CHAPTER II

THEORETICAL ASPECTS

This chapter emphasizes on various theoretical models and mechanisms that are used to explain different experimental results in the present thesis. Different circuit modelling of charge transfer that are employed for the calculation of electron transfer resistance (R_{et}), capacitance (C_{dl}) and phase angle (Φ) using electrochemical impedance spectroscopy have been discussed. This is followed by the discussion of theories of cyclic voltammetry and Randles-Sevcik that are employed for the analysis of electro-catalytic behaviour of synthesized electrodes. The theory of Laviron's method of calculating heterogeneous rate constant and transfer coefficient has been discussed. Finally, the theory governing enzyme kinetics and its immobilization method has been explained.

2.1 Electrochemical Impedance Spectroscopy:

Electrochemical Impedance spectroscopy is a powerful analytical technique applied to study the resistance to the flow of charges between the counter and the working electrodes on application of small amplitude voltage signal. The hindrance to the charge flow may arise due to the different electrode processes such as electrode reaction at the electrode/electrolyte interface, the migration of charge carrying species through the electrolyte and interfaces. Impedance spectroscopy is a perturbative characterization of the dynamics of the electrochemical processes for unravelling complex non-linear processes occurring at the electrode/electrolyte interface [221, 222].

The heterogeneous reaction responsible for the Faradaic current flowing at an electrified interface due to any electrochemical reaction is given by [3]:

$$O + ne' \rightarrow R \qquad 2.1$$

Where n is the number of electron transferred, O is the oxidant and R is the reduced product. The current is always accompanied by a non-faradaic or capacitive component, no matter how well the measurement is made. The non-faradaic current arises due to the accumulation of the charged species (consumption of oxidant or production of reductant) near the electrode surface. The resistance to the flow of current when potential E is applied across the electrodes in an electrical cell [223, 224]:

$R = {}^{E}/_{I}$,

Both faradaic and non-faradaic components encountered complex resistances when an AC potential (E_t) is applied to an electrochemical cell given by:

$E_t = E_0\ Sin(\omega t)$ and $I_t = I_0\ Sin\ (\omega t + \varphi)$,

where the radial frequency is $''\omega = 2\pi f''$.

The total impedance Z is given by:

$$Z = Z_0 \frac{Sin\ (\omega t)}{Sin\ (\omega t+\varphi)} \qquad 2.2$$

The frequency-dependent impedance, Z(ω), at the interface that has the circuit made of resistors, capacitors, or inductors, or any combination of these, the total impedance is represented as the sum of the real (Z') and imaginary ($-Z''$) components [$Z = Z' + j\ (-Z'')$ [221, 225, 226], where $j = \sqrt{-1}$] with Φ (the phase angle) = $\tan^{-1}$ [$Z''(\omega)/Z'(\omega)$] where

$$Z\ (\omega) = R_s + \frac{R_p}{1+\omega^2 R_p^2 C_d^2} - j\frac{\omega R_p^2\ C_d}{1+\omega^2 R_p^2 C_d^2} = Z'\ (\omega) - j\ Z''\ (\omega) \qquad 2.3$$

at high frequencies $\omega \rightarrow \infty$.

At low frequencies ($\omega \rightarrow 0$), it becomes

$$Z(\omega) = R_s + R_p + \sigma\omega^{-1/2} - j\left(\sigma\omega^{-1/2} + 2\sigma^2 C_d\right) \qquad 2.4$$

The Warburg impedance is related to σ by the equation:

$$Z_w = \left(\frac{2}{\omega}\right)^{1/2}\sigma \qquad 2.5$$

Equation 2.3 is represented by a semicircle with a radius of $R_{p/2}$ which intersects the X-axis at Rs and (R_s+R_p) at higher and lower frequency side on the Z'(ω). According to equation 2.4, a straight line with intercept ($R_s + R_p - 2\sigma^2 C_d$) representing Wargburg impedance is obtained [227].

2.1.1 Different Impedance components in an electrochemical cell system:

(a) Solution or Electrolyte Resistance (R_s):

The solution resistance between the working electrode and the counter electrode arising due to the presence of different species in solution contributes to the impedance of an electrochemical cell. The resistance of an ionic solution depends on the concentration of redox species, temperature, and the electrode area [227]. The resistance of an electrode with area A and length l carrying a uniform current is defined as:

$$R = \rho \frac{l}{A} \tag{2.6}$$

where ρ is the solution resistivity.

(b) Double layer capacitance (C_{dl}):

A changing potential causes rearrangement and accumulation of charged particles on the interface between an electrode and its surrounding electrolyte resulting in an electric double layer. Therefore an insulating layer develops in the interface causing hindrance to the flow of current and the system behaves like a capacitor [228].

(c) Polarization Resistance (R_p):

Perturbation of the system results in deviation of the electrochemical process from equilibrium referred to as polarizing the electrode and the transition resistance between electrode and electrolyte is called polarization resistance (R_p). The polarization of electrode results in current flow which can be controlled by the kinetics of the heterogeneous reactions and the mass transfer of redox species both towards and away from the electrode [229].

(d) Charge transfer Resistance (R_{ct}):

The resistance formed by a kinetically-controlled electrochemical reaction given by equation 2.1. It is the polarization resistance (R_p) at standard electrode potential (η) [230].

(e) Warburg Impedance (Z_w):

Diffusion of the charged species across the electrolyte also can create impedance called Warburg impedance. At high frequencies of potential perturbation, the Warburg impedance is negligible as since diffusing reactants don't have to move very far. At lower frequency, the Warburg impedance increases as the reactants have to diffuse [231].

2.1.2 Common Equivalent Circuit Models:

The plot of change of the real and imaginary parts of impedance with the frequency *f* has been theoretical represented in form of circuit models. In this section some common equivalent circuits models have been discussed which can be used to interpret simple impedance spectroscopy data.

(a) Purely Capacitive System:

For a metallic electrode with a semi conducting coating has very high impedance. Figure 2.1 depicts the equivalent circuit model which consists of a resistor (R_s) (due to electrolyte) and a capacitance in series with the Nyquist Plot ($-Z''$ *vs.* Z') [232]. The value of R_s can be determined by the intercept of the curve with the X axis and the capacitance can be estimated by a curve fit.

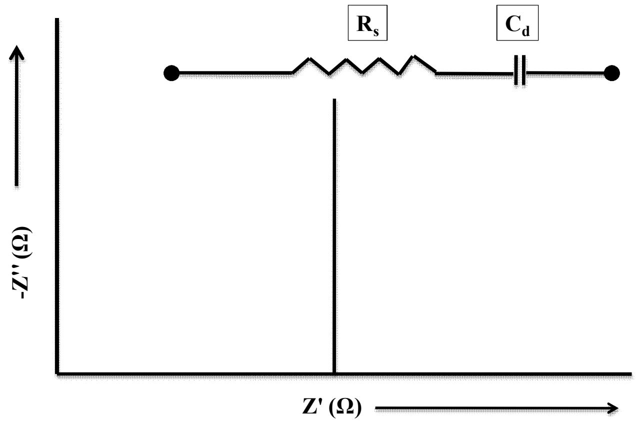

Figure 2.1: Typical Nyquist Plot for a capacitor in series with the resistor circuit and the correspondingequilvalent circuit model.

(b) Simplified Randles Cell:

The Randles equivalent circuit is one of the simplest possible models describing processes at the electrochemical interface. For a given electrochemical reaction the transfer of electron is dominated by two process (a) capacitive behaviour of the interface (C_d) and (b) the resistive behaviour of the heterogeneous transfer of electrons (R_p). Considering these two parameters in a parallel as shown in Figure 2.2, a semi-circle response on Nyquist plot is obtained. The equivalent circuit model named as Simplified Randles cell and it includes a solution resistance in series, a double layer capacitor and a charge transfer (or polarization resistance) in parallel [233]. The intercept intersecting the X axis at low frequency side is the sum of the polarization resistance (R_p) and solution resistance (R_s). The real axes value near the origin at high frequency side gives the value of R_s.

(c) Mixed Kinetic and Diffusion Control:

This circuit models a cell where polarization is due to kinetics of charge transfer accompanied by diffusion processes. At lower frequencies side, the mass transport of the electro active species (as shown in Figure 2.3) contributes to the total impedance by hindering the electron-transfer process. In this model Warburg impedance (Z_w) is added in series with the R_p which is the impedance due to the diffusion of charged particles across the electrolyte. The linear spike at low frequency side in the Nyquist Plot making an angle 45^0 with the real axes is due to the presence of Warburg impedance. For an electrode with more capacitive behavior this slope tends towards 90^0.The complete randles cell representing the kinetics and diffusion controlled reaction and the corresponding Nyquist Plot is shown in Figure 2.3 [234, 235].

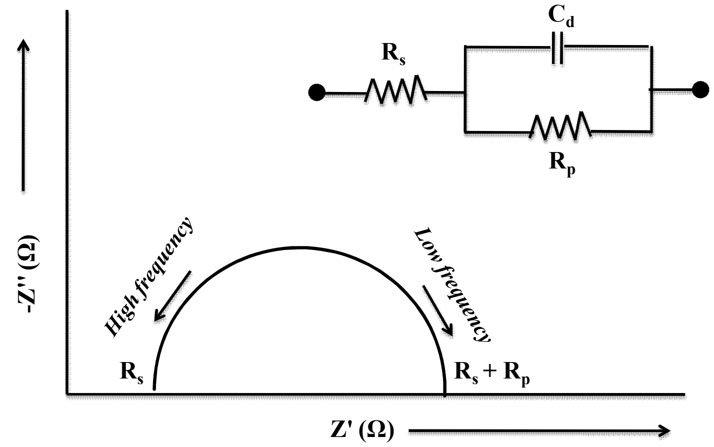

Figure 2.2: Nyquist Plot for a simplified Randles cell and the corresponding equivalent circuit model.

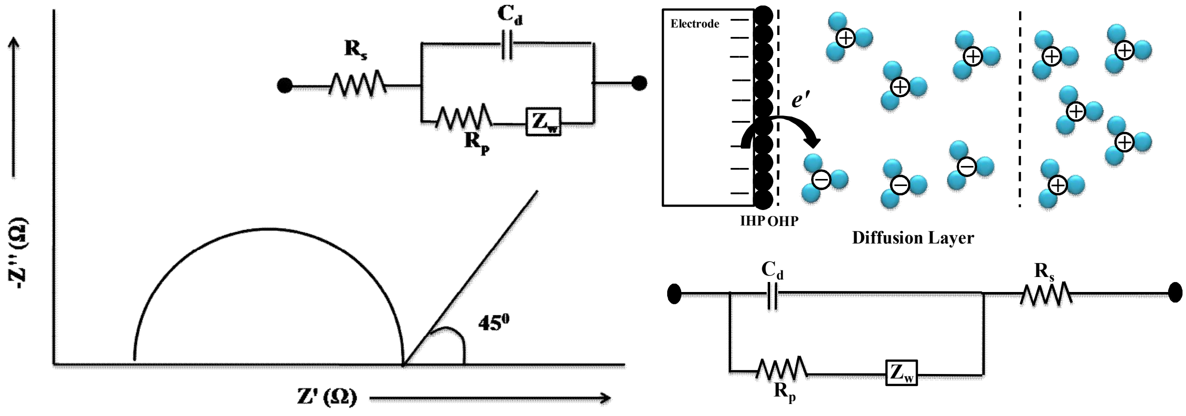

Figure 2.3: Nyquist Plot for a mixed Randles cell and the corresponding circuit model.

2.2 Cyclic Voltammetry:

Cyclic voltammetry is a method applied for study electrochemical performance of a system and it is equivalent to double potential step chronoamperometry [236]. In 1938, the theory of cyclic voltammetry was first reported and described by Randles and Sevcik [237, 238]. It is the most extensively used technique for acquiring qualitative information about the reactions occurring in an electrochemical cell system and the potentials at which they occur**.** A cyclic voltammogram (CV) can be acquired by applying a predetermined linear potential to an immersed, stationary electrode, sweeping between starting potential (E_i) to a final value (E_f) which increases or decreases with time (Figure 2.4) [239]. The applied potential results in flow of current through the electrode that either oxidizes or reduces the species allowing cyclic voltammetry an ideal method of determining concentration. This analytical method indicates the potential at which redox process occurs and the magnitude of this current is proportional to the concentration of the analyte in solution [239].

Fundamental two processes occurring at electrode electrolyte surface can be distinguished as:

(a) Capacitive Current:

Capacitive current, is also called "double-layer" current is the result of assembling or removal of charge particles in the electrode electrolyte interface when the electrode is perturbed, it does not include any actual transfer of charge [240]. As a result of depletion of the electro-active material at the electrode surface and a concentration gradient is set up. According to Fick's Law [241] (equation 2.7), the diffusion of reactant towards the electrode surface and the corresponding reaction product diffuse away from the electrode surface.

$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial x^2} \qquad 2.7$$

(b) Faradaic Current:

Faradaic current is a result of non-adsorptive electrochemical reactions ($A + ne' \rightarrow B$) occurring at the electrode interface at the electrode surface. The redox reaction is controlled by Faraday's Law (equation 2.8) [242], that is, the amount of charged species (oxidant) transfer is proportional to the number of moles of reactant converted.

$$\frac{[A]_0}{[B]_0} = e^{nF/RT} \qquad 2.8$$

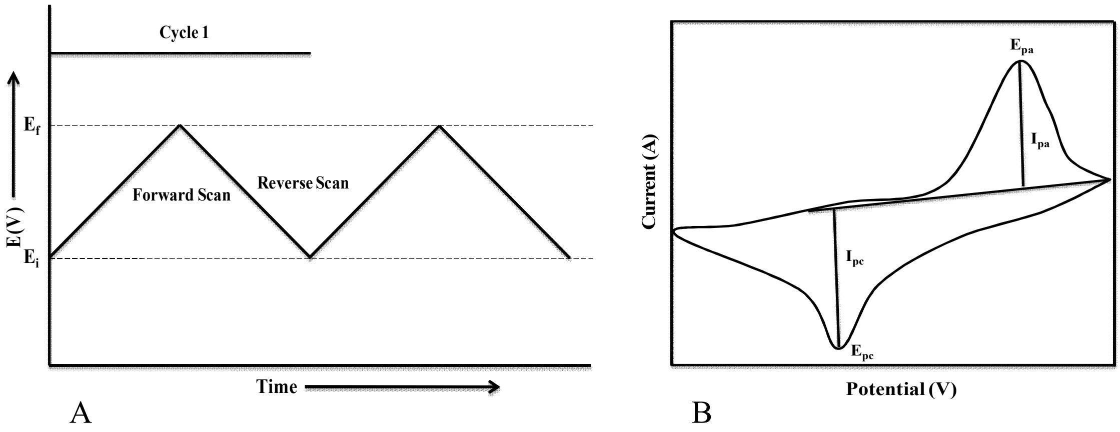

Figure 2.4: (A) Potential scan from an initial value (E_i) to final value (E_f) with respect time and (B) The cyclic voltammogram of a Nernstian electrochemical reaction.

In an electrochemically reversible process the electron transfer is not rate dependent and the rate of electron transfer is fast enough to maintain equilibrium of oxidized and reduced form of redox species at the electrode surface [243]. Quasi-Reversible process is an intermediate between reversible and irreversible systems and the redox current is controlled by both the mass transport and charge transfer [243]. The parameters I_p (I_{pa} and I_{pc}), E_p (E_{pa} and E_{pc}) and ΔE_p (E_{pa}-E_{pc}) for both oxidation and reduction reaction under a particular experimental condition can be determined using

CV [245]. The significance of evaluating these parameters is to understand the electrode process and the reversibility of the electrochemical reactions given by [246, 247]:

1. Reversible reaction:

(a) $\frac{I_{pa}}{I_{pc}} = 1;$

(b) $\Delta E_p = E_{pa} - E_{pc} = \frac{59}{n} mV$

(c) $I_p \propto \vartheta^{\frac{1}{2}}$

(d) E_p is independent of ν.

2. Irreversible reaction:

(a) No reverse peak is observed referring chemical irreversibility.

(b) E_p shifts to $\frac{30}{\alpha n} mV$, for each decade increase in ν where α is charge transfer coefficient.

(c) $\Delta E_p = E_p - E_{\frac{p}{2}} = \frac{47}{\alpha n} mV$

3. Quasi reversible reaction:

(a) $\frac{I_{pa}}{I_{pc}} = 1;$ Provided $\alpha_a = \alpha_c \sim 0.5$.

(b) $\Delta E_p = E_{pa} - E_{pc} > \frac{59}{n} mV$ and increases with scan rate

(c) E_p shifts negatively with increasing scan rate

2.2.1 Randles- Sevcik theory:

Working electrode held at potential E_i acts a simple donor or acceptor of electrons participating in general electrode reaction given by [240]:

$$o + ne' \leftrightarrow R \tag{2.9}$$

Assuming semi infinite linear diffusion, the potential swept at a scan rate υ is given by: 2.10

$$E(t) = E_0 - \vartheta t$$

For stationary electrode voltammetry; the boundary value problem can be written as

$$\frac{\partial C_O}{\partial t} = D_O \frac{\partial^2 C_O}{\partial x^2} \tag{2.11}$$

$$\frac{\partial C_R}{\partial t} = D_R \frac{\partial^2 C_R}{\partial x^2} \qquad 2.12$$

where C_o and C_R are the concentration of oxidized and reduced species., respectively.

At condition where $t = 0; x \geq 0$

$$C_o = C_o^*;\ C_R = C_R^*$$

And at $t \geq 0; x \rightarrow \infty$

$$C_o \rightarrow C_o^*;\ C_R \rightarrow 0$$

At $t > 0; x = 0$

$$D_o\left(\frac{\partial C_o}{\partial x}\right) = -D_R\left(\frac{\partial C_R}{\partial x}\right)$$

$$\frac{C_o}{C_R} = exp\left[\left(\frac{nF}{RT}\right)(E - E^0)\right] \qquad 2.13$$

Where C_o^* and C_R^* are the bulk concentration of *O* and *R* species, respectively. E^0 is the formal potential, *E*, *R*,*T* and *F* have their usual meaning and significance. The problem of solving the above equation was first considered by Randles and Sevcik [237, 238], later it has been modified by Nicholson and Shain [239].

The boundary condition (equation 2.13) can be re-written as:

$$C_o/C_R = \theta S_\lambda(t)$$

where $\theta = exp\left[\left(\frac{nF}{RT}\right)(E_i - E^0)\right]$ and $S_\lambda(t) = e^{-\alpha t}$

The surface concentration can be derived by taking Laplace Transformation of diffusion equation given by equation 2.9 to 2.12 and then applying convolution theorem [240, 247], the expression of C_o and C_R can be written as:

$$C_o(0,t) = C_o^* - \frac{1}{\sqrt{\pi D_o}}\int_0^t \frac{f(\tau)\partial\tau}{\sqrt{t-\tau}}\ ;\ C_R(0,t) = \frac{1}{\sqrt{\pi D_R}}\int_0^t \frac{f(\tau)\partial\tau}{\sqrt{t-\tau}}\ ; \qquad 2.14$$

where $f(t) = D_o\left(\frac{\partial C_o}{\partial x}\right)_{x=0} = \frac{i}{nFA}$

Again using the boundary condition $\frac{C_o(0,t)}{C_R(0,t)} = \theta e^{-\sigma t} = \theta S(t)$ to equation 2.14

$$\int_0^t f(\tau)(t-\tau)^{-1/2}\,\partial\tau = \frac{C_o^*}{\theta S(t)(\pi D_R)^{-1/2} + (\pi D_o)^{-1/2}} \qquad 2.15$$

It is assumed that $f(\tau) = g(\sigma\tau)$, $Z = \sigma\tau$ where $\tau = Z/\sigma$ and using this in equation (2.15)

$$\int_0^t f(\tau)(1-\tau)^{-1/2}\partial\tau = \int_0^{\sigma t} g(Z)\left(t - Z/\sigma\right)^{-1/2}\frac{\partial Z}{\sigma} \qquad 2.16$$

Equation 2.16 can be re-written as

$$\int_0^{\sigma t} g(Z)\sigma t Z^{-1/2}\sigma^{-1/2}\partial Z = \frac{C_O^*(\pi D_O)^{1/2}}{1+\xi\theta S(\sigma t)} \qquad 2.17$$

$$\int_0^{\sigma t} \frac{\chi(Z)\partial Z}{(\sigma t-Z)^{1/2}} = \frac{1}{1+\xi\theta S(\sigma t)} \qquad 2.18$$

$$\xi = \sqrt{\frac{D_O}{D_R}} \text{ and } \sigma t = \left(\frac{nF}{RT}\right)(E_i - E) \qquad 2.19$$

And $\chi(Z)$ is given by

$$\chi(Z) = \frac{i(\sigma t)}{nFAC_O^*(\pi D_O\sigma)^{1/2}} \qquad 2.20$$

Rearranging equation; we can obtained the expression for current

$$i = nFAC_O^*\pi D_O\sigma^{1/2}\chi(\sigma t) \qquad 2.21$$

$\chi(\sigma t)$ is a pure number at any point on the cyclic voltammetry curve and equation (2.21) represents the specific relationship between the current, which is proportional to the bulk concentration of the redox species and square root of scan rate [240]. At 25^0 C, for A in cm^{-2}, D_0 in cm^2s^{-1}, C_0^* in $molcm^{-3}$, υ in Vs^{-1} and i_p in ampere is given by

$$i_p = 0.446\left(\frac{F^3}{RT}\right)^{\frac{1}{2}} n^{\frac{3}{2}} A D_0^{1/2} C_0^* \vartheta^{1/2} \qquad 2.22$$

2.2.2 Surface concentration of adsorbed electro active species:

The Cyclic Voltammetry measurement can be used to calculate the surface concentration of adsorbed species at the electrode surface [248, 249]. The surface concentration can be defined as the coverage of adsorbed species in mol over per unit area (m^2, cm^2) of electrode surface. The adsorbed species is assumed as electro active in nature. An electrochemical reaction is taken under consideration and within a potential range, Γ's are surface concentration is independent of applied potential E [249].

Under this condition

$$\frac{\partial \Gamma_O(t)}{\partial t} = \frac{\partial \Gamma_R(t)}{\partial t} = \frac{i}{nFA} \qquad 2.23$$

Where Γ_0 (t) and Γ_R (t) ($molcm^{-2}$) are the surface concentration of oxidized and reduced species after time t and it is given by

$$\Gamma_O(t) = \frac{\beta_0 C_O \Gamma_O(0,t)}{1+\beta_O C_O(0,t)+\beta_R C_R(0,t)}\ ;\ \Gamma_R(t) = \frac{\beta_R C_R \Gamma_R(0,t)}{1+\beta_O C_O(0,t)+\beta_R C_R(0,t)} \qquad 2.24$$

The total surface concentration of adsorbed species is given by

$$\Gamma_0^* = \Gamma_o(t) + \Gamma_R(t) \qquad 2.25$$

Using equation 2.23

$$\frac{\Gamma_O(t)}{\Gamma_R(t)} = \frac{b_O C_O(0,t)}{b_R C_R(0,t)} \qquad 2.26$$

If the reaction is Nernstian, so that

$$\frac{C_o(0,t)}{C_R(0,t)} = exp\left[\left(\frac{nF}{RT}\right)(E - E^0)\right]$$

Therefore equation 2.26 becomes

$$\frac{\Gamma_O(t)}{\Gamma_R(t)} = \frac{b_O}{b_R} exp\left[\left(\frac{nF}{RT}\right)(E - E^0)\right] \qquad 2.27$$

Using equation 2.25 and 2.26 in 2.27, the relation between the current (i) and scan rate (υ)

$$\frac{i}{nFA} = -\frac{\partial \Gamma_O(t)}{\partial t} = \frac{\partial \Gamma_O(t)}{\partial E} \qquad 2.28$$

With $= E_i - \vartheta t$, the equation for the i-E curve can be written [250]:

$$i = \frac{n^2F^2\vartheta A\Gamma_o^* \left({}^{b_o}/_{b_R}\right) exp[({}^{nF}/_{RT})(E - E^0)]}{RT\left\{1 + \left({}^{b_o}/_{b_R}\right) exp[({}^{nF}/_{RT})(E - E^0)]\right\}^2}$$

The above equation can be re-written as

$$i_p = \frac{n^2F^2\vartheta A\Gamma_o^*}{4RT}$$

Hence the value of surface concentration (Γ_o^*) can be calculated using the linear slope of peak current (i_p) versus scan rate (υ).

2.2.3 The variation of E_p with pH (Pourbaix diagram):

At electrochemical equilibrium, the variation of potential as a function of pH of the operating solution can be diagrammatically represented by Pourbaix diagram. It has been named after a Belgium electrochemist and corrosion scientist, Pourbaix (1963) [251]. The effect of pH on the electro chemical behaviour of reactive species can be understood by plotting Pourbaix diagram between the peak potential E_p and pH.

The heterogeneous reaction involving m protons and n electrons can be written as [252]:

$$A + mH^{+} + ne' \leftrightarrow B$$

A standard Gibb's free energy ΔG^0 is associated with this equation and it is given by

$$\Delta G^0 = -RTlnK$$

K is the equilibrium constant;

$$K = [A][H^{+}]^{m} \qquad 2.29$$

Using this in equation 2.29

$$\Delta G^0 = -(RT)\ln\frac{[B]}{[A][H^{+}]^{m}}$$

According to Nernst Equation

$$\Delta G^0 = -FE$$

$$E = E^0 + \frac{RT}{nF}ln[H^{+}]^{m} - \frac{RT}{nF}\ln\frac{[B]}{[A]}$$

$$E = E^0 - \frac{2.303\,mRT}{nF}pH - \frac{RT}{nF}ln\frac{[B]}{[A]}$$

Considering $D_A = D_B$

$$E_f^0 = E^0 - \frac{2.303mRT}{nF}\,pH$$

For equal transfer of electrons

$$m = n \text{ for } E^0 = 0$$

$$E = 0.059\,pH$$

The potential shifts towards lower potential side with the increasing pH and shows a linear variation with regression equation of $E_{pa} = m\,(pH) + c$ with a slope of 59mV/pH [252, 253].

2.3 Laviron's Theory:

In the year 1979, Laviron published mathematical formulations to calculate the heterogeneous charge transfer rate constant, k_s, and the charge transfer coefficient α of a redox couple [254]. It has been done from voltammetry experiments based on using the variation of anodic and cathodic peak potentials as a function of logarithm of scan rate.

A general representation of heterogeneous reaction equation;

$O + ne' \leftrightarrow R$

The applied potential is given by

$$E = E_i + \vartheta t \qquad 2.30$$

Where E_i the initial potential in Volt (V) and ν is is the scan rate in Vs^{-1}.

In surface voltammetry [255, 256], the oxidized and reduced species gets strongly adsorbed and the surface standard potential is given by

$$E = E^0 - \frac{RT}{nF} ln \frac{a_0}{a_R} \qquad 2.31$$

Where a_o and a_R are the adsorption coefficient of oxidized (O) and reduced (R) species, respectively.

And equation representing the current is given by

$$i = nFAk_s \left\{exp\left[-\alpha nF \frac{(E-E^0)}{RT}\right] - \Gamma_R exp\left[(1-\alpha)nF\frac{(E-E^0)}{RT}\right]\right\} \qquad 2.32$$

$$\Gamma_o + \Gamma_R = \Gamma_T \qquad 2.33$$

$$i = -nFA\frac{\partial \Gamma_o}{\partial t} \qquad 2.34$$

Γ_0 and Γ_R are the surface concentrations of O and R species, respectively in $molcm^{-2}$, k_s is the heterogeneous rate constant in s^{-1}, A, n, F, R and T have their usual meaning and significance.

Further current can be defined by a dimensionless quantity Ψ given by equation 2.35 [257]:

$$\Psi = i\left(\frac{F^2}{RT}\right)n^2\vartheta A\Gamma_T = m\left[\left(\frac{\Gamma_O}{\Gamma_T}\right)\eta^{-\alpha} - \left(\frac{\Gamma_R}{\Gamma_T}\right)\eta^{1-\alpha}\right] \qquad 2.35$$

Where $m = \left(\frac{RT}{F}\right)\left(\frac{k_s}{\eta\vartheta}\right)$ and $n = \exp\left[\left(\frac{nF}{RT}\right)(E - E^0)\right]$ 2.36

Let us substituteΓ_o, Γ_R and Γ_T by χ_o, χ_R and χ_T and considering equation (2.36)

Then from equation 2.30 and (2.32-2.34), we derived a differential equation given by

$$\left.\begin{aligned} \frac{\partial\left(\frac{\chi_o}{\chi_T}\right)}{\partial\eta} + m(1+\eta)\eta^{-(1+\alpha)}\left(\frac{\chi_o}{\chi_T}\right) = m\eta^{-\alpha} \\ \frac{\partial\left(\frac{\chi_R}{\chi_T}\right)}{\partial\eta} + m(1+\eta)\eta^{-(1+\alpha)}\left(\frac{\chi_R}{\chi_T}\right) = m\eta^{-(1+\alpha)} \end{aligned}\right\} \qquad 2.37$$

Expression for $\Psi$for cathodic variation of potential can be calculated by using the values of $\frac{\chi_o}{\chi_T}$ or $\frac{\chi_R}{\chi_T}$ (obtained from equation 2.37) in equation 2.35

$$\Psi_C = m\eta^{-\alpha}\left\{1 - m(1+\eta)exp[f(\eta)]\int_{\infty}^{\eta} Z^{-(1+\alpha)}\, exp[-f(Z)]dZ\right\} \qquad 2.38$$

For anodic variation of potential Ψ can be written as

$$\Psi_a = -m\eta^{-\alpha}\left\{\eta - m(1+\eta)exp[f(\eta)]\int_{\infty}^{\eta} Z^{-\alpha}\, exp[-f(Z)]dZ\right\} \qquad 2.39$$

Where $f(\eta) = \left[\frac{m}{\alpha(1-\alpha)}\right]\eta^{-\alpha}[1 - \alpha(1+\eta)]$ and m is negative for the cathodic curve and positive for the anodic curve. The dimensionless function representing cathodic and anodic currents is given by 2.38 and 2.39. Further Ψ_C and Ψ_a can be written in more simplified form by considering $m \to 0$ for irreversible or quasi reversible reactions [257].

Therefore taking $m \to 0$,

$\eta^{1-\alpha} \ll \eta^{-\alpha}$ and $\eta \ll 1$ for cathodic curve

And equation 2.38 becomes

$$\Psi_c = m\eta^{\alpha} exp\left(\frac{m\eta^{-\alpha}}{\alpha}\right)$$

Similarly for anodic curve $n^{-\alpha} \ll \eta^{1-\alpha}$ and $\eta \gg 1$ and equation 2.39 becomes

$\Psi_a = -m\eta^{1-\alpha} exp\left(\frac{-m\eta^{1-\alpha}}{1-\alpha}\right)$

For maximum value of Ψ_c , $\eta^{-\alpha} = \frac{\alpha}{|m|}$ and the peak potential for cathodic reaction can be written as [37]:

$E_P = E^0 - \frac{RT}{\alpha nF} ln\left[\frac{\alpha}{|m|}\right]$ or $E_p = E^0 - 2.3RT\frac{log\upsilon}{\alpha nF}$

Similarly for anodic reaction $\eta^{1-\alpha} = (1-\alpha)m$

$E_P = E^0 + \frac{RT}{(1-\alpha)nF} ln\left[\frac{1-\alpha}{|m|}\right]$ or $E_{pa} = E^0 + 2.3RT\frac{log\upsilon}{(1-\alpha)nF}$

For a reversible reaction $m \rightarrow \infty$, the cathodic and anodic peaks are symmetrical with respect to the potential axis [258-260]. The peak potential becomes independent of scan rate (υ).

2.3.1 Determination of k_s and α:

According to Laviron's theory [254], the variation of ΔE_p with scan rate yields two straight lines with cathodic slope equal to $\frac{2.3\,RT}{nF\alpha_c}$ and anodic slope equal to $\frac{2.3\,RT}{nF(1-\alpha_a)}$. The heterogeneous rate can be calculated by putting the value of α in the equation:

$Logk_s = \alpha \log(1-\alpha) + (1-\alpha)log\alpha - log\frac{RT}{nF\vartheta} - \frac{\alpha(1-\alpha)nF\Delta E_P}{2.3\,RT}$

This equation is valid for $\Delta E_P > 200mV$. For $E - E^0 = 0$; k_s can be evaluated using

$$k_s = \frac{\alpha nF\vartheta_c}{RT} = \frac{(1-\alpha)nF\vartheta_a}{RT}$$

Larger value of k_s indicates that the equilibrium between O and R will be re-established quickly after application of potential. Smaller the value of k_s denotes slow kinetic and longer time requirement for equilibrium [261]. The accepted range of k_s for different electron transfer rate is given by Table 2.1 [262]. Transfer Coefficient (α) is used to describe the symmetry between the forward and reverse electron transfer steps [263]. The charge transfer coefficient is the measure of symmetry between electrochemical reactions and signifies the fraction of the potential at the electrode interface that helps in lowering energy barrier of the redox reaction. Figure 2.5 displayed the graphically demonstration showing the slope of the forward and reverse energy barriers being related to the magnitude of the respective rate constant [264].

Table 2.1: The value of heterogeneous rate constant (k_s) derived for different electron transfer processes.

Parameter	**Reversible**	**Quasi-Reversible**	**Irreversible**
Heterogeneous rate constant	$k_s > 0.020$ cms^{-1}	$0.020 > k_s > 5\times10^{-5}$ cms^{-1}	$k_s < 5\times10^{-5}$ cms^{-1}

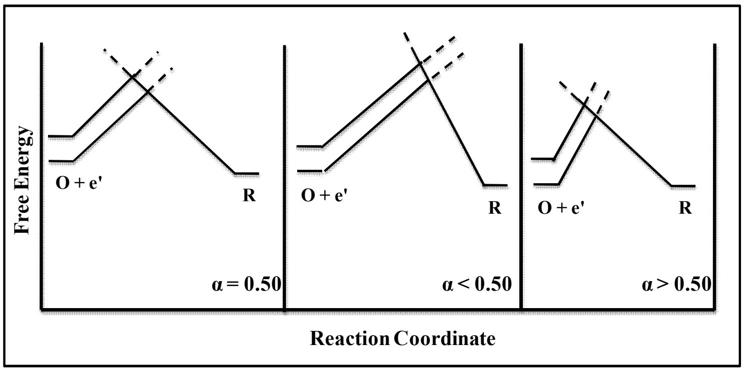

Figure 2.5: The graphically display showing the slope of the forward and reverse energy barriers being related to the magnitude of the respective rate constant.

2.4 Enzyme Kinetics Theory:

2.4.1 Michaelis-Menten Kinetics

The rate of an enzymatic chemical reaction is the speed or velocity at which the reaction proceeds. Since the presence of enzyme can cause enhancement in the kinetics of the reaction therefore the rate of chemical reaction is proportional to the enzyme activity [265]. The reaction is monitored as the change in the concentration of a reactant or product during a given interval of time. In the year 1913, Leonor Michaelis and Maud Leonora Menten proposed a theoretical model known as Michaelis-Menten kinetics [266] aiming to explain enzyme dynamics for a simple single substrate enzymatic reaction. According to this theory, an enzyme catalyzed reaction is always accompanied by a transition state of formation of enzyme substrate complex and the binding of enzyme-substrate is reversible. The theory is based on the reaction of enzyme with only one substrate. This theory assumes that the enzyme *E* first combine with the substrate *S* and results in the enzyme-substrate complex (*ES*), which in turn breaks down to form the product *P* and releasing the free enzyme. Initially the rate of formation of product P increases slowly and after an interval of time saturation occurs as shown in Figure 2.6.

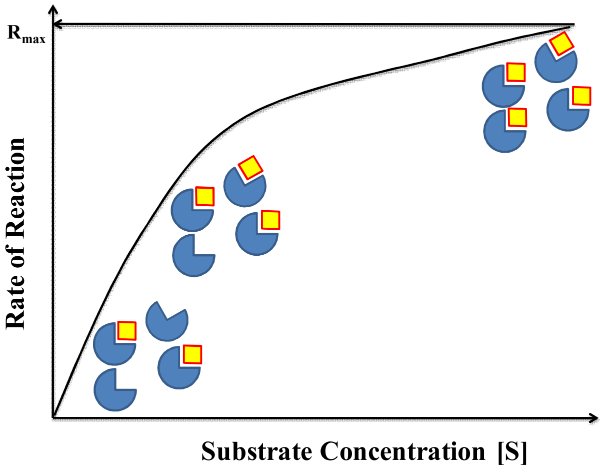

Figure 2.6: Michealis Menten Plot of rate of reaction versus concentration of substrate.

To understand this theory we have to consider a single substrate chemical reaction given by:

$$E + S \overset{k_1}{\rightarrow} ES \overset{k_2}{\rightarrow} E + P \qquad 2.40$$

$$E + S \overset{k_3}{\leftarrow} ES \overset{k_4}{\leftarrow} E + P \qquad 2.41$$

where E represents the *free enzyme*; S is the *substrate* of the enzyme; ES is the intermediate *enzyme-substrate complex*; P is the *product* of the reaction; and the $k_{n's}$ are the individual forward and backward rate constants.

The rate of formation and breakdown of the enzyme-substrate complex *ES* can be derived using the two step reaction mechanism (equation 2.40 & 2.41). The initial velocity (reaction rate) is equal to the rate of breakdown of the enzyme-substrate complex *ES*, according to the equation (2.40) for which we can write the first order rate equation.

$$V_0 = k_2[ES] \qquad 2.42$$

where $[ES]$ is the concentration of enzyme-substrate complex. However, we cannot determine k_2 or $[ES]$ directly using the equation (2.42). On the other hand, the second order rate equation for the formation of $[ES]$ from E and S can be divided in to two equations (2.43 & 2.44) [267-269]. Considering the initial time interval of the reaction in the forward direction, when [S] is very high and the concentration of product can be considered very less or zero ([P] → zero).

Therefore the rates of formation and breakdown of the intermediate product ES complex are given by two reactions:

$$\text{Rate of formation of ES} = k_1\,[E][S] \qquad 2.43$$

$$\text{Rate of breakdown of ES} = k_2[ES] + k_3\,[ES] \qquad 2.44$$

At steady state,

$$\frac{d[ES]}{dt} = k_1[E][S] + k_2[ES] + k_3[ES] = 0$$

Therefore the rate of formation is equal to the breakdown of substrate [ES]

$$k_1\,[E][S] = (k_2 + k_3)[ES] \qquad 2.45$$

$$[E][S] = \frac{(k_2+k_3)}{k_1}[ES] \text{ (Dividing by } k_1\text{)}$$

$$[E][S] = K_m[ES]$$

where K_m is the Michaelis-Menten constant

The total enzyme concentration is the sum total of free and bound form and it is given by

$$E_T = E + [ES]$$

The equation 2.45 can be derived as

$$[E_T - ES]\,[S] = K_m[ES]$$

$$[E_T][S] - [ES]\,[S] = K_m[ES]$$

$$[E_T][S]] = K_m[ES] + [ES][S]$$

$$[E_T][S] = [ES]\{K_m + [S]\}$$

$$[ES] = \frac{[E_T][S]}{([S] + K_m\,)}$$

The rate of product formation

$$V_o = k_2\left(\frac{[E_T][S]}{([S] + K_m\,)}\right)$$

As the substrate concentration increases, the concentration of *ES* complex increases and maximum enzyme activity is achieved called saturation and the corresponding rate of reaction V_{max}, given by

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

This equation is the mathematical expression known as Michaelis-Menten equation, which established a mathematical expression for enzymatic reaction.

$$\text{At } V_0 = \frac{V_{max}}{2}$$

$$K_m = [S]$$

Therefore K_m can be defined as the concentration of the substrate at which the initial reaction velocity is half of the maximum velocity and is independent of the enzyme concentration.

2.4.2 Lineweaver-Burk (LB) Plot:

The LB [270] plot is another approach of calculating the value of important parameters of enzyme kinetic i.e. K_m and V_{max}. The equation can be rearrange by taking reciprocal of M.M equation which results in straight line equation $y = mx + C$

By taking the reciprocal of both sides of Michaelis-Menten equation, we get

$$\frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]} \qquad 2.46$$

Rearranging the equation, we have

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \qquad 2.47$$

Equation (2.47) is known as the Lineweaver-Burk equation. Comparing equation 2.47 with equation of straight line, we can write;

$y = \frac{1}{V_0}$; $x = \frac{1}{[S]}$ and slope is given by $m = \frac{K_m}{V_{max}}$. Therefore the plot of $\frac{1}{V_o}$ versus $\frac{1}{[S]}$ (Figure 2.7) results in a straight line with an intercept of $\frac{1}{V_{max}}$ on the $\frac{1}{V_o}$ axis, and an intercept of $\frac{-1}{K_m}$ on the $\frac{1}{[S]}$ axis and slope '*m*' which can be used to determine the value of K_m. Using Lineweaver Burk reciprocal plot, more accurate values of V_{max} and K_m can be calculated.

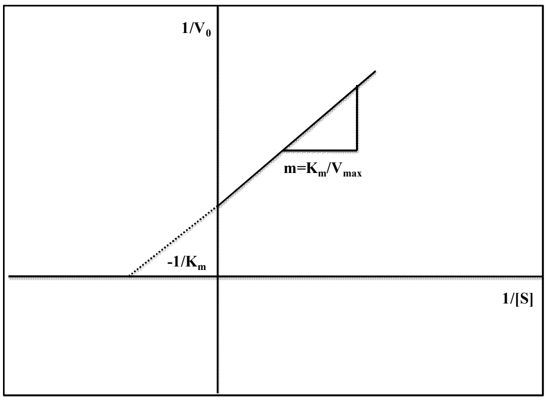

Figure 2.7: The graph of double reciprocal equation or Line-weaver Burk Plot.

2.5 Theory of Enzyme immobilization or carbodiimide coupling:

The carbodiimide coupling between primary amine and carboxylic groups using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), a zero-length cross-linker, is a versatile tool for immobilizing enzymes, antibodies, proteins and macromolecules to prepare bioactive probe. The method of cross linking has been used in various applications viz. Biosensors, cell biology detection and analysis methods. The conjugation mechanism requires two sequences of reactions. The initial step includes the reaction of EDC with carboxyl group as a result of which an intermediate product O-acylisurea which susceptible to amide reaction. The unstable product O-acylisurea will be displaced by nucleophillic attack from primary amine group and undergoes hydrolysis. Finally a stable amide bond is formed with carboxylic group as shown in figure 2.8. the coupling efficiency can be increased by the addition of NHS (N-hydroxysuccinimide or its more water soluble analogue Sulfo NHS) which stabilizes the intermediate and converts it onto a semistable amine-reactive NHS ester [271, 272].

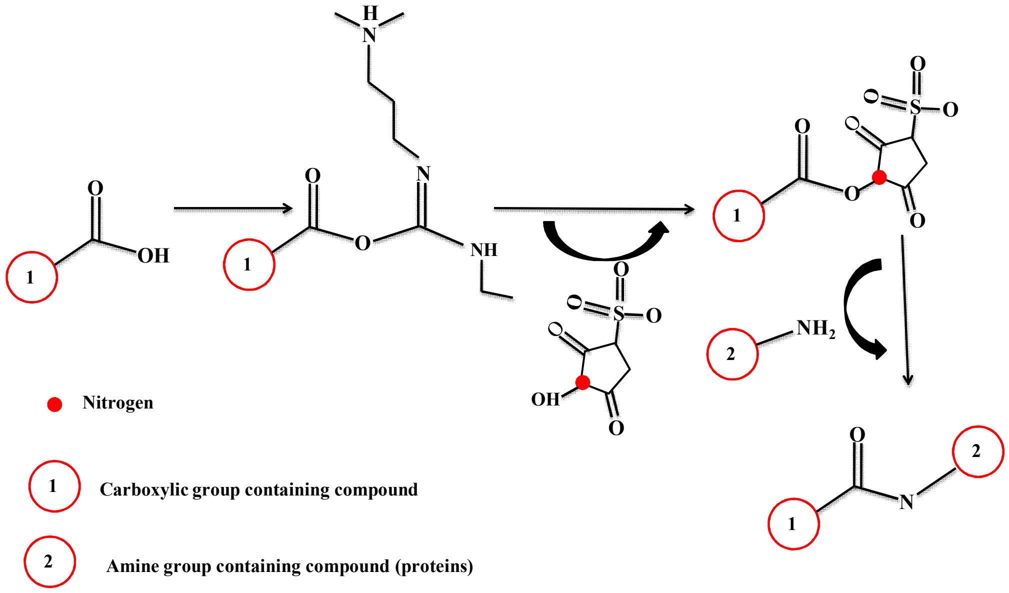

Figure 2.8: Schematic representation of covalent bonding using carbodiimide cross linking.