CHAPTER 3

CHARACTERIZATION TECHNIQUES

Overview of the Chapter

Performance characteristics of fabricated biosensor are controlled by achieving an understanding and control over the properties and behavior of the materials used. To accomplish the same physical and chemical characterization of materials used in addition to response characteristics of biosensor after each modification step becomes pertinent. Current biosensor technology exploits nanostructures and their varied optical, electronic, magnetic and other properties for enhancing performance and efficiency. Morphological characterization of these nanostructures is of paramount importance since their properties vary drastically with their size and shape. Microscopic techniques like Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and optical microscopy (OM) are exploited for morphological analysis. Moreover, chemical and electrochemical characterization of the nanostructures and biosensors, in the present work, is done through spectroscopic techniques (UV-Visible spectroscopy, energy dispersive X-ray spectroscopy, electrochemical impedance spectroscopy), zeta potential analysis and cyclic voltammetry. Present chapter gives a brief description of these characterization techniques. This chapter is structured as follows;

3.1 Microscopy
   3.1.1 The Optical Microscopy
   3.1.2 The Electron Microscopy
      3.1.2.1 Scanning Electron Microscopy
      3.1.2.2 Transmission Electron Microscopy

3.2 Zeta Potential Analysis
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3.3 UV-Vis Spectroscopy
   3.3.1 Principle of Absorption Spectroscopy
   3.3.2 Instrumentation

3.4 Electrochemical Impedance Spectroscopy
3.5 Cyclic Voltammetry
   3.5.1 Reversible Process
   3.5.2 Irreversible and Quasi-Reversible Systems

3.1 Microscopy

Microscopy allows investigation of entities that are difficult to be observed with unaided eyes. Microscopic techniques are employed for examination of microscopic (micrometer; $10^{-6}$ m) to submicroscopic (nanometer; $10^{-9}$ m) scale objects. Depending on the source of illumination, microscopy is classified as:
   ➢ Optical Microscopy and
   ➢ Electron Microscopy

3.1.1 The Optical Microscopy

Some four hundred years ago, the Dutch scientist Anton van Leeuwenhoek, observed bacteria, blood cells and different structures within the animal cells with the help of a glass lens accurately placed in between the eye and the object. A simple microscope was based on one focusing lens. With the evolvement in the field of microscopy, researchers have incorporated multiple lenses in order to enhance resolution between the two points. The basic principle of any optical microscopy is structural image formation as a result of phase difference between the diffracted light passing through the specimen and the undeviated light either passing through the specimen or around the specimen. Subsequently, the problem of out of focus light rays, in case of conventional optical microscopy (OM), was eliminated from the image by use of a confocal “pinhole” in confocal/multiphoton microscopy. Advancement in the field led to development of fluorescence microscopy through which both reflected and fluorescence light can be studied. It is especially useful for biological samples - for identification of specific molecules in complex structure, to locate their spatial distribution, to study the biochemical dynamics etc.

The basic design of an optical microscope is shown in Figure 3.1. The optical microscope shown in Figure 3.1, incorporates two lenses; the lens placed in proximity of the specimen to be investigated is called an objective lens while the other placed in vicinity of eye is known as an ocular lens. The variation in the above mentioned lenses allows control over magnification of image of the specimen. The resolution of optical microscope is limited by
wavelength of visible light (400-700 nm) and at best could be ~ 0.2 μm. The coarse and fine adjustment knobs allow the appropriate focusing of the object.

In an optical microscope, light from the lamp source passes through the condenser and then specimen. A part of light passes around the specimen and while some through the specimen undisturbed in its path, i.e., no phase change. This is referred to as direct or undeviated light. However, a major part of light passing through the specimen undergoes diffraction resulting in phase change. The diffracted light and undeviated light are projected by the objective at intermediate image plane located at fixed diaphragm of the eyepiece. The difference in phase of the two light beams results in destructive interference leading to decreased intensity of light. Depending on the extent of phase change experienced by the light after passing through different parts of the specimen, the intensity of light after destructive interference varies leading to more or less dark areas. These different intensity patterns are recognized as image of the specimen.

**Figure 3.1:** Schematic diagram showing different components of an Optical Microscope
Advantages

✓ No sample pre-treatment required for imaging.
✓ Easy integration with digital camera systems for analysis of specimens.
✓ Fast and adaptable to all kinds of sample systems, from gas, to liquid, and to solid sample systems, in any shapes or geometries.

Disadvantages

✓ Low resolution, usually down to only sub-micron or a few hundreds of nanometers, mainly due to the light diffraction limit.

3.1.2 The Electron Microscopy

The underlying principle behind the working of an electron microscope is similar to that of the optical microscope studied in previous section with the exception of the illumination source that is a focused high energy electron beam in case of electron microscopy as opposed to a visible light source in OM. The limitations including low achievable magnification (500x or 1000x) and low resolution limit (maximum 0.2 µm) of the optical microscopes were the major driving force behind the development of first electron microscope. The electromagnetic lenses and the high energy electron beam allows the achievable magnification values more than 10,000 x in electron microscope permits morphological, topographical and crystallographic information of specimen of nanometer length scales as well.

Depending on type of electrons captured for imaging (see Figure 3.2), the electron microscope are of two types.

✓ Transmission electron microscope.
Scanning electron microscope.

Resolution (R) of image is given by Abbe’s equation as

\[ R = \frac{0.612\lambda}{\text{n} \sin \alpha} \]

and the wavelength of imaging radiation, i.e., electron is given by

\[ \lambda = h(2meV)^{-0.5} \]

where nsina is numerical aperture, h is Planks constant, m is mass of electron with charge e and accelerating voltage V. In electron microscopy, very short wavelength ~0.04 A° can be deflected by magnetic field.

3.1.2.1 Scanning Electron Microscopy

The first Scanning Electron Microscope (SEM) based on emission of secondary electrons was developed at the RCA Laboratories in New Jersey. The first commercial model was delivered in 1958 by AEI Company to Pulp and Paper Research Institute of Canada.

The basic components involved in working of a SEM are shown in Figure 3.3. A focused beam of electrons from a fine probe is rastered over the surface of the specimen. The scan coils and objective lens allows the horizontal and vertical deflection of electron beam to scan the specimen surface. The interaction of electron beam with the surface leads to expulsion of electrons or photons from the specimen surfaces which are further collected with the help of detectors. Thus the formed image is a representation of the corresponding point where the electron beam strikes the specimen surface. The SEM generally operates on a voltage ranging between 2 to 50kV and its beam diameter that scans the specimen is 5nm-2μm.

The low energy secondary electron detection gives topographic contrast (surface texture and roughness) and can resolve surface structure up to 10 nm. However, detection of high energy backscattered electrons gives both compositional and topographic information, good atomic number contrast but poor lateral resolution as compared to secondary electron image. Moreover, X-ray detection gives chemical information of the sample through Energy Disperse X-ray Spectroscopy (EDS).
Figure 3.3: Schematic representation of a scanning electron microscope

**Advantages**

 ✓ Analysis of conducting material is possible.
 ✓ Based on interaction with surface only so no requirement of electron-transparent sample.
 ✓ 3D imaging of the specimen is possible.

**Disadvantages**

 ✓ Low resolution, usually above a few tens of nanometers.
 ✓ Staining of non-conducting material is required.
3.1.2.2 Transmission Electron Microscopy

The first transmission electron microscope was developed by two German researchers Max Knoll and Ernst Ruska in the year 1931. This microscopy involves focusing and magnification of emitted electrons using a pair of magnetic lenses. In TEM, we utilize the electrons that go through a very thin specimen (<200nm) and detect scattered electrons as a result of strong interaction between electrons and matter. Recording image, diffraction pattern, x-ray spectrum and electron energy loss spectrum gives topographic, compositional and crystallographic information. Figure 3.4 show different components of a TEM. It consists of three types of lenses - condenser, objective, and projector lenses which are involved in the formation of final image. The condenser lens controls the brightness of the beam and confines the electron beam coming from the filament onto the specimen. The objective lens forms the

![Figure 3.4: Schematic outline of a transmission electron microscope](image-url)
preliminary enlarged image of the illuminated portion of the specimen whereas the projector lens facilitates the further enlargement of illuminated portion. The electrons that are transmitted through the specimen are involved in image formation while the electrons that can’t pass through or get deflected by dense atoms in the specimen are deducted from the image. An aperture in objective lens can be used to eliminate the effect of these scattered electrons on the formed image. Thus, a contrast image is formed. Finally, the enlarged image is projected onto the phosphor screen or photographic film with the help of projector lens. In order to further enhance the magnification, an intermediate lens can be placed between the objective and projector lens.

Another important parameter of TEM is the requirement of an ultra-high vacuum and a high voltage during the operation of the instrument. Ensuring vacumm condition is of paramount importance to avoid collision of electron beams with stray molecules present in atmosphere. These collisions not only lead to spreading of the beam but also volatilization of these stray molecules leading to contamination of the microscope column. Both these phenomenon lead to poor image quality.

*Sample Preparation:* The specimen should be very thin, less than 100 nm as TEM allows strong interaction between electrons and matter. The thin specimens can be achieved by mechanical thinning.

**Advantages**

- It allows determination of structure and phase and identification of defect.
- High resolution (approx 0.2 nm) and magnification (over one million times).
- No requirement of metallic stain coating for sample observation.
- Allows imaging of crystalline lattice.

**Disadvantages**

- Trained manpower requirements for operation and analysis.
- Expensive capital cost as well as maintenance.
- Does not allow analysis of samples that are not electron transparent.

### 3.2 Zeta potential analysis

The net charge on nanostructures is the major parameter contributing to the long term stability of the synthesized colloidal system. The physical stability of the colloidal suspension depends
on uniformity of charge distribution on surface of nanostructures, higher electrostatic repulsion between the particles results in higher long term stability of the system. Zeta potential can be defined as the electrostatic potential near the surface of a particle; it is quantified by studying the electrophoretic mobility of the particles in an applied d.c. electric field. The particle velocity is determined by the Doppler shift in the laser light scattered by the moving particles.

Zeta potential

The net charge on the surface of particles in colloidal system could be because of the ionization of chemical groups present on the surface of particle or at times the surface of particles itself favorably adsorbs ions of positive/negative charge of the opposite sign from the solution.

The degree of dissociation of chemical groups and thus the net charge on the particle surface is highly dependent on the pH of the suspension, therefore the value of zeta potential depends on the pH of the solution. The charge at the particle surface greatly affects the ions present in the region close to particle surface. This results in formation of electrical double layer around each particle due to attraction of oppositely charge ions and counter ions near the particle surface as shown in Figure. 3.5. The ions in the inner layer called as Stern layer are attached firmly because of high attraction, between opposite charges whereas the ions in outer layer

![Figure 3.5: Schematic presentation of formation of a double layer on the surface of a particle. (Reproduced from Book III -Electrophoresis by R. P. W. Scott)](image-url)
known as diffuse layer are less firmly associated. The diffused layer stretches out up to few nm from the particle surface and is believed to form a hypothetical boundary within which the particles are stable. Thus the ions present within the boundary are only believed to move with the movement of particles in the solution. The potential at this boundary of diffused layer is termed as zeta potential. The degree of stability of particles in suspension is highly dependent on the value of zeta potential. Thus higher value of zeta potential represents lower tendency of particles coming together to form agglomerates. The differentiation of the system stability on the basis of value of zeta potential is outlined in the Table 3.1 shown below.

Table 3.1: Stability of the system with corresponding value of zeta potential

<table>
<thead>
<tr>
<th>Stability of the colloidal system</th>
<th>Value of zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid agglomeration</td>
<td>0 to ± 5</td>
</tr>
<tr>
<td>Incipient Stability</td>
<td>±10 to ± 30</td>
</tr>
<tr>
<td>Moderate Stability</td>
<td>±30 to ± 40</td>
</tr>
<tr>
<td>Good Stability</td>
<td>±40 to ± 60</td>
</tr>
<tr>
<td>Excellent Stability</td>
<td>More than ± 61</td>
</tr>
</tbody>
</table>

3.3 UV-Visible Spectroscopy

The absorption and emission of energy at a particular wavelength under specific conditions by materials is the underlying principle behind any spectroscopic technique. It provides the
detailed information about the optical properties of samples including the nanomaterials. Since the electronic transitions require the radiation from the UV and visible region of the electromagnetic radiation spectrum, it is often called UV-visible or UV-vis spectroscopy. This technique provides a convenient platform for detection of analytes and their concentration in a solution as compared to the classical analytical techniques including gravimetric and volumetric methods.

3.3.1 Principle of Absorption Spectroscopy

Principle of absorption spectroscopy is based on Beer-Lambert Law which states that the fraction of incident radiation absorbed is proportional to the number of absorbing molecules in its path. The amount of light absorbed or transmitted by a solution, directly depends on the concentration of the solute and length of the path travelled by radiation through the sample. The peak intensity of absorption spectrum is directly proportional to the number of molecules absorbing light of a specific wavelength.

Hence,

\[ \log \frac{I_o}{I} = \varepsilon c l \]

where, \( c \): concentration of the solute in mol l\(^{-1}\), \( l \): path length of the sample in cm, whereas \( I_o \) and \( I \) are the intensity of the incident light and intensity of light transmitted through the sample solution, respectively. \( \varepsilon \) represents the molar extinction coefficient dependent on absorbing species in a particular solvent at a particular wavelength. The ratio \( I / I_o \) is known as transmittance \( T \) while the logarithm of the inverse ratio \( I_o / I \) indicates the absorbance \( A \). Thus,

\[ - \log \frac{I}{I_o} = - \log T = \varepsilon c l \]

\[ \log \frac{I_o}{I} = A = \varepsilon c l \]

A = \varepsilon c l

From the absorption spectra one can easily calculate the wavelength at which the maximum absorption occurs. This wavelength is represented as \( \lambda_{\text{max}} \). With known values of \( \lambda_{\text{max}} \) and \( \varepsilon \) the concentration of the solution could be easily determined. The absorption spectrum of a compound is one of its most useful physical characteristics, both as a means of identification (qualitative analysis) and of estimation (quantitative analysis).
3.3.2 Instrumentation

The basic components of a UV-vis spectrophotometer are described in the Figure 3.7 shown below,

![Figure 3.7: Schematic representation representing different components of an absorption spectrophotometer.](image)

- **Light source**: The most general source of Ultraviolet light is a deuterium arc that offers light in the range of 190 - 380 nm. While a tungsten lamp or a quartz-iodine lamp is used as a source for obtaining Visible light. Xenon lamp source also serve as a potential candidate for light source as it covers the UV as well as visible range. Moreover, it ensures a long lifetime as it is consumed only during the actual measurement cycle. The major requirement of any source is its stability or in other words its intensity should not fluctuate during the measurements.

- **Wavelength selector**: In order to enhance the selectivity and specificity of the technique a narrow band of wavelength must be supplied. Most of the instruments generally use a filter for desired beam of light and thus are unable to provide a narrow band of the wavelengths. Most of the sophisticated instruments utilize a monochromator. A monochromator can be defined as a device used to provide light of single wavelength out of range of wavelengths emitted by the source. It comprises of an entrance slit, a collimating device which facilitates production of parallel light, a wavelength selection system, a mirror or a focusing lens and finally an exit slit. The two types of monochromators are prism and diffraction grating.

- **Sample handling**: Generally samples in solution or vapor phase are analyzed in a cell called cuvette which must be transparent to the wavelength of light passing through it. Various cuvettes are available for the spectral determination, but generally they are made of glass or quartz. A cuvette must have the following characteristics;
  - The entrance and exit surfaces must be exactly parallel and orthogonal.
  - Light path must be tightly controlled.
  - Optical windows of cuvette must be highly smooth, parallel and flat.
Generally, cuvettes of path length 1.0 cm are used, though cuvettes with smaller path lengths (0.1 mm or 0.05 mm) are also available.

- **Detectors:** The function of a detector is to convert a received light signal into an electrical signal which can be transformed into a readable output. The signal generated by the detector is linear in transmittance. The most commonly used detectors are the photomultiplier, the silicon diode and the diode array.

- **Signal Processing and Output Devices:** Finally the transducer sends the generated electrical signal to the recorder. The spectrum of solvent is electronically subtracted from the solution so as to determine the spectrum of particles under investigation only. Thus the output plot between the wavelength and the intensity of absorption is characteristic of the absorbing species.

### 3.4 Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy is a non-destructive technique used for exploration of electrical properties of materials and interfaces of conducting electrodes. Impedance can be defined as the measure of the ability of a circuit to resist the flow of electrical current; this resistance is a combined effect of resistors, capacitors and inductors. In impedance measurements a small excitation signal is used in order to obtain pseudo-linear response of the cell. In a linear system for an applied sinusoidal potential, the corresponding current is also sinusoidal at the same frequency but shifted in phase as shown in Figure 3.8 below,

![Figure 3.8: Current response in a linear system](image)
A linear system can be defined as the system in which the output of the system is the weighted sum of the responses from the individual input signals i.e., the output is simply the superposition. Therefore, the excitation signal can be expressed as,

$$E(t) = E_0 \cos(\omega t)$$

where, $E_0$ is the amplitude of the excitation signal, $\omega$ is the angular frequency, and $E_t$ is the potential at time $t$. Here angular frequency $\omega$ (radians/sec) is directly proportional to frequency, $f$ (in hertz),

$$\omega = 2\pi f$$

As observed from Figure 3.8, the response current at a time $t$ ($I_t$) in a linear system, is shifted in phase ($\phi$) and has different amplitude, $I_0$:

$$I(t) = I_0 \cos(\omega t - \phi)$$

Further, following Ohm's Law the AC resistance (admittance) can be calculated by the expression:

$$Z(t) = \frac{E(t)}{I(t)} = \frac{E_0 \cos(\omega t)}{I_0 \cos(\omega t - \phi)} = Z_0 \frac{\cos(\omega t)}{\cos(\omega t - \phi)}$$

Using Euler's relationship,

$$\exp(i\phi) = \cos \phi + i \sin \phi$$

Thus, the potential and corresponding current response can be described as follows

$$E(t) = E_0 \exp(j\omega t)$$

$$I(t) = I_0 \exp(i\omega t - i\phi)$$

Therefore, Impedance of the system can be described as a complex number, comprising of a real ($Z'$) and an imaginary part ($Z''$).

$$Z = \frac{E}{I} = Z_0 \exp(i\phi) = Z_0 (\cos \phi + i \sin \phi)$$

The plot between real part ($Z'$) and imaginary part ($Z''$) is known as "Nyquist plot".

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Electrochemical transformations occurring at the interface can be investigated by fitting it to an equivalent electrical circuit model shown in above Figure. 3.9. The most common electrical elements included in the circuit are resistors, capacitors, and inductors. Table 3.2 shows impedance value of the different electrical components,

Table 3.2: Impedance value of different electrical components.

<table>
<thead>
<tr>
<th>Electrical component</th>
<th>Relationship b/w I and E</th>
<th>Impedance (Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistor</td>
<td>$V = IR$</td>
<td>$Z = R$</td>
</tr>
<tr>
<td>Capacitor</td>
<td>$I = C , \frac{dE}{dt}$</td>
<td>$Z = \frac{1}{i\omega C}$</td>
</tr>
<tr>
<td>Inductor</td>
<td>$E = L , \frac{di}{dt}$</td>
<td>$Z = i\omega L$</td>
</tr>
</tbody>
</table>

From the Nyquist plot, the impedance spectra can be interpreted using the equivalent circuit (Randles, 1947), schematically displayed in Figure 3.9 (inset). The above circuit includes four major parameters: $R_s$ which represents the ohmic resistance of the electrolyte solution; $Z_w$ indicates the Warburg impedance, which is an outcome of the diffusion of ions from the bulk of the electrolyte to the electrode interface; $R_{ct}$ characterizes the resistance to electron transfer while the impedance $Z_{CPE}$ denotes the constant phase element (CPE). Since, $R_s$ and $Z_w$ represents the diffusion feature of redox probe in solution and bulk properties of the electrolyte solution, thus ideally they are not altered by modifications on electrode surface.
Whereas, the modification on electrode surface highly effects the $R_{ct}$ and $Z_{CPE}$ as their value corresponds to the insulating and dielectric features at the interface of electrode. The diameter of the semicircular part of the electrochemical impedance spectra corresponds to the charge transfer resistance at the electrode ($R_{ct}$), while the linear part represents the diffusion limited process or Warburg impedance ($Z_w$).

**Advantages**

✓ Non-destructive technique.
✓ Provides quantitative data.
✓ Provides time dependent information.
✓ Can be used in number of applications including fuel cells development, characterization of electroactive polymer layers, etc.

**Disadvantages**

✓ Costly.
✓ No information about the frequency of plotted data points can be obtained from the Nyquist plot.
✓ Complex data analysis.

### 3.5 Cyclic Voltammetry

Cyclic Voltammetry (CV) is one of the most extensively used electro-analytical technique for characterizing the electrochemical behavior of electrochemically active species. It is often the primary experiment done in any electrochemical study. In the year 1958, Kemulla et.al first practiced this technique at a hanging mercury drop electrode for detection of concentrations of Cu, Tl, Pb, Cd and Zn ions [111]. CV is a multifaceted technology giving information about thermodynamic parameters like redox potentials and equilibrium constants as well as kinetic parameters such as rate constants for reactions involving electro active species. The electrochemical behavior of any system is studied by scanning the potential at a working electrode relative to a reference electrode and monitoring the subsequent current flowing through a counter electrode in a quiescent solution.

The repeating triangular potential excitation signal shown in Figure 3.10, facilitates the potential of working electrode to sweep back and forth between two selected potential values known as switching potentials. The scan rate is reflected by the slope of the plot. The potentiostat monitors the current corresponding to changing applied voltage. The plot between
recorded current and potential is known as cyclic voltammogram.

Figure 3.11 represents a typical cyclic voltammogram of an electron transfer reaction in which the rate is governed by diffusion of the electroactive species to electrode surface. As observed from the plot, no flow of electrons or current is witnessed in the beginning of the scan. During the potential scan, when the applied potential achieves a positive potential value which is more than that of redox couple present in the solution, the anodic current starts flowing in the circuit due to oxidation of redox species at the electrode surface. The anodic current increases rapidly until the concentration of redox species getting oxidized near the surface of electrode becomes zero, as indicated by peak marked as $I_{pa}$ in Figure 3.11. After the

Figure 3.10: Cyclic voltammetry potential waveform with repeating potentials

Figure 3.11: Cyclic voltammogram of a reversible redox couple during a single potential cycle.
complete oxidation of redox species near the electrode surface, resulting anodic current starts decreasing. Similarly, after switching the direction of potential, the high negative potential at working electrode as compared to reduction potential of redox species, results in reduction of redox species accumulated near the electrode surface during the positive potential scan. The reduction of redox species is marked by appearance of cathodic current. Again, the current increases with increasing scan further towards high negative potential.

The potential value at which the species getting reduced is almost depleted in the vicinity of electrode is marked by \( I_{pc} \) in Figure 3.11. The critical parameters obtained from cyclic voltammogram, i.e., the magnitude of anodic peak current \( (I_{pa}) \), magnitude of cathodic peak current \( (I_{pc}) \) and their corresponding potentials at which peaks occur, \( E_{pa} \) and \( E_{pc} \) can be exploited further to know the reaction kinetics occurring at the working electrode. The rate of reaction could be determined by

- Mass transfer rate of the electro-active species.
- Rate of electron transfer between electrode and electro active species.
- Rate of adsorption or de-sorption at the surface of electrode.

**Nernst Equation**

For a redox reaction,

\[
\text{Red} \rightleftharpoons \text{Ox} + ne^{-}
\]

the electrode potential at equilibrium is given by Nernst equation in terms of the concentration of electroactive species involved in redox reaction as

\[
E = E^0 + \frac{0.059}{n} \log_{10} \left( \frac{[O_s]}{[R_s]} \right)
\]

Where, \( E^0 \) is the formal potential; \( E \) is the applied potential; \( O_s \) and \( R_s \) represent the concentration of the redox species at the interface, respectively.

In CV measurements, since the solution is static (no stirring) hence electron transfer from electroactive species to electrode surface takes place via diffusion only. The concentration gradient created as a result of conversion of redox species from one form to other is the driving force behind electron transfer via diffusion. The concentration-distance profile is shown in Figure 3.12. Hence the current response of the electrode would depend on
concentration of the electroactive species, the surface area of the electrode and the voltage scan rate, in addition to the diffusivity of the electroactive species.

Figure 3.12: Schematics showing concentration--distance profile at various stage of the cyclic voltammogram; the blue lines correspond to the reducing species and the red lines to the oxidizing species.

3.5.1 Reversible Process:

When both redox species exchange electrons with the electrode they are known as an electrochemically reversible couple. For a reversible system, the maximum current or the peak anodic/cathodic current is given by Randles–Sevcik equation:

\[ i_p = (2.69 \times 10^5)n^{3/2}AD^{1/2}\nu^{1/2}C^* \]

where \( i_p \) is the peak current (Ampere), \( n \) is the number of electron equivalent exchanged during the redox process, \( A \) represents the active area of the working electrode (cm\(^2\)), \( D \) is the diffusion coefficient(cm\(^2\)s\(^{-1}\)) and \( C_0 \) indicates the bulk concentration of the electroactive species(mol cm\(^{-3}\)); \( \nu \) is the scan rate (Vs\(^{-1}\)). Since the redox products are stable for a reversible system hence;
- Magnitude of $I_{pa}$ and $I_{pc}$ should be same and therefore, ratio of the two is unity. However, in case of slow or chemically coupled redox reaction the ratio is farther away from unity.
- The peak currents $I_{pa}$ and $I_{pc}$ are proportional to the square root of the scan rate.
- The corresponding peak potentials $E_{pa}$ and $E_{pc}$ do not alter as a function of voltage scan rate.
- The peaks position on the potential axes ($E_p$) is related to the formal potential of the redox process. The formal potential $E^\circ$ is centered between $E_{pa}$ and $E_{pc}$
  \[ E^\circ = \frac{E_{pa} + E_{pc}}{2} \]
- The peak separation $\Delta E_p$ can be used to determine the number of electrons transferred.
  \[ \Delta E_p = E_{pa} - E_{pc} = \frac{59}{n} \]
  where, $n$ is the number of electrons transferred at all scan rates. Thus, a reversible (fast) one electron process has $\Delta E_p$ value of 59 mV. However, the uncompensated solution resistance and non-linear diffusion are the two major sources incorporating errors in measured values for a reversible process. The process involving slow electron transfer, are characterized by a peak potential ($\Delta E_p$) greater than the above mentioned value and moreover, $\Delta E_p$ increases further with increasing scan rate.

But a large variation can be observed in electrochemical behavior or cyclic voltammogram in cases where system is irreversible or quasi-reversible as compared to their reversible counterparts.

### 3.5.2 Irreversible and Quasi-reversible Systems

The irreversibility in any system is a result of slow electron transfer between working electrode and redox species which can be characterized by a shift of the peak potential with the scan rate ($\nu$) as well as the value of $\Delta E_p$ is greater than 59 mV for one electron process.

\[ E_p = E^\circ - \frac{RT}{\alpha n_a F} \left[ 0.78 - \ln \frac{k^\circ}{D^{1/2}} + \ln \left( \frac{\alpha n_a F \nu}{RT} \right)^{1/2} \right] \]

Where $\alpha$ is the transfer coefficient and $n_a$ is the number of electrons involved in the charge-transfer step. Thus, $E_p$ occurs at potentials higher than $E^\circ$, with the overpotential related to $k^\circ$ (standard rate constant) and $\alpha$. Displacement in peak potential can be taken care of by
changing scan rate \( \nu \) irrespective of the rate constant \( k^\circ \). At 25°C temperature the peak potential and half peak potential differ by

\[
E_P - E^\circ = \frac{48}{\alpha n}
\]

Hence, with increase in the number of electron transfer, \( \alpha n \) increases resulting in more drawn out voltammogram.

A quasi-reversible system can be defined as any process or system which shows reversibility at low scan rates while shows irreversibility at higher scan rates. The shift in state from reversible to irreversible process is a result of comparatively slower mass transfer rate than the rate of electron transfer at the electrode surface. This in turn disturbs the Nernstian equilibrium and brings about quasi-reversibility in the system.

So far introduction to biosensors in general and glucose biosensors in particular have been discussed in chapter 1. Next chapter we have discussed the need for development of glucose biosensors, loop holes in existing literature and technology and historical development of glucose biosensors while present chapter dealt with the characterization techniques that have been exploited in the current work. In the following chapters, fabrication of glucose biosensors through synthesis of different nanostructures of various materials has been presented. Characterization of these nanostructures and fabricated biosensor has been carried out using the techniques discussed in the current chapter 3. Following chapter focuses on optimization of parameters for synthesis of monodispersed nanostructures of different materials.