CHAPTER 1

Introduction

- General Overview
- Electrode Process
- Electroanalytical Techniques
- Modification of Electrodes
- Carbon nanotubes and Graphene
- Characterization
- Aim and Scope of the present Investigation
Abstract

Electrochemical techniques are less time consuming and more accurate than the conventional methods like chemical and spectroscopic methods. This chapter deals with the introduction of biosensors and the general overview of electrochemistry and electrochemical techniques like Cyclic voltammetry, Chronoamperometry, Differential pulse voltammetry and Chronocoulometry which are used for the detection of molecules like NADH, H2O2, AA, DA, UA, ethanol and formaldehyde. This chapter also discusses the various methods for the modification the electrodes and the methods to characterization of the chemically modified electrodes by electrochemical methods, microscopic and spectroscopic techniques.
1.1. **General Overview**

The ability to measure accurately molecules of environmental, industrial, pharmaceutical, clinical and biomedical importance is crucial to the understanding of the various roles they play in human systems. The use of analytical methods such as chromatography and spectroscopy for the detection and measurement of pollutants are difficult and time consuming. Electrochemical sensors however, hold an outstanding ability among analytical methods available for environmental applications because of their simplicity and low-cost, hence the need to design and fabricate smart and highly selective electrochemical sensing devices is of major importance. The success of electrochemical sensing depends on the type of electrode used, the electrocatalyst used to modify the electrode or sensor as well as the construction technique.

A chemical sensor is a small device that can be used for direct measurement of the analyte in the sample matrix. Chemical sensor consists of a transduction element covered with a chemical or biological recognition layer. This layer interacts with the target analyte and the chemical changes resulting from this interaction and translated by the transduction element into electrical signals.

Electrochemical sensors represent an important subclass of chemical sensors in which an electrode is used as the transduction element. Electrochemical biosensors combine the analytical power of electrochemical technique with the specificity of biological recognition processes. The aim is to biologically produce an electrical signal that relates to the concentration of analyte. For this purpose, a biospecific reagent is either immobilized or retained at a suitable electrode, which converts the biological recognition event into a quantitative amperometric or potentiometric response.
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Biosensor consists of

a) Bioreceptor; that specifically binds to the analyte.

b) An interface; where a specific biological event takes place and gives rise to a signal.

c) The transducer element; which converts to an electronic signal.

d) Computer software; which converts to a meaningful physical parameter describing the process being investigated.

1.2. Electrode Processes

FARADAIC PROCESSES: All processes that obey Faraday’s law are termed faradaic processes; these processes involve electron transfer at an electrode / electrolyte interface. These reactions are also called electron / charge transfer reactions. Electrodes at which these processes occur are called charge transfer electrodes [1, 2].

Investigation of electrochemical behavior involves holding certain variables constant while observing the trends in others. Typical variable shown in diagram 1.1

Figure 1.1: Electrochemical behavior involves holding certain variables constant while observing the trends in others.
**NON FARADAIC PROCESSES:** Sometimes changes exist in the electrode / electrolyte interface without charge transfer taking place. These changes are due to processes such as adsorption and desorption. Such processes are called non faradaic processes. No electrons flow through the electrode / electrolyte interface during non faradaic processes. However, transient external currents can be generated by non faradaic processes [1, 2].

1.2.1. Mass Transport Processes

The movement of charged or neutral species in an electrochemical cell to the electrode surface is governed by three processes namely, diffusion, migration and convection [1-7].

**DIFFUSION** is the motion of species under the influence of chemical potential gradient. (i.e., from the region of high concentration to regions of lower concentrations).

**MIGRATION** is the motion of a charge body (such as an ion) under the influence of an electrical potential gradient.

Finally **CONVECTION** is hydrodynamic transport either due to density gradients (natural convection) or due to external means such as stirring or vibration of the electrode (forced convection).

1.3. Electrochemical Techniques

1.3.1. Cyclic Voltammetry

Cyclic voltammetry (popularly known as CV) is a type of potentiodynamic electrochemical measurement. It is the most performed electrochemical technique to study the electrochemical reactions as well to provide insight on the reversibility and kinetics of such reactions [8]. In cyclic voltammetry experiment, the potential of an electrode is
ramped linearly from an initial potential to a final potential and then back to the initial potential. The potential is applied between the reference electrode and the working electrode and the current is measured between the working electrode and the counter electrode. This data is then plotted as current \((i)\) vs. potential \((E)\). The potential at which the peak current occurs is known as the peak potential \((E_p)\) where the redox species have been depleted at the electrode surface. If the electronic transfer at the surface is fast and the current is limited by the diffusion of species to the electrode surface, then the current peak will be proportional to the square root of the scan rate. The magnitude of the faradaic current \((I_{pa}\text{-anodic peak current})\) or \((I_{pc}\text{-cathodic peak current})\), gives an indication of the rate at which electrons are being transferred between the redox species and the electrode. Cyclic voltammetric processes can be of three types reversible, irreversible and quasi-reversible.

**Reversible Process**

A reversible process is one in which the electron transfer is rapid, and the electroactive oxidized (or reduced) species in the forward scan is in equilibrium with the electroactive reduced (or oxidized) species in the reverse scan as per the (equation 1.1)

\[
\text{Red} \leftrightarrow \text{Ox} + \text{n}e^- \quad \text{1.1}
\]

Typical cyclic voltammogram of a reversible process consists of two symmetrical peaks, where the charges (the areas under the peaks) for oxidation and reduction are equal as all the adsorbed species undergo oxidation/reduction and the current peaks \(I_{pa}\) and \(I_{pc}\) are equal and proportional to the concentrations of the active species. \(\Delta E_p = (E_{pa} - E_{pc})\) should be independent of the scan rate \((v)\), but in practice \(\Delta E\) increases slightly with increasing \(v\), this is due to the solution resistance \((R_s)\) between the reference electrode and the working electrodes [9, 10]. Theoretically, the potential difference between the oxidation and
reduction peaks is 59 mV for one-electron reversible redox reactions. However, in practice, 
ΔE is sometimes found in the range 60-100 mV.

Below is a list of the diagnostic criteria used to characterize a reversible voltammogram.

\[ i_p \propto v^{1/2} \]

\[ E_p \text{ is independent of } v \]

\[ \Delta E_p = (E_{pa} - E_{pc}) = \frac{59}{n} \text{ mV at } 298 \text{ K} \]

At 25°C, the peak current is given by the Randles-Sevick equation [1, 2].

\[ i_p = (2.69 \times 10^5)n^{1/2}ACD^{1/2}v^{1/2} \]

where, \( i_p \) = peak current (A)
\( n \) = number of electrons transferred
\( A \) = electrode area (cm²)
\( C \) = concentration (mol cm⁻³)
\( D \) = diffusion coefficient (cm²s⁻¹)
\( v \) = scan rate (Vs⁻¹)

These parameters make CV most suitable for characterization and mechanistic studies of redox reactions at electrodes.

A linear plot of \( i_p \) vs. \( v^{1/2} \) indicates that the currents are controlled by diffusion to the electrode surface [9]. The ratio \( i_{pa} / i_{pc} = 1 \) for a totally reversible process. The potential where the current is half of its limiting value is known formal potential (\( E^0 \)). The formal potential of a reversible couple is given by the average of two peak potentials, \( E^0 = E_{pa} + E_{pc}/2 \), where \( E_{pa} \) and \( E_{pc} \) are the anodic and cathodic peak potentials respectively.

The separation between the peak potentials, \( \Delta E_p \) for reversible couple is given by the equation 1.3 and can be used to determine the number of electrons transferred. \( \Delta E_p \) is independent of scan rate, and at 298 K equation is 1.3


\[ \Delta E_p = E_{pa} - E_{pc} = 2.303 \frac{RT}{nF} = 0.0591/n \text{ V} \]

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**Irreversible Process**

For an irreversible process, only forward oxidation (reduction) peak is observed but at times with a weak reverse reduction (oxidation) peak, as the rate of mass transport increases, the reverse peak becomes less and less pronounced, resulting in slow electron exchange at the electrode surface [7].

Below is a list of the diagnostic criteria used to characterize an irreversible voltammogram

\[ i_{pc} \propto v^{1/2} \]

No reverse peak

\( E_{pc} \) shifts to higher overpotentials as \( v \) increases

The peak current, \( i_p \) for irreversible process is given by equation 1.4

\[ i_p = (2.99 \times 10^5)n(au)^{1/2}ACD^{1/2}v^{1/2} \]

Where, \( \alpha = \) coefficient of electron transfer,

\( i_{pv} = \) peak current (A)

\( n = \) number of electrons transferred

\( A = \) electrode area (cm\(^2\))

\( C = \) concentration (mol cm\(^{-3}\))

\( D = \) diffusion coefficient (cm\(^2\)s\(^{-1}\))

\( v = \) scan rate (Vs\(^{-1}\))

For a totally irreversible system the shift of the peak potential with the scan rate is characterized by equation 1.5

\[ \Delta E_p = E^{\circ} - \frac{RT}{anF} \left[ \frac{0.78 - ln \left( \frac{k}{D^{1/2} ln \left( \frac{anE^{C}v}{RT} \right) ^{1/2}} \right)}{D^{1/2} ln \left( \frac{anE^{C}v}{RT} \right) ^{1/2}} \right] \]
Where all the symbols are defined above. The peak potential and the half peak potential differ by \(0.048/\alpha\) n mV.

**Quasi-Reversible Process**

When the electrode process is neither very facile nor very sluggish, we speak of quasi-reversible behavior. In reversible process the current is purely mass-transport controlled, but in quasi-reversible process the current is controlled by both mass transport and charge transfer kinetics [1, 11]. For quasi reversible process, \(i_p\) increases with \(v^{1/2}\) but not in linear relationship and \(\Delta E > 0.059/\) n V increases with increase in \(v\) [2].

**1.3.2. Chronoamperometry**

Chronoamperometry is a simplest most-short lived electrochemical technique in which the potential of the working electrode is stepped and the resulting current from faradaic processes occurring at the electrode is monitored as a function of time. When using these techniques, it is very importance that the potential is stepped from a value where the species of interest is not reduced (or oxidized) to a value where the current is diffusion-controlled. Furthermore, the step potential must be selected so that only the redox species is electrolyzed. Cyclic voltammetry is useful for selecting these potentials. This uncomplicated potential step wave form monitors the current response following the electrode potential being stepped from an initial electrode potential at which the oxidized (reduced) species is stable in solution, to the first step potential where redox reaction occurs forming the reduced (oxidized) species and held at this value for the first step time in a single potential experiment. In a double potential step experiment, the potential is stepped again after the first step time and now changed to the second step potential, and it is then held at this value for the second step time.
In the analysis of CA data is based on the Cottrell equation 1.6 [12-14].

\[ i = nFACD^{1/2} \pi^{-1/2} t^{-1/2} \]  

Where, \( n \) = number of electrons transferred
\( F \) = Faraday’s constant (96485 Cmol\(^{-1}\))
\( A \) = electrode area \((\text{cm}^2)\)
\( C \) = concentration \((\text{mol cm}^{-3})\)
\( D \) = diffusion coefficient \((\text{cm}^2 \text{s}^{-1})\)
\( t \) = time \((\text{s})\)

A plot of \( i \) versus \( t^{-1/2} \) is often referred to as cottrell plot which defines the current – time dependence for linear diffusion control and can be used to calculate the diffusion coefficient \((D)\) resulting from the slope of the plot.

### 1.3.3. Chronocoulometry

Chronocoulometry belongs to the family of step techniques. Instead of varying current with time after application of a potential step perturbation in chronoamperometry, In Chronocoulometry a pulse of potential is applied and the amount of the charge passed as a function of time is observed. Chronocoulometry gives practically the same information that is provided by chronoamperometry, since it is just an integrated form of the current–time response. However, chronocoulometry offers important experimental advantages like

- Unlike the current response that quickly decreases the measured signal usually increases with time, and hence the later parts of the transient can be detected more accurately.
- A better signal-to-noise ratio can be achieved.
- Contributions of charging/discharging of the electrochemical double layer and
any pseudocapacitance on the surface (charge consumed by the electrode reaction of adsorbed species) to the overall charge passed as a function of time can be distinguished from those due to the diffusing electroreactants.

Chronocoulometry is useful for measuring electrode surface areas, diffusion coefficients, adsorption of electroactive species and the mechanisms and rate constants for chemical reactions coupled to electron transfer reactions [15, 16]. The total charge ($Q_{\text{total}}$) measured in response to the potential step comes from three sources as shown by integrated Cottrell equation 1.7

a) Charge of the double layer ($Q_{\text{dl}}$) which dominates at shorter times

b) Charge of the adsorbed species ($Q_{\text{ads}}$)

c) Charge of the species diffusing at the electrode ($Q_{\text{diff}}$) which dominates at longer times

$$Q_{\text{total}} = Q_{\text{diff}} + Q_{\text{dl}} + Q_{\text{ads}}$$

or

$$Q_{\text{total}} = 2nFAC(Dt)^{\frac{1}{2}}/\pi^{\frac{3}{2}} + Q_{\text{dl}} + Q_{\text{ads}} \quad \text{1.7}$$

Where, $n =$ number of electrons transferred

$F =$ Faraday's constant (96485 Cmol$^{-1}$)

$A =$ electrode area (cm$^2$)

$C =$ concentration (mol cm$^{-3}$)

$D =$ diffusion coefficient (cm$^2$s$^{-1}$)

$t =$ time (s)

The surface coverage of the electrode was calculated by adopting the method due to Laviron [17]. According to this method, the total amount of charge ($Q$) is related to the surface concentration of electroactive species ($\Gamma_{s}$) by the equation 1.8.
According to equation Cotrell the plot of $Q_{\text{diff}}$ vs. $t^{1/2}$ should be linear and the slope proportional to the concentration of the reactant, as well as to $n$, $A$ and $D^{1/2}$. This behavior is shown in Figure 1.2 and has been observed experimentally in a large number of cases. The intercept of $Q$ vs. $t^{1/2}$ plot gives $Q_{\text{di}}$ or $Q_{\text{di}} + Q_{\text{ads}}$ (in the case of adsorption of R). In the latter case, $Q_{\text{di}}$ can be estimated from the charge passed in the same experiment performed with supporting electrolyte only. It is evident that at short times $Q$ vs. $t^{1/2}$ curves are not linear, since neither the charging process nor the oxidation of the adsorbed amount of the reactant is instantaneous, under well-designed experimental conditions.

1.3.4. Differential Pulse Voltammetry

A third technique is differential pulse voltammetry as depicted in Figure 1.3 [2], a technique similar to CV. In CV, the potential across the electrodes is varied linearly with time up and down, in cycles. In DPV, the potential across the electrodes is varied in a step pattern. The start of each subsequent step is higher than the previous. The current is measured before and after each step, and the difference is returned. This allows the charging current to be removed from the output, yielding a more accurate result than CV.
1.3.5. Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy is a powerful technique employed for understanding electrochemical system including those involved in corrosion [18], batteries [19], fuel cells [20] and investigating electrochemical properties of electrodes or systems and their interfaces [21]. Impedance is measured by applying a small sinusoidal AC voltage voltage $V(t)$ probe (2-10 mV) to an electrochemical cell and measuring resultant sinusoidal current $I(t)$[21-24]. The AC probe voltage should be small enough so that the system response linear and allowing simple equivalent circuit analysis. The applied sinusoidal potential and the resultant sinusoidal current are represented as a function of time. These measurements are done over a suitable frequency range and the results are capable for characterizing physiochemical processes of the materials [2, 21-25]. The relationship is as shown in the equation 1.9

$$Z = \frac{V(t)}{I(t)} \quad \text{equation 1.9}$$

Where $V(t)$ is the sinusoidal applied voltage at time $t$, $V(t) = V_o \sin(\omega t)$, $V_o$ is the maximum potential amplitude, $\omega$ is the radial frequency in rad.s$^{-1}$ and can be related to frequency $f$(Hz) as $\omega = 2\pi f$. At the same frequency as the applied sinusoidal potential the
current response $I(t)$ is also sinusoidal but with a shift in phase $I(t) = I_0(\sin \omega t + \theta)$, where $I_0$ is the maximum current applied and $\theta$ represents the phase shift by which the voltage lags the current [2, 21, 22]. The in-phase current response corresponds to the real (resistive) component of the impedance and the out-of-phase current response determines the imaginary (capacitive) component. The complex notation of the impedance is shown in equation 1.10

$$Z = Z' + jZ'' = Z_{\text{real}} + jZ_{\text{imaginary}} \quad \text{equation 1.10}$$

Where $Z'$ and $Z''$ are the real and the imaginary parts of the impedance respectively and $j$ is a complex number [21].

**Data Interpretation of EIS**

The electrochemical impedance results are analyzed using electrical equivalent circuits, and within the circuit, simple electric elements such a resistance ($R$) and capacitance ($C$) are connected to model the electrochemical processes [29, 32]. Randles circuits which is often used to interpret simple electrochemical systems and it is shown in the Figure 1.4.

![Randles equivalent circuit](image)

**Figure 1.4: Randles equivalent circuit**

The resistance ($R_{ct}$) in the equivalent circuit represents the charge–transfer resistance, ($R_s$) represents the solution or the electrolyte resistance and the double layer capacitance ($C_{dl}$). In Randles circuit $R_s$ is connected in series to the parallel combination of
$R_{ct}$ and $C_{dl}$ and $Z_w$ is the Warburg impedance, which arises from reaction rate which is controlled by mass-transfer phenomenon, this component is connected in series to $R_{ct}$.

Impedance data can be graphically shown by either Nyquist or Bode plot [21, 22-24]. A Nyquist plot shown in the Figure 1.5. Shows the plot of the imaginary part ($Z''$) versus the real part ($Z'$) of the impedance data. Electrochemical impedance spectroscopy offers many benefits over the other techniques, some of them are given: (i) the use of low sinusoidal voltage makes the system remain at equilibrium, (ii) accurate and repeatable measurements and (iii) the characterizing of interfacial properties in the absence of a redox reaction.

![Figure 1.5: Nyquist Plot for Randles equivalent circuit.](image)

The following part of the discussion briefly describes the various methods reported or adopted in literature for the modification of electrodes.

### 1.4. Modified Electrodes

Electrode surfaces are modified in order to achieve an electrochemical function which is not possible or difficult to achieve using conventional electrodes. Chemically modified electrode is expected to have improvements on increased selectivity, sensitivity, chemical and electrochemical stability, as well as a larger usable potential window and decreased fouling effects. Chemically modified electrode is defined as a conducting or semiconducting material that is coated with a film of monomolecular, multi-molecular,
ionic, or polymeric film (Chemical modifier) which alters the electrochemical, optical, and other properties of the interface. In other words, the attachment of chemical modifier to the surface of an electrode imparts some chemical, electrochemical or desirable properties that are not available at the unmodified electrode. The conductive and semi conductive substrates are derived from conventional electrode materials.

1.4.1. General Methods of Modifying Electrodes

Electrode surfaces are modified by following methods

1. Chemisorption
2. Covalent Bonding
3. Composite
4. dip-dry coating
5. Drop-dry coating
6. spin-coating
7. Electro polymerization

Chemisorption

In this method, the film is strongly adsorbed or chemisorbed on the surface of the electrode yielding mono layer coverage. Electrode modification using self assembled monolayer falls in this category [25-27].

Covalent Bonding

This method employs a linking agent like organosilanes for stronger attachment to the substrate surface on which one of the several monomolecular layers of the chemical modifier can be attached covalently [28,29].
Composite

A method in which the chemical modifier mixed with an electrode matrix material to form a composite. An example for this method is having an electron-transfer mediator (the chemical modifier) mixed with carbon particles in a carbon paste electrode (the electrode matrix) [30].

Dip-dry Coating

The electrode is dipped in a solution of chemical modifier (polymer, modifier, catalyst) for a period such that a uniform film formation occurs on the electrode surface through adsorption. Then, the electrode is taken out of the solution and allowed to dry the solvent [31].

Drop-dry Coating

A known volume of the chemical modifier (polymer, modifier, catalyst) are dropped on to the electrode surface and allowed to stand so that the solvent gets evaporated [32].

Spin-Coating

Spin coating is done by applying uniform thin films of chemical modifier (polymer, modifier, catalyst) in excess on to flat electrode surface, which is then rotated at high speed in order to spread the chemical modifier by centrifugal force and evaporating to dry. A machine used for spin coating is called a spin coater, or simply spinner. Rotation is continued while the chemical modifier spins off the edges of the electrode surface, until the desired thickness of the film is achieved. The applied solvent is usually volatile, and simultaneously evaporates. So, the higher the angular speed of spinning, the thinner the film. The thickness of the film also depends on the concentration of the solution and the solvent [33].
Electro Polymerization

In this method of modifying the electrode, the electrode is dipped in a chemical modifier (polymer, modifier, catalyst) and the layers of electro polymerized materials builds on the electrode surface. Generally, the peak current increases with each voltammetric scan such that there is a observable difference between each scans indicating the presence of the polymerized material. For example, electro polymerization of aniline [34].

1.5. Carbon Nanotube Modified Electrodes

Carbon nanotubes were accidentally discovered by Japanese microscopist, Sumio Iijima in 1991 while investigating the synthesis of fullerenes by using electric arc discharge technique [35]. CNTs have a finite carbon structure, an allotrope of carbon with a cylindrical nanostructure. These nanotubes have diameters in the range of few nanometers and their lengths are up to several micrometers. Each nanotube is a single molecule made up of a hexagonal network of covalently bonded carbon atoms. Carbon nanotubes are fullerene related structures which consist of graphitic cylinders closed at either end. Carbon nanotubes are of two types: single-walled and multi-walled. A single-walled carbon nanotube consists of a single graphene cylinder of 1-2 diameter as depicted in Figure 1.6(a) [36], whereas a multi-walled carbon nanotube comprises several concentric graphene cylinders ranging from 2-50, with the tubes separated by a distance of 0.34 nm and the innermost tube having a diameter of 2 nm as shown in Figure 1.6(b) [35].
A carbon nanotube can be considered as a large molecule consisting of carbon atoms, forming a hexagonal mesh. It may also be regarded as a one atom thick sheet of graphite rolled into a tube, with high aspect ratio. Such cylinder can be observed as a fundamental structural unit, known as single walled carbon nanotube. Using that fundamental structural unit, a multi walled carbon nanotube can be formed. MWNTs are in fact concentrically nested SWNTs, with distance between two layers, or walls, of 0.34 nm. Each atom in a single layer has three nearest neighbouring atoms, and they are bonded by covalent bonds, which have characteristic properties (bond length and bond angle). Atoms on different layers of MWNT are not connected by covalent bonds and only interaction between them is through van der Waals forces. Van der Waals forces are rather weak compared to covalent bonds. The structure of carbon nanotubes are shown in Figure1.6 (b). A carbon nanotube is characterized in terms of diameter and chiral angle $\theta$, or with chirality of a tube. Chirality is defined by chiral vector,

$$C_h = na_1 + ma_2$$  

1.11
Chiral vector is direction along which the graphite sheet is rolled up to form a nanotube and it defines a carbon nanotube, through three basic patterns: zig-zag, with chiral angle $\theta = 0^\circ$, armchair with chiral angle $\theta = 30^\circ$ and basic chiral nanotube with chiral angle in range from $0^\circ < \theta < 30^\circ$. In expression (1) $n$ and $m$ are integers which represent the number of steps along the carbon bond of hexagonal lattice, and $a_1$ and $a_2$ are unit vectors (Figure 1.7) [37].

![Figure 1.7: Definition of roll-up vector as linear combinations of base vectors $a$ and $b$](image)

**Properties**

Carbon nanotubes have gained immense interest by the researchers, because of the unique properties such as high electrical conductivity, high thermal conductivity, adsorption properties, high surface area, excellent mechanical strength and good chemical stability so as nanoscale materials for the development of batteries [38], nano electronic devices [39-41] and for construction of electrochemical sensors [42].

**Applications**

CNTs have been studied for there various potential applications, considering to their remarkable properties [43]. Carbon nanohorns are the spherical aggregates of CNTs with irregular horn like shape. Research studies have proved CNTs and CNHs as a potential carrier for drug delivery system [44]. The outstanding mechanical properties and the high surface-to-volume ratio (due to their small diameter) make carbon nanotubes
potentially useful as anode materials in lithium-ion battery systems [45]. Oxygen reduction reaction is important for fuel cells and MWCNTs have the ability to electro-catalyze this reaction [46]. Extraordinarily high and reversible hydrogen adsorption of CNTs has attracted much consideration in the possibility of being used as high-capacity hydrogen storage media [47]. CNTs have the capability to function as electro catalysts which permits them to operate as electrochemical sensors. The fabrication of electrochemical sensors will be discussed in more details.

1.6. Graphene Modified Electrodes

Graphene was discovered by Andre Geim and Konstantin Novoselov in 2004, which led to their winning the 2010 Nobel Prize in Physics. It is a single layer of carbon and one atom thick. The carbon atoms are connected in a hexagonal lattice that looks like a honeycomb, with carbon–carbon distance of 0.142 nm.

Graphene is a flat monolayer of carbon atoms tightly packed into a two-dimensional (2-D) honeycomb lattice, and is a basic building block for graphitic materials of all other dimensionalities as shown in Figure 1.8. The single-layered honeycomb structure of graphene makes it the “mother” of all carbon-based systems: the graphite we find in our pencils is simply a stack of graphene layers; carbon nanotubes are made of rolled-up sheets of graphene; and Buckminster fullerene molecules, or “buckyballs”, are nanometre-size spheres of wrapped-up graphene [48]. A single 2-D sheet of graphene is a hexagonal structure with each atom forming 3 bonds with each of its nearest neighbors. These are known as the σ bonds oriented towards these neighboring atoms and formed from 3 of the valence electrons. These covalent carbon-carbon bonds are nearly equivalent to the bonds holding diamond together giving graphene similar mechanical and thermal properties as diamond. The fourth valence electron does not participate in covalent bonding. It is in the 2pz state oriented perpendicular to the sheet of graphite and forms a conducting π band.
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![Figure 1.8: Illustration of Graphene](image)

Properties

Graphene, a two-dimensional, single-layer sheet of sp$^2$ hybridized carbon atoms, has attracted tremendous attention and research interest, owing to its exceptional physical properties, such as high electronic conductivity, good thermal stability, and excellent mechanical strength so as nanoscale materials for the development of batteries [49] and for construction of electrochemical sensors [50].

Applications

Graphene have been studied for there various potential applications, considering to their remarkable properties [48]. One of the most extensively explored applications of graphene is the field-effect transistor (FET) [51]. Owing to its good electronic conductivity, optical transparency, and large specific surface area, graphene-based materials have been extensively explored in the field of photovoltaic’s applications [52]. Graphene-based materials possess large specific surface area, excellent conductivity, and availability for surface functionalization, which are important characteristics in the electrochemical applications [53]. Until now, many graphene-based electrochemical sensors have been reported to detect glucose [54, 55], ascorbic acid [56], dopamine [57] and H$_2$O$_2$ [58]. The fabrication of electrochemical sensors will be discussed in more details.
1.7. Microscopic Techniques

The following microscopic techniques have been used in this work to characterize the electrodes and confirm the presence of suspected or unknown structures.

1.7.1. Scanning Electron Microscopy

Scanning Electron Microscopy is a technique used to scan the dry surfaces of a material where one is only interested in the surface details [59]. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample’s surface topography, composition, and other properties such as electrical conductivity. The operation of the SEM is as follows: The SEM generates a beam of incident electrons in an electron gun above the sample chamber. The electrons are produced by a thermal emission source, such as a heated tungsten filament, or by a field emission cathode. The energy of the incident electrons can be as low as 100 eV or as high as 30 keV depending on the evaluation objectives. The electrons are attracted through the anode, condensed (condenser lens) and focused (objective lens) as a fine point onto the sample. High-energy electrons that are ejected by an elastic collision of an incident electron, typically with a sample atom’s nucleus, are referred to as backscattered electrons. Secondary electrons can be formed by collisions with the nucleus where substantial energy loss occurs or by the ejection of loosely bound electrons from the sample atoms, which are collected by secondary detector. The secondary electron detector produces a clear and focused topographical image of the sample where as the backscatter electron detector reflects an elemental composition of sample and used for energy X-ray analysis [60].

1.7.2. Atomic Force Microscopy

Atomic force microscope was invented by Binning et al in 1986. AFM provides 3D profile on nanoscale, by measuring the forces acting between a fine tip and a sample. The tip is attached to the free end of the cantilever (cantilever is made of Si or Si$_3$N$_4$) and is
bought very close to a surface. Attractive or repulsive forces resulting from interactions between the tip and the surface will cause a positive or negative bending of the cantilever. The bending is detected by a laser beam, which is reflected from the back side of the cantilever onto a position sensitive photodiode detector. The measured cantilever deflections are used to generate a map of the surface topography.

AFM is operated in three primary imaging modes.

a) Contact mode AFM: When the spring constant of cantilever is less than surface, the cantilever bends. The force on the tip is repulsive. By maintaining a constant cantilever deflection the force between the probe and the sample remains constant and the image of the surface is obtained.

b) Tapping mode AFM: In this mode the cantilever is oscillating close to its resonance frequency. The probe lightly taps on the sample surface during scanning, contacting the surface at the bottom of its swing. Forces that act between the sample and the tip will not only cause a change in the oscillation amplitude, but also change in resonant frequency and phase of the cantilever. The amplitude is used for the feedback and the vertical adjustments of the piezoscanner are recorded as a height image. Simultaneously, the phase changes are presented in the phase image.

c) Non contact mode AFM: In this mode, the probe does not contact the sample surface. The force on the tip is attractive. By maintaining a constant cantilever deflection changes in the amplitude due to attractive Vander Waals forces the surface topography can be measured [10].

1.7.3. Transmission Electron Microscopy

Transmission electron microscopy is a microscopy technique operated at a high resolution whereby a beam of electrons is transmitted through the sample owing to the
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interest in internal details thus revealing the morphology, crystallographic information and
the compositional information of the material examined. TEMs are capable of imaging at a
significantly higher resolution than light microscopes, owing to the small de Broglie
wavelength of electrons. This enables the instrument's user to examine fine detail even as
small as a single column of atoms, which is tens of thousands times smaller than the
smallest resolvable object in a light microscope. TEM forms a major analysis method in a
range of scientific fields, in both physical and biological sciences.

The operation of TEM is similar to that of the slide projector; however, it shines a
beam of electrons instead of light, generated from an electron gun. This stream of electrons
is focused by a coherent beam that is restricted by a condenser aperture. The beam striking
the sample transmits portions of the sample which are focused by the objective lens into an
image that is projected onto the screen. These are dark and light areas of the image
representing the more densely packed section which allowed fewer electrons to pass
through and the lens densely packed section which allowed more electrons to pass through,
respectively [61].

1.8. Spectroscopic Techniques

1.8.1. Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique used to study vibrational,
rotational and other low-frequency modes in a system [62]. It relies on inelastic scattering
or raman scattering of monochromatic light, usually from a laser in visible, near infrared or
near ultraviolet range. A sample is illuminated with a laser beam. Light from the
illuminated spot is collected with a lens and sent through a monochromator. Wavelengths
close to the laser line due to elastic Rayleigh scattering are filtered out while the rest of the
collected light is dispersed onto a detector. The Raman effect occurs when light impinges
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upon a molecule and interacts with the electron cloud and the bonds of that molecule. For the spontaneous Raman effect, which is a form of light scattering, a photon excites the molecule from the ground state to a virtual energy state. When the molecule relaxes it emits a photon and it returns to a different rotational or vibrational state. The difference in energy between the original state and this new state leads to a shift in the emitted photon's frequency away from the excitation wavelength.

1.8.3. Fourier Transform Infrared Spectroscopy

Infrared spectroscopy deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It can be used to explore the chemical properties of a sample, including chemical bonds, molecular geometries, molecular energy levels and molecular interactions.

Performing infrared spectroscopy with a steady state light source can provide analytical information on a sample with regard to its chemical composition and respective concentrations. If interferometer is used to encode the spectrum in the measured signal than it is called Fourier transform infrared spectroscopy.

A FTIR spectrometer uses a Michelson interferometer to modulate the optical signal and encode the spectrum information.

The advantages of using FTIR is

a) All the wave length is measured simultaneously with one detector.

b) Allows the spectrometer to produce a spectrum very quickly so it is able to combine multiple scans [63, 64].

1.8.3. Powder X-ray Diffraction Spectroscopy

Powder X-ray diffraction is a rapid analytical technique primarily used for phase identification of crystalline materials [65]. X-ray diffraction is based on constructive
interference of monochromatic X-rays and a crystalline sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law \( n\lambda = 2d \sin \theta \). These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

1.9. Aim and Scope of the Present Investigation

The aim of this work was the investigation of different types of electrode materials such as functionalized graphene modified graphite electrode, Multiwalled carbon nanotubes - graphene modified graphite electrode by layer-by-layer technique for the detection of NADH. Graphene - (polyaniline-polystyrene sulphonate) composite modified graphite electrode for detection of NADH and hydrogen peroxide. Functionalized graphene-alcohol oxidase modified electrode by layer-by-layer technique for the detection of ethanol, polystyrene sulphonate wrapped multiwalled carbon nanotubes modified graphite electrode for simultaneous determination of ascorbic acid, dopamine and uric acid, poly(diallyl dimethyl ammonium chloride) and polystyrene sulphonate - potassium hexacyanoferrate (II) composite by layer-by-layer technique on graphite electrode for the detection of ascorbic acid and electrodeposited gold particles on functionalized graphene modified graphite electrode for the determination of formaldehyde in alkaline medium.
1.10. References


