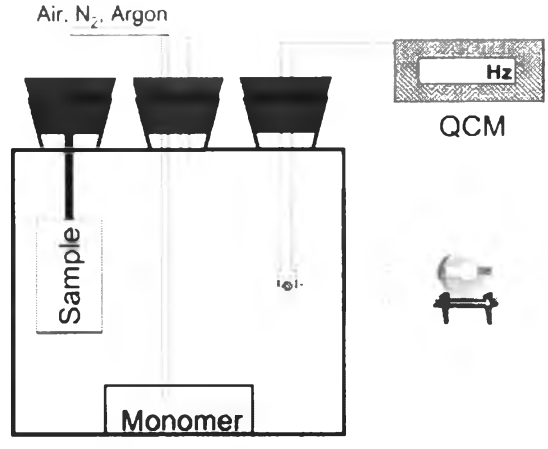


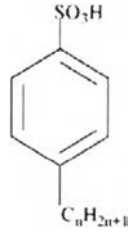
Figure 2.5 Schematic drawing of polymerization chamber

of vapor-phase polymerization

Mohammadi et.al. described as a VPP process using $FeCl_3$ or H_2O_2 as oxidants for polymerization of polypyrrole films. It was later adapted for the formation of well-defined surface patterns of polypyrrole using copper converted to $CuCl_2$ asoxidants. VPP has also been used for in situ polymerization of polypyrrole inside a number of different The homogeneity reported for surface films made by VPP has generally been inferior compared to films made by solvent processing. This is seen either in the topography of the films or in a percolation-like behavior of the conductivity as a function of thickness, where the conductivity suddenly rises when individual grains coalesce. One reason for the poor film quality is the preference for use of $FeCl_3$ as the oxidant. $FeCl_3$ crystallizes readily when the solvent evaporates, and the grainy structure of the deposit is then transferred to the polymer film formed in VPP process. The use of $FeCl_3$ was originally advocated because the presence of trace amounts of chlorine showed up to be essential for production of conducting polypyrrole (PPy) under VPP conditions. To get smooth films it is, however, necessary to suppress crystallite formation in the dried layer of oxidant, and it will be shown here that the use of organic sulfonates, that does not readily crystallize, is a versatile route to both smooth and highly conducting films. Nonconducting polymers

and rubbers, as first reported by Ueno et al., who made a conducting composite by exposing PVC blended with $FeCl_3$ to pyrrole vapors.

de Leeuw *et al.* found that ferric *p*-toluenesulfonate (Fe(III) tosylate) and their derivatives, were a well-suited oxidant for chemical polymerization of 3,4-ethylenedioxythiophene (EDT) as shown in **Fig 2.6** . Imidazole was used as inhibitor to suppress the polymerization until the solvent was evaporated. As Fe(III) tosylate did not crystallize under these conditions, smooth and well-conducting films were obtained. Fe(III) tosylate was tested in VPP of PPy in preformed polyurethane foams. Although the results were promising, it appears that no other attempts to utilize Fe(III) sulfonates as oxidants in VPP processes have been reported. The present paper demonstrates how conducting films of PPy and polythiophenes can be prepared by VPP using various organic ferric sulfonates. These conjugated polymers systems are chosen because they are insoluble and cannot be prepared by conventional solvent processing. VPP appears to be the only alternative to electrochemical oxidation for the synthesis of well-conducting films of PPy.



Where n = 0, benzenesulfonic acid
n = 1, p-toluenesulfonic acid
n = 2, p-ethylbenzenesulfonic acid
n = 12, p-dodecylbenzenesulfonic acid

Figure 2.6 Derivative of sulfonic acid used as oxidizing agents for vapor-phase polymerization

2.1.5 Enzymes

The 20 different amino acids found in living organisms are the building blocks of peptides/proteins and they play important roles in metabolism. Peptides and proteins are macromolecules made up from long chains of amino acids shown in **Fig 2.7** and they are joined head-to-tail via peptide bonds **Fig 2.8.** Shorter chains of up to a few hundred amino acids are referred to as peptides; on the other hand, proteins may consist of thousands of amino acids. The sequence of the amino acids within the molecule is essential for the structure and function of peptides/proteins in biological process. Moreover, enzyme can form double bond character of the C-N bond peptide as shown in **Fig 2.9.**

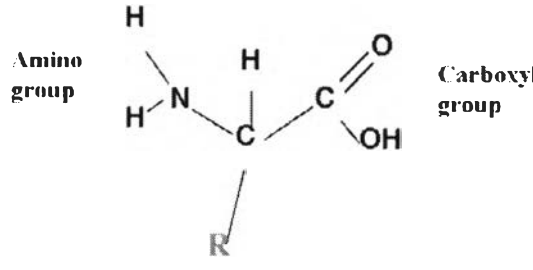


Figure 2.7 General structure of an amino acid; the substituent group (R) varies from one amino acid to another

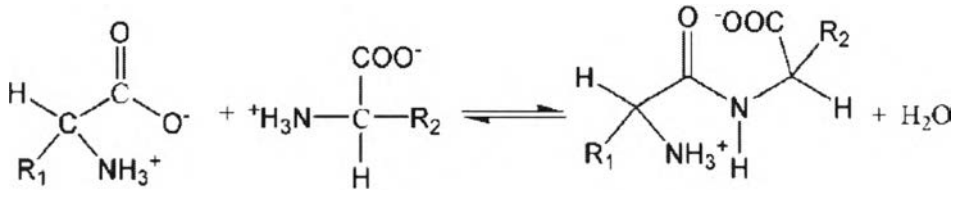


Figure 2.8 Peptide bond formation from two amino acids

The C-N bond cannot rotate due to its partial double character giving to the peptide unit NH-CO rigidity. However, the bonds to the neighboring C atoms can rotate within steric constraints allowing to folding of proteins

Figure 2.9 Double bond character of the C-N bond in peptide

The three-dimensional structure of a protein is very well defined and essential for it to function. Proteins are found in all forms of living organisms and perform a wide variety of tasks. There are mainly two types of proteins: 1. Fibrous, elongated proteins which are insoluble in water and provide structural support. 2. Globular, spherical proteins which are water soluble and have specific functions in the immune system and metabolism. Globular proteins have compact structure with very characteristic grooves and peaks on their surface. Analogous to a key fitting into a lock, other molecules fit into these grooves and peaks. Enzymes and antibodies are example of such specific proteins.

2.1.5.1 General Properties of Enzymes

Enzymes are biological catalysts that speed biochemical reactions without being permanently changed. Every enzyme is very specific in its action and can increase the rate of one particular reaction or one type of reaction. The name of an enzyme is often formed by adding *ase* to the name of its substrate. **Fig 2.10** compares energy of activation (E_a) when an enzyme is not present to when an enzyme is present; illustrating that enzyme lowers the amount of energy required for activation.

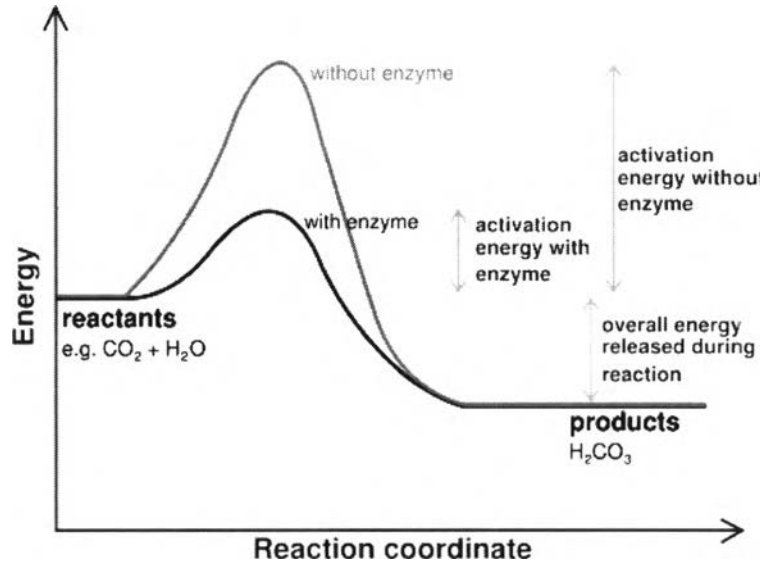


Figure 2.10 The utilization of enzyme in catalytic chemical reactions.

Enzymes, as all catalysts, increase the reaction rate lowering energy of activation region of the protein called active site. The characteristics of active sites can be summarized as follows:

1. The active site constitutes a small portion of the overall protein structure
2. The active site is a three-dimensional niche in the protein
3. The specificity of the enzyme depends on the arrangement of atoms in the active site
4. The substrate-enzyme binding process involves a relatively small amount of energy

The mechanism by which the active site takes part in the reaction was first postulated by Emil Fischer in 1860. The specificity of the reaction, according to Fischer, was the result of the "lock and key" fit of the enzyme and substrate. However, in 1962 the "induced fit model" by Daniel Koshland was proposed because the enzyme is induced to undergo a structural rearrangement upon substrate binding to

accommodate it more. The change in shape of the active site facilitates the reaction. After the reaction has been completed, the products are released, and the active site returns to its original state. Only a small amount of enzyme is actually needed in a cell since enzymes are repeatedly used. **Fig 2.11** depicts an enzymatic reaction.

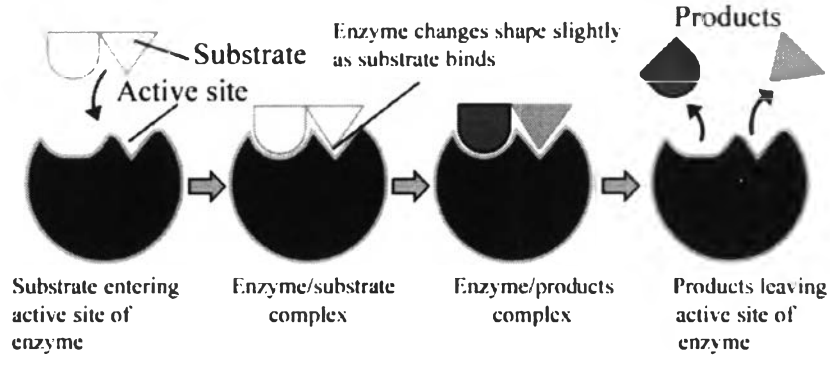


Figure 2.11 Diagrams to show the induced fit hypothesis of enzyme Reaction

The decrease in the rate of a reaction brought about by the addition of a substance, inhibitor, is called inhibition. In competitive inhibition in **Fig 2.12**, another molecule is so close in shape to the enzyme's substrate inhibits the reaction. In noncompetitive inhibition, a molecule binds to an enzyme, but not at the active site, leading to a shift in the three-dimensional structure of the enzyme.

Denaturation is defined as the breakdown of the numerous interactions which maintain the biologically active conformation. Due to cooperative nature of the forces which sustain the ordered structure, denaturation generally results in essentially random conformation. Many enzymes require a nonprotein "cofactor" to assist them in carrying out their function. Some cofactors are ions; magnesium (Mg^{2+}), potassium (K^{+}) and calcium (Ca^{2+}) are often involved in enzymatic reactions. Some other cofactors, called "coenzymes", are organic molecules that bind to enzymes and serve as carriers for chemical groups or electrons

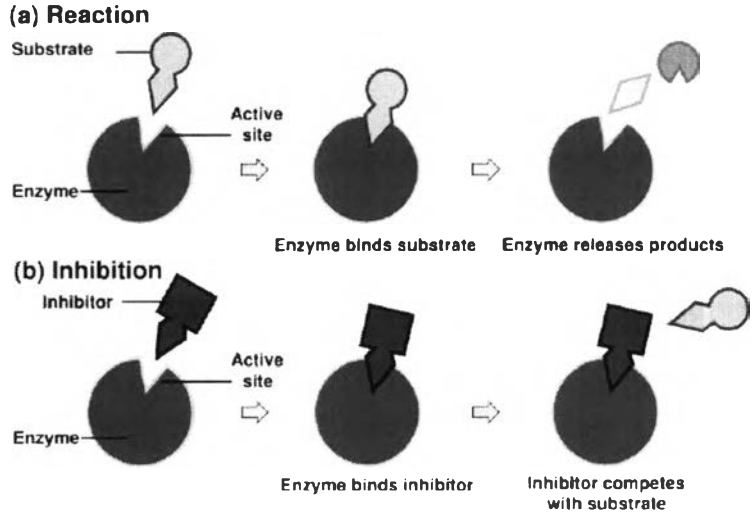


Figure 2.12 Competitive inhibitors bind reversibly to the enzyme, preventing the binding of substrate

2.1.5.2 Factors Affecting Enzymatic Speed

Rates of enzymatic reactions depend on amount of enzyme and substrate as well as temperature and pH in absence of inhibitors. To achieve maximum product per unit time substrate should fill active sites most of the time. To study the effect of enzyme concentration on the reaction rate the substrate is kept in excess amount. Hence, the order of reaction becomes zero, in other words the reaction becomes independent of substrate concentration. **Fig 2.13** depicts that any change in the amount of product formed over a specified period of time is dependent upon the level of enzyme present. When the amount of enzyme is kept constant and the substrate concentration is gradually increased, the reaction velocity will increase until it reaches a maximum **Fig 2.12.**

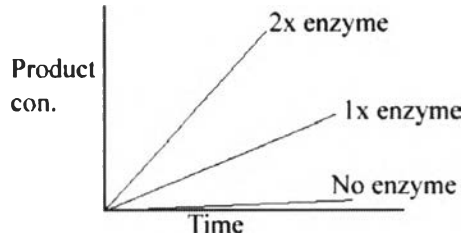


Figure 2.13 "Zero order" reaction rate involved with independent of substrate concentration

A higher temperature generally results in an increase in enzyme activity. As the temperature rises, the movement of both enzyme and substrate increases, and there are more effective collisions between them. If the temperature rises beyond a certain point, the enzyme is denaturated and no enzyme activity is observed. At that temperature, the energy introduced to the system begins to overcome the energy of the active forces holding the enzyme in its 3D form. For the majority of the commercial enzymes, the optimal temperature range is between 40 °C and 60 °C. Effect of temperature on reaction rate is shown in **Fig 2.15**. The ability of the amino acids at the active site of an enzyme to interact with the substrate depends on their electrostatic state, i.e. whether they are properly charged or uncharged, as well as their spatial orientation. Enzymes, with some exceptions, generally work in the pH range of 6.0-8.0. If the pH is not right, the charge on one or all of the required amino acids is such that the substrate can neither bind nor react to produce product. In addition, the static forces holding the amino acid chain may be altered and the chain may unfold. Each enzyme has an optimal pH that helps maintain its normal configuration.

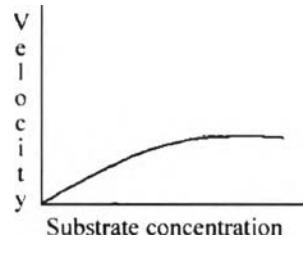


Figure 2.14 Effect of substrate concentration on the velocity of an enzyme

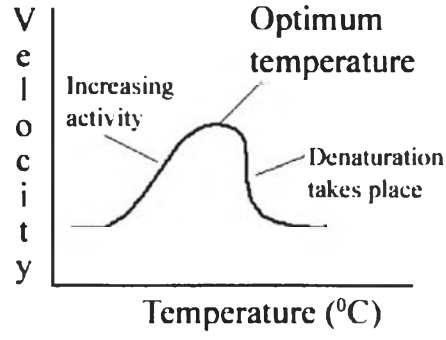


Figure 2.15 Effect of temperature on reaction rate

2.1.5.3 Basic Enzyme Kinetics

The kinetic feature that most distinguishes enzyme-catalyzed reactions from simple chemical reactions is that they show saturation. Nearly all enzyme catalyzed reactions show first-order dependence of rate on substrate concentration at very low concentrations, but instead of increasing indefinitely as the concentration increases, the rate approaches a limit at which there is no dependence of rate on concentration and the reaction is zero order with respect to substrate. **Fig 2.16** illustrates this behavior

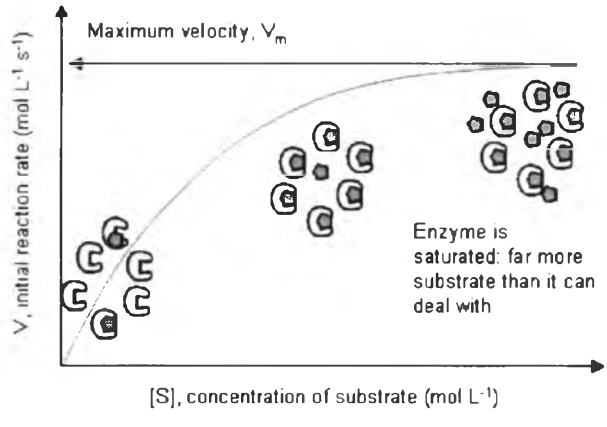


Figure 2.16 Dependence of rate on substrate concentration for a typical enzyme catalyzed reaction

In 1913 Michaelis and Menten proposed a mechanism to explain these observations. Their mechanism supposes that the first step in the reaction is the binding of the substrate "S" to the enzyme "E" to form an "enzyme-substrate complex" (ES) which then reacts to give the product "P" with the regeneration of the free enzyme:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad \text{Eq. 1}$$

"ES" can be denoted as "X". Since [E] + [X] = [E]0 and [S] + [P] = [S]0, there are two independent rate equations for this mechanism:

$$\frac{d[X]}{dt} = k_1[E][S] - (k_2 + k_3)[X] \qquad \text{Eq. 2}$$

$$\frac{d[P]}{dt} = k_3[X] \qquad \text{Eq. 3}$$

These two equations cannot be solved to obtain analytical expressions for [E], [S], [X] and [P] as function of time. Since enzymatic reactions are generally studied with enzyme concentrations much lower than the concentrations of substrates, it is a good approximation to assume that the enzymatic reaction is in a steady state in which d[X]/dt = 0. By introducing the equation for the conservation of enzyme, $[E] = [E]_0 - [X]$, in equation 2, we obtain

$$[X] = \frac{k_1[E]_0[S]}{k_1[S] + k_2 + k_3} \qquad \text{Eq. 4}$$

Substituting this expression in equation 3 yields

$$\frac{d[P]}{dt} = \frac{k_3[E]_0}{1 + (k_2 + k_3)/k_1[S]} \qquad \text{Eq. 5}$$

The steady-state equation for the overall reaction is frequently written

$$v = \frac{k_{cat}[E]_0}{1 + K_m/[S]} \qquad \text{Eq. 6}$$

Where "k_{cat}" is the "turnover number", in this case "k_3", and "K_m" is the "Michaelis constant" in this case "$(k_2+k_3)/k1$". The turnover number is the number of product molecules produced per enzyme molecule (strictly per catalytic site) per second. The equation 6 can be written as shown:

$$v = \frac{v_{max}[S]}{K_m + [S]} \qquad \text{Eq. 7}$$

The most natural way of plotting steady-state kinetic data is to plot the rate against the substrate concentration as in **Fig 2.17**. However, determination of kinetic parameters is difficult since the line is curved. To transform the Michaelis-Menten equation into the equation for a straight line there are three ways. The most popular is plotting Michaelis-Menten plot relating the reaction rate v to the substrate concentration

as illustrated in **Fig 2.18**. This is obtained by taking reciprocals of both sides of **Eq.7**.

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_m}{v_{max}}\frac{1}{[S]} \qquad \text{Eq. 8}$$

The reaction rate is the number of reactions per second catalyzed per mole of the enzyme. Since the reaction rate asymptotically increases with increasing substrate concentration approaching the maximum rate V_{max}, there is not a clearly-defined substrate concentration at which the enzyme can be said to be saturated with substrate. A more appropriate measure is to characterize an enzyme is the substrate concentration at which the reaction rate reaches half of its maximum value ($V_{max}/2$). This concentration is equal to the Michaelis constant, K_m .

A small Km indicates that the enzyme requires only a small amount of substrate to become saturated, and vice versa. Hence, the maximum velocity is reached at relatively low substrate concentrations. K_m and affinity are inversely proportional, in other words an enzyme with small K_m shows high affinity towards its substrate.

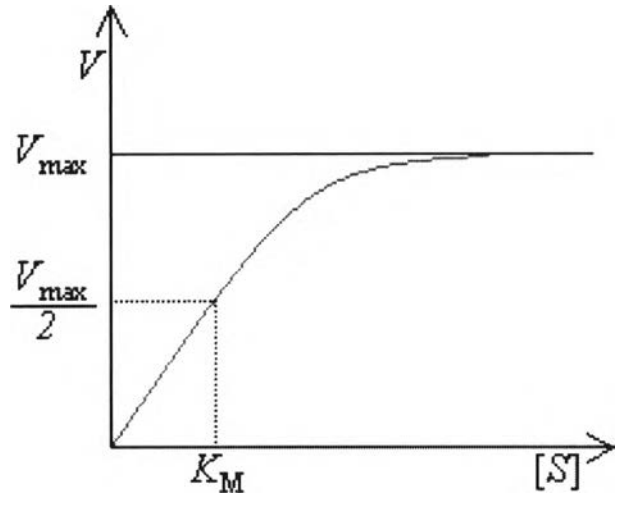


Figure 2.17 Michaelis-Menten plot relating the reaction rate v to the substrate concentration

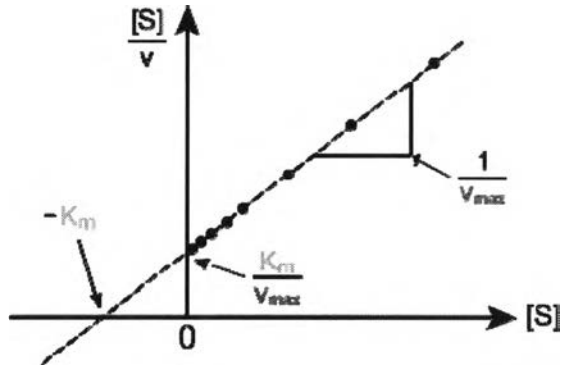


Figure 2.18 Hanes–Woolf plot relating the substrate/v to 1/substrate concentration

2.1.6 Biosensors

A biosensor can be defined as a device consisting of a biological recognition system and a transducer **Fig 2.19**. A biosensor includes two steps: a recognition step and a transducing step. In the recognition step the biological element can recognize the analyte either in solution or in the atmosphere. Bioreceptors can be

classified according to the biorecognition elements embedded; bioreceptors may be biological molecular species (e.g. antibodies, enzymes, proteins, or nucleic acids) or living biological systems (e.g cells, tissue, or whole organisms) that utilize a biochemical mechanism for recognition. The transduction element converts the analyte-receptor binding event into a quantitative optical or electrical signal. The signal can be a change in (a) the resonance unit (surface plasmon resonance), (b) the optical properties (UV-Vis-IR absorption), (c) the mass (piezoelectric biosensors), (d) the electrical properties.

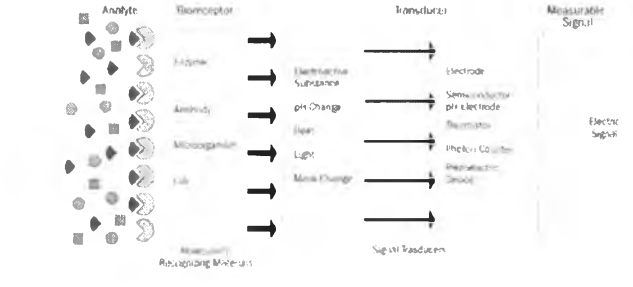


Figure 2.19 Schematic representation of a biosensor and the factors defining the sensor signal

The first demonstration of enzyme integration into an electrode was performed in the early 1960s and a term "enzyme electrode" was derived. The enzyme electrode can be defined as a miniature chemical transducer which functions by combining an electrochemical procedure with immobilized enzyme activity. Sensors need to be specific, sensitive, stable, easy to use, portable and inexpensive. Hence, several factors should be considered before choosing an appropriate immobilization technique aiming on the preservation of a maximum of enzyme activity and stability, a sufficient enzyme loading on the sensor surface, and a proper design of the sensor architecture to enable a productive communication between the biocatalytic recognition process and the transducer surface.

Thanks to their bulkiness, biomolecule immobilization in or on electro synthesized polymers is carried out following various strategies involving affinity interactions, electrostatic adsorption or incorporation, chemical grafting or entrapment

process during the electrochemical growth of the polymer. Electrochemically deposited polymer films used for biomolecule immobilization are conducting polymers such as polyacetylene, polythiophene, polypyrrole, polyaniline and polyindole. Although the most used conducting polymer, polypyrrole is a good immobilization matrix, its derivatives are also widely used to enhance its properties and biocompatibility.

2.1.7 Amperometric Biosensors

Electrochemical techniques are frequently used in transduction of chemical information into an understandable signal. Voltammetry, amperometry, potentiometry and impedometry are commonly utilized in sensing. Voltammetry refers to the measurement of current resulting from potential application, whereas in amperometry, a uniform potential is applied and the change in current is monitored as a function of time. On the other hand, potentiometry is based on the potential measurement under no current flow; the potential developed is the result of change in free energy. In the case of impedometry, the approach is to perturb the cell with an alternating potential in small magnitude and to observe the way in which the system follows the perturbation at steady state. Amperometry is extensively used in biosensor applications where an oxidation-reduction reaction is involved.

Amperometric biosensors measure the current produced during the oxidation or reduction of a product or reactant at a constant applied potential. The most important factor affecting the functioning of amperometric biosensors is the electron transfer between catalytic molecule, usually oxidase or dehydrogenase, and the electrode surface most often involving mediation or conducting polymer. Oxidoreductases produce a flow of electrons directly to the electrode in the process of turning a substrate into a product. Those enzymes contain various active sites, including flavin adeneine dinucleotides (FADs), metal centers that allow them to take part in oxidation-reduction reactions. The active sites are capable of existing at multiple oxidation states, permitting the enzymes to gain electrons from their substrate, followed by transfer of those electrons to an electrode via some intermediary. Since, oxidoreductases use oxygen in the reaction and produces H_2O_2 as side product either oxygen depletion using a Clark electrode or production of H_2O_2

can be monitored. Additionally mediated sensing employing charge mediator sites within the sensor to shuttle electrons is also used

As seen in **Fig 2.20**, the substrate reacts with the immobilized enzyme and reduces its active site by transferring electrons. The reduced enzyme then reacts with molecular oxygen present in the medium and returns to its oxidized state while generating hydrogen peroxide. H_2O_2 subsequently diffuses to electrode surface where it is oxidized generating electrons. The current change created by these electrons is measured and correlated with amount of substrate. The drawback is the variable oxygen amount in body for *in-vivo* applications. Mediated sensing in **Fig 2.21** is predicated on a signal transducer other than hydrogen peroxide that serves to "shuttle" electrons from the enzyme-substrate reaction to the electrode surface. Charge mediators, often referred as redox polymers, may be free or immobilized. In this manner, electrons may be transferred at a higher rate such that oxygen interference can be minimized. The possible leaching of mediator *in-vivo* applications is the disadvantage.

A commercially available glucose biosensor is available which the enzymatic reaction were shown in **Eq. 9.** Follow by the reaction in **Eq. 10**, the H_2O_2 will be oxidized due to the two of electrons produced: A drop of blood is applied to a disposable electrode strip, which can be inserted into the device. The electrical current is read after few seconds and the signal is converted to a glucose concentration, which is then displayed on the instrument.

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{(GOX)} \text{gluconic acid} + H_2O_2 \qquad \text{Eq. 9}$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \qquad \text{Eq. 10}$$

GOx is the glucose oxidase enzyme

Conducting polymers have the ability to efficiently transfer electric charge produced by the biochemical reaction to electronic circuit. They provide good detectability and fast response as the redox reaction of the substrate, catalyzed by an appropriate enzyme, taking place in the bulk of polymer layer. Hence, they are widely used in amperometric sensor applications. To illustrate, single-walled carbon

nanotube/polypyrrole composite film were recently used for amperometric glucose, cholesterol, urea, and alcohol sensing.

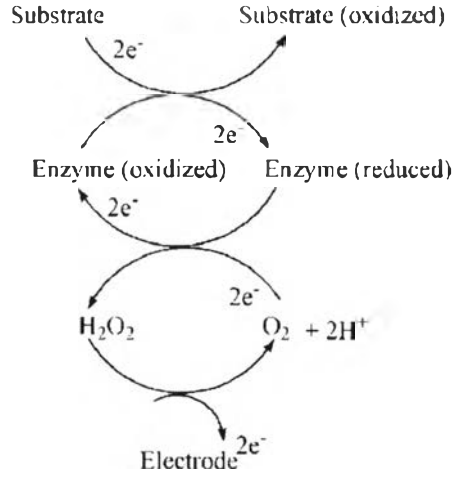


Figure 2.20 Operating principle of the amperometric detection without mediator

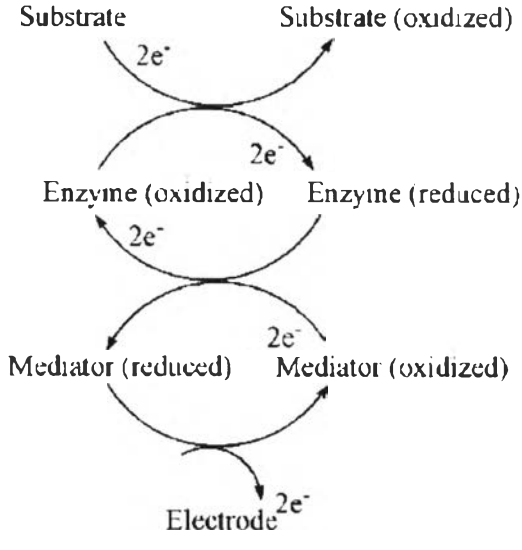


Figure 2.21 Operating principle of the amperometric detection with mediator

2.1.8 Applications of Biosensors

A wide variety of applications for qualitative and quantitative analysis is listed in **Table 2.2** Only a few biosensors have been made commercially available, such as a blood glucose sensor for home monitoring diabetics.

Table 2.2 The applications of biosensor

Field	Applications
Health care	Markers of diseases Monitoring of administered drugs Diagnosis of infectious diseases Analysis of glucose/urea/cholesterol, etc. and hormone levels
Environmental	Water and soil analysis Detection of pesticides and other toxic substances Industrial effluent control
Agriculture	Pesticides, crop diseases
Food control	Food freshness Determination of fruit ripeness by glucose content Quantification of cholesterol in butter Pathogenic organisms like E. coli
Process control	Fermentation monitoring
Microbiology	Bacterial and viral analysis

2.1.9 Cyclic Voltammetry

Cyclic voltammetry provides information about the properties and characteristics of electrochemical systems and also gives insight into any complicating side processes, electron transfer reactions, and kinetic properties. This method has been widely used to examine the redox behavior of surface deposited electroactive films qualitatively as a function of specific experimental conditions (sweep rate, solution composition, pH, temperature, etc.).

In this method, a triangular potential waveform is the input and the current/potential response to this perturbation is monitoredIn a typical experiment, the potential starts at a reducing value, and is scanned anodically until monomer oxidation and polymerization occurs. Subsequently, the potential is scanned cathodically back to the original value, where the reduction of polymer which has deposited on the electrode occurs

A typical voltammogram of a reversible process, standard ferrocene solution is shown in **Fig 2.22**. Reversibility involves the oxidized and reduced forms are in equilibrium and their concentrations can be predicted by the Nernst equation. A test for reversibility of the redox reaction is the difference in potential between the anodic (E_{pa}) and cathodic (E_{pc}) peaks aregiven with the following equation : $E_{pa} - E_{pc} = 2.203\ RT/nF = 0.056/n$

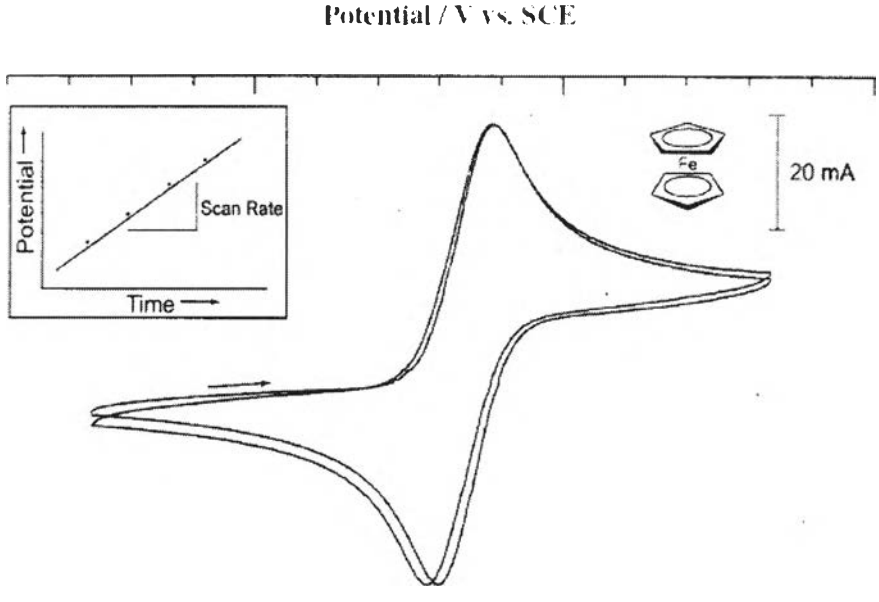


Figure 2.22 Cyclic voltammogram of ferrocene.The inset shows the definition of scan rate.

2.1.10 Square-wave Voltammetry

Squarewave voltammetry (SWV) is a further improvement of staircase voltammetry which is itself a derivative of linear sweep voltammetry as show in **Figure 2.23.** In linear sweep voltammetry the current at a working electrode is measured while the potential between the working electrode and a reference electrode is swept linearly in time. In squarewave voltammetry, a squarewave is superimposed on the potential staircase sweep. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species

begins to be oxidized or reduced. In staircase voltammetry the potential sweep is a series of stair steps. The current is measured at the end of each potential change, right before the next, so that the contribution to the current signal from the capacitive charging current is minimized. The differential current is then plotted as a function of potential, and the reduction or oxidation of species is measured as a peak or trough. Due to the lesser contribution of capacitative charging current the detection limits for SWV are on the order of nanomolar concentrations.

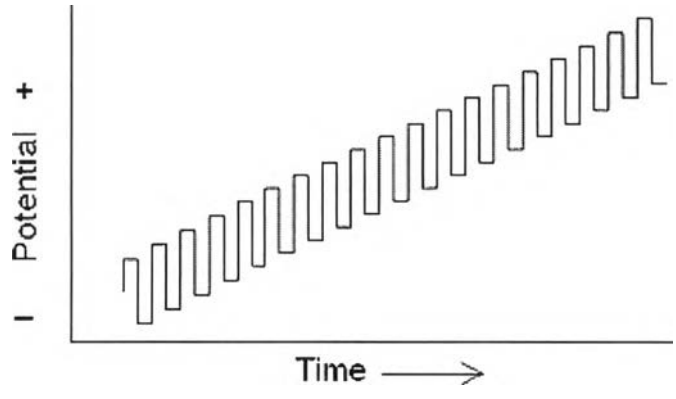


Figure 2.23 Squarewave potential sweep

2.1.11 Differential Pulse Voltammetry

Differential Pulse Voltammetry, DP, (Differential Pulse Polarography, DPP) is often used to make electrochemical measurements. It can be considered as a derivative of linear sweep voltammetry or staircase voltammetry, with a series of regular voltage pulses superimposed on the potential linear sweep or stair steps. The current is measured immediately before each potential change, and the current difference is plotted as a function of potential. By sampling the current just before the potential is changed, the effect of the charging current can be decreased. By contrast, in normal pulse voltammetry the current resulting from a series of ever larger potential pulses is compared with the current at a constant 'baseline' voltage. Another type of pulse voltammetry is square wave voltammetry, which can be considered a special type of differential pulse voltammetry in which equal time is spent at the potential of the ramped baseline and potential of the superimposed pulse.

2.1.12 Electrode Reactions

A typical electrode reaction involves the transfer of charge between an electrode and a species in solution. The electrode reaction usually referred to as electrolysis, typically involves a series of steps:

1. Reactant (O) moves to the interface: this is termed mass transport

2. Electron transfer can then occur via quantum mechanical tunneling between the electrode andreactant close to the electrode (typical tunnelling distances are less than 2 nm)

3. The product (R) moves away from the electrode to allow fresh reactant to the surface

The 'simplest' example of an electrode reaction is a single electron transfer reaction, e.g. Fe^{3+} +e− = Fe^{2+}. Several examples are shown in **Fig. 2.24**

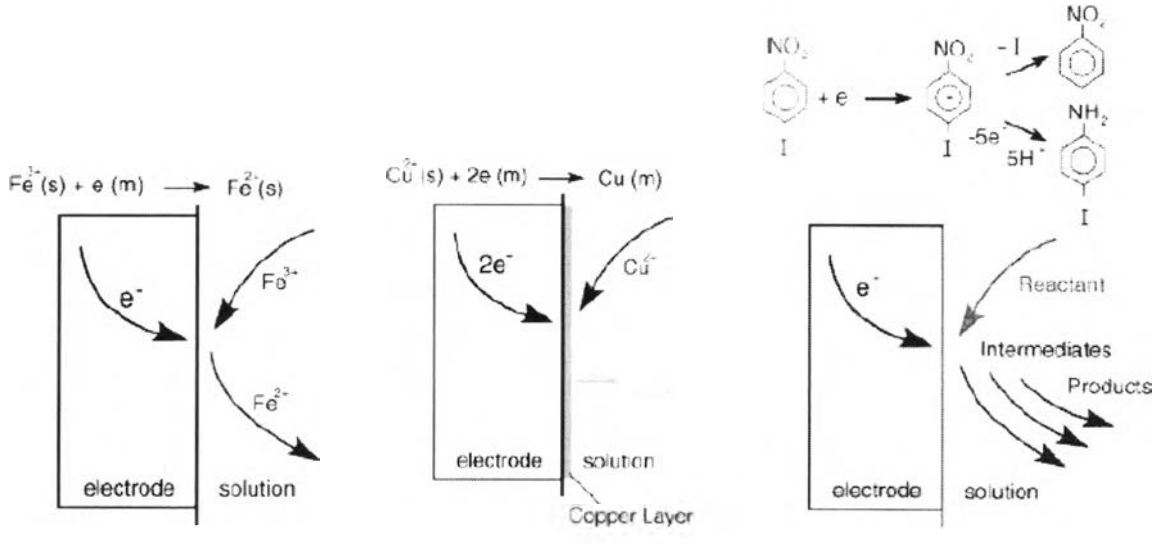


Figure 2.24 Simple electrode reactions

In **Fig 2.24** , Simple electrode reactions (left) A single electron transfer reaction. Here the reactant Fe^{3+} moves to the interface where it undergoes a one electron reduction to form Fe^{2+}.

The electron is supplied via the electrode which is part of a more elaborate electrical circuit. For every Fe^{3+} reduced a single electron must flow. By keeping track of the number of electrons flowing (ie the current) it is possible to say

exactly how many Fe^{3+} molecules have been reduced. (middle) Copper deposition at a Cu electrode. In this case the electrode reaction results in the fomation of a thin film on the orginal surface. It is possible to build up multiple layers of thin metal films simply by passing current through appropriate reactant solutions. (right) Electron transfer followed by chemical reaction. In this case an organic molecule is reduced at the electrode forming the radical anion. This species however is unstable and undergoes further electrode and chemical reactions.

2.1.13 Graphene

Graphene is a 2-dimensional, crystalline allotrope of carbon. In graphene, carbon atoms are densely packed in a regularsp^2-bonded atomic-scale chicken wire (hexagonal) pattern. Graphene can be described as a one-atom thick layer ofgraphite. It is the basic structural element of other allotropes, including graphite, charcoal, carbon nanotubes and fullerenes. It can also be considered as an indefinitely large aromatic molecule, the limiting case of the family of flat polycyclic aromatic hydrocarbons. High-quality graphene is strong, light, nearly transparent and an excellent conductor of heat and electricity. Its interactions with other materials and with light and its inherently two-dimensional nature produce unique properties, such as the bipolar transistor effect, ballistic transport of charges and large quantum oscillations.

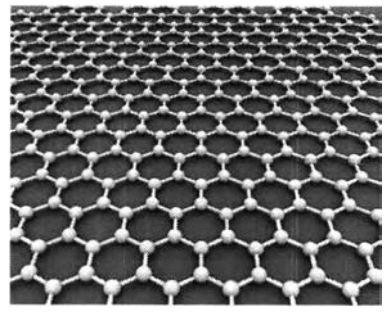


Figure 2.23 Graphene is an atomic-scale honeycomb lattice made of carbon atoms.

2.2 Literature Survey

2.2.1 Screen-printed Carbon Electrode

Much attempt has been find the new material for use as the inexpensive electrode. Disposable screen-printed carbon electrodes (SPCEs) fabricated via thick-film (screen-printing) technology offers the most promising route for the development of miniaturized sensors for the detection of different analytes including microorganisms. The low cost of the material (SPC) and its compatibility for mass production make SPCEs the primary choice for researchers and diagnostic companies, particularly for electrochemistry driven applications. The great interest in the SPCEs is also due to attractive features of the carbon: chemically inert, low background currents and wide potential window. The surface pretreatment methods for SPCE employed to-date including electrochemical, chemical treatments prior to electrochemical, and exposure to UV and oxygen plasma are usually based on either increasing the density of oxygenated groups on the electrode surface or increasing the exposure of the electroactive graphitic particles. Further improvement in the heterogeneous charge transfer rate constant and electrochemical reversibility by modification of SPCE surface with MWCNT, garphene and conductive polymer.

Elmorsy *et al.* (2008) fabricated SPCE by using the printed disposable potentiometric strips containing both working and reference electrodes are utilized as end-point indicator electrodes for the potentiometric titration of ionic surfactants in different samples The printed electrodes show very short response time reaching 3 s with adequate shelf-life (6 months). The proposed disposable strips have been successfully used for the potentiometric titration of cationic and anionic surfactants in their analytical grade solutions, pharmaceutical preparations, detergents and water samples with sensitivity comparable with the official method and ability of field measurement.

Ledru *et al.* (2006) studied the preparation and performances of screen-printed carbon electrodes modified in their bulk with HRP (HRP-SPCE) is reported. The resulting modified HRP-SPCE was prepared in a one-step procedure, and then was optimised as an amperometric biosensor operating at [0–100] mV versus Ag/AgCl in flow injection mode for hydrogen peroxide.. The best performing HRP-

SPCE in terms of sensitivity and operational stability was obtained when graphite powder was modified with HRP previously oxidised by periodate ion (IO_4^-)

María *et al,* (2005) fabricated immunosensor for Mycobacterium tuberculosis on screen-printed carbon electrodes with 2 methods. The immune complexes between *M. tuberculosis* antigens and monoclonal antibodies against *M. tuberculosis* were formed out of the electrode surface. And then, the *M. tuberculosis* antigens were captured by monoclonal antibodies against *M. tuberculosis*, which were immobilised on the electrode surface through the reaction with rabbit IgG passively adsorbed on the SPCEs

Béatrice *et al.* (2001) modified SPCEs by trigger luminol electrochem iluminescence at 450 mV versus printed Ag/AgCl. Choline oxidase (ChOD) is immobilized on the working electrode surface by ionic interactions with a diethylaminoethyl (DEAE) modified polymer (Sepharose or dextran) before entrapment in a poly(vinyl alcohol) bearing styrylpyridinium groups (PVA-SbQ) photocrosslinked matrix. The use of DEAE-dextran instead of DEAE-Sepharose allows to easily control the amount of deposited ionic polymer, but the operational stability of the sensors is decreased

2.2.2 Vapor-phase Polymerization

Recently, conducting polymer thin films have been investigated as transparent electrodes in photovoltaic devices and organic light emitting diodes. Due to its relatively high conductivity and excellent transmission in the visible region

Christopher *et al,* (2011) work with poly (3, 4-ethyelenedioxythiophene) (PEDOT) which has been shown to be a viable option for such applications. Herein described is a method for the vapor phase polymerization (VPP) of transparent PEDOT thin film electrodes on flexible polyethylene naphthalate (PEN) substrates and the comparison of this VPP method with two current approaches to PEDOT deposition: solution-based in situ polymerization and spin coating a dispersion of PEDOT:PSS. Electrical conductivities and UV–vis transmittances were measured for films produced by each of these methods, with VPP PEDOT showing both the highest conductivity (approx. 600 S/cm) and transmittance (>94% at 550 nm). The stability of these PEDOT films, stored under ambient conditions, was

investigated by monitoring the conductivity and transmittance of the thin films over time(Madl *et al.*, 2011).

Joonhyuk *et al,* (2010) synthesized conducting polymer on flexible substrates by injet printing. Complex polypyrrole patterns were obtained via oxidation polymerization of vaporized monomer on the inkjet-printed oxidant patterns. The patterned lines are readily controlled by inkjet printing with the high resolution of micrometer scale. This process provided highly conductive polymer patterns of polypyrrole with the sheet resistance of 2.8×10^3 Ω, the minimum line width of *ca.* 60 μm and the film thickness of *ca.* 450 nm. Furthermore, metallic copper pattern was prepared on previously patterned polypyrrole architectures by electroless plating as a practical application(Cho *et al.*, 2010).

Jinyeol *et al,* (2003), The conductive thin films of ferric chloride doped polypyrrole (PPy) were obtained by in situ vapor-phase polymerization method under ambient conditions. Homogeneous and thin conductive PPy films were uniformly fabricated at nano-level thickness on the plastic film substrates by continuous roll process. The conductivity of the films typically 600 nm thick rapidly increases with the increasing deposition time of the pyrrole monomer (up to 6×10^2 S/cm). These films have good electromagnetic shielding and microwave absorption properties. The change of light-transmittance follows a sine curve with the variation of the PPy film thickness. The transmittance of the film increased with thickness from 35 to 45 nm (max. 90%) and decreased with thickness from 55 to 65 nm (min. 60%). Highly ordered conductive PPy surfaces were observed by an atomic force microscopy (AFM)(Kim *et al.*, 2003).

2.2.3 Carbonization of Electrospun Nanofiber

Sungho *et al*, (2012) The effect of structural evolution polyacrylonitrile (PAN) on mechanical properties was investigated in stabilization and carbonization. PAN spun fibers were stabilized in a convection oven with a constant tension for various times at 250°C. These structural changes by stabilization resulted in the significant decrease of tensile properties of fibers. In Raman spectra with heat treated fibers from 400°C up to 1200°C, the intensity ratio of the D to G bands (I_D/I_G) decreased as heat treatment temperature increased, indicating an increase of basal

plane of graphitic layer of heat treated fibers. Tensile strength of heat treated fibers at 1200°C was found to be as high as 2.2 GPa.

Karacan *et al*, (2012) Thermal stabilization of polyacrylonitrile (PAN) precursor fiber was performed with a pretreatment of an aqueous guanidine carbonate solution and its structure. The use of guanidine carbonate pretreatment of polyacrylonitrile precursor fiber was found to be very useful for the acceleration of thermal stabilization of polyacrylonitrile precursor fiber prior to the carbonization stage. An accelerated thermally stable aromatic ladder structure formation resulting in much reduced thermal stabilization time. A totally disordered amorphous phase which seemed to be a direct consequence of the crosslinked and cyclized structure present in the stabilized fibers. Guanidine carbonate pretreated and thermally stabilized PAN precursor fibers showed a carbon yield of 52.5% at 1100 °C. The use of guanidine carbonate pretreatment is expected to significantly increase the productivity of carbon fiber manufacturing at a substantially reduced cost by significantly reducing the time necessary for thermal stabilization of polyacryonitrile fiber.

Zussman *et al*, (2005) The mechanical and structural properties of individual electrospun PAN-derived carbon nanofibers are presented. EELS spectra of the carbonized nanofibers shows the Catoms to be partitioned into 80% sp^2 bonds and 20% sp^3 bonds which agrees with the observed structural disorder in the fibers. Mechanical testing was performed on individual carbonized nanofibers a few microns in length and hundreds of nanometers in diameter. The bending modulus was measured by a mechanical resonance method and the average modulus was 63 GPa.

2.2.4 Dopamine Biosensor

(Tong *et al.*, 2013) An electrochemical method for the detection of dopamine based on a glass carbon electrode modified with electrospun CeO_2/Au composite nanofibers was investigated in this article. The CeO_2/Au composite nanofibers were prepared by the electrospinning technique and then annealed in air. Cyclic voltammetry (CV) showed that the electrospun CeO_2/Au composite nanofibers modified carbon glass electrode exhibited an excellent electrocatalytic response to the dopamine (DA). The detection limit (S/N = 3) was as low as 0.056 μM and the sensitivity could reach 127 μA mM^{-1} cm^{-2}. All these demonstrated that the

electrospun CeO_2/Au composite nanofibers were good electrocatalyst for the oxidation of dopamine.

(Rattanarat *et al.*, 2012) report the development of an electrochemical paper-based analytical device (ePAD) for the selective determination of dopamine (DA) in model serum sample. The ePAD device consists of three layers. In the top layer, SU-8 photoresist defines a hydrophilic sample application spot on the filter paper. The middle layer was made from transparency film and contained two holes, one for sample preconcentration and the other for the surfactant to allow transfer to the third layer. Furthermore, only the SDS-modified paper showed the selective shift in oxidation potential for DA. DA determination was carried out using square-wave voltammetry between −0.2 and 0.8 V vs. Ag/AgCl, and this ePAD was able to detect DA over a linear range of 1–100 μM with a detection limit (S/N = 3) of 0.37 μM. The ePAD seems suitable as a low cost, easy-to-use, portable device for the selective quantitation of DA in human serum samples.

(Fooladsa *et al*, 2012) presented a biosensor designed based on catalase (CAT) and modified carbon paste electrode (CPE) with zinc oxide (ZnO) nanoparticles for Determination level of dopamine by electrochemical methods. A pair of well-defined redox peaks was observed at the CAT / ZnO Nps/ CPE with The formal potential (E_o) equal to + (92.5 ± 1) mV. Zinc oxide nanoparticles could play a key role in creates the CAT CV response and facile electron transfer between CAT and CPE. The CAT / ZnO Nps/ CPE showed a good sensitive state towards oxidation of DA. The designed biosensor showed a good stability and retains its 91% activity after 21 days.