Chapter 2
Experimental
Setup and Instrumental Techniques

2.1 Introduction

In this chapter, details of the experimental setups will be presented. In addition, the instrumentation and characterization methods used throughout this work are provided.

2.2 Experimental Setup

2.2.1 Monomer Distillation

All the monomers used in this thesis were distilled before polymerization. The experimental setup for monomer distillation is given in Figure 2.1

Figure 2.1 Schematic diagram of the apparatus setup for distillation of monomers.
2.2.2 Sample Filtration

In this thesis, samples obtained by chemically polymerization were filtered. The relevant experimental setup is presented in Figure 2.2.

![Figure 2.2 Schematic diagram of the apparatus setup for sample filtration.](image)

2.2.3 Electrochemical Polymerization Cell Setup

In chapter 6, PEDOT thin films have been polymerized electrochemically. The synthesis of PEDOT films was performed using a CH instrument electrochemical workstation (CHI 6005D). A three electrode cell setup was employed for the electropolymerization as shown in Figure 2.4

The working electrode used in a polymerization should be conductive and stable at the polymerization potential employed, to avoid oxidation of the working electrode itself. In this work, glassy carbon (GC) employed as the working electrode.
A platinum wire used as the counter electrode and Ag/AgCl (3M KCl) was used as the reference electrode in the aqueous solution.

2.2.4 Electrochemical Instrumentation Setup

All electrochemical measurements were performed with a CHI6005D electrochemical workstation (Austin, USA) interfaced to a Lenovo computer system (Intel E7300, 2.66 GHz, 1.00 G.B Ram, 300 G.B hard disk space) as shown in Figure 2.3.

![Figure 2.3: Picture of CHI6005D electrochemical analyzer interfaced to a Lenovo computer system.](image)

Electrochemical experiments were carried out with a conventional three-electrode system as portrayed in Figure 2.4. Comprising a bare and modified gold and/or glassy carbon working electrode, a platinum wire auxiliary electrode and an Ag/AgCl (3.0 M KCl) reference (CH Instrument, Austin, USA) with a three-electrode
system, the potential of the working electrode is controlled relative to the reference electrode which is placed as close to the working electrode as possible to reduce the ohmic potential drop. The current flows between the working electrode and auxiliary electrode. All potentials are reported vs. Ag/AgCl reference at room temperature.

![Diagram of an electrochemical cell consisting of the working gold and/or glassy carbon electrode, Ag/AgCl reference electrode and an auxiliary platinum electrode.](image)

Figure 2.4 Diagram of an electrochemical cell consisting of the working gold and/or glassy carbon electrode, Ag/AgCl reference electrode and an auxiliary platinum electrode.

All the solutions were deaerated with inert nitrogen gas for about 15 minutes prior to data acquisition and the electrochemical cell (Figure 2.4) was blanketed under the inert gas during the entire experimental period.
2.2.4.1 Reference and Auxiliary Electrodes

The Ag/AgCl reference electrode consists of a silver wire, coated with silver chloride which is immersed in a solution containing 3.0 M KCl as shown in Figure 2.5. Since, there is a large chloride concentration gradient across the reference electrode frit, there is slow diffusion of chloride ions from the reference electrode solution into the same solution. Therefore, the reference electrode must be stored with the frit immersed in a 3.0 M KCl (which is identical in composition and concentration to the reference electrode solution) between experiments. Sometimes, air bubbles will form in the solution next to the frit and these can be removed by gently flicking the end of the electrode otherwise artifacts (e.g. excessive noise) may be seen in the experimental data while voltammetric experiments are performed.

Figure 2.5 Photos of (A) Ag/AgCl reference electrode and (B) platinum auxiliary electrode.
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The platinum wire auxiliary electrode (Figure 2.5) provides a surface for a redox reaction to balance the one occurring at the surface of the working electrode, and does not need special care, such as polishing. In order to support the current generated at the working electrode the surface area of the auxiliary electrode must be equal to or larger than that of the working electrode.

2.3 Instrumental Techniques

2.3.1 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy is one of the most intensively used method to characterize the structural properties of CPs and interaction between polymer and DNA [166,167]. In this thesis, the FT-IR spectra of CP composites were measured in the range 400-4000 cm\(^{-1}\) on polymer pellets made with KBr at nexus-670 FT-IR spectrometer, taking 64 scans at a resolution of 10 cm\(^{-1}\). The FT-IR spectra of PPy, PPy-PANI, PPy-PANI-AuNP composites were dropcasted on individual gold coated silicon substrate and electrochemically polymerized thin film (PEDOT & PEDOT-AuNP) were measured directly on the films at nexus-670 FT-IR spectrometer.

2.3.2 UV-Visible Spectroscopy

UV-Visible spectroscopy is a very useful technique for probing the molecular and conformational structures of CPs [168,169]. The particular absorption bands indicate the different forms of the polymer such as oxidation state, the extent of doping as well as the conjugation length of the polymer backbone. In this thesis the UV-Visible spectra of CPs (in m-cresol and N-methyl-2-pyrrolidone (NMP)) was characterized using Shimadzu Pharmaspect UV-1700.
2.3.4 Raman Spectroscopy

Raman spectroscopy is based on monitoring the intensity and wavelength of light that is scattered inelastically from molecules or crystal. It is used to study vibrational, rotational and other low-frequency modes in a system. Typically, a sample is illuminated with a laser beam at a certain frequency. The scattered light from the illuminated spot, which consists of Rayleigh and Raman scattering, is collected with a lens and pass through a monochromator. Rayleigh scattering has the same frequency as the laser light and is filtered out, while Raman scattering is dispersed onto a detector. The difference in frequency of the laser light and the observed Raman scattering corresponds to the vibrational frequencies of the sample. The Raman spectrum is the plot of an optical intensity of Raman scattering versus vibration frequency. Raman spectroscopy has been widely used to characterize the structural property of CP [170,171]. The Raman frequency shifts have been used to investigate oxidation states [172,173], conductivity [174], and degradation product from thermal treatments [175] or electrochemical oxidation of CPs [176]. It has also been used for the identification of DNA attachment [177-179]. In this thesis, a Reinshaw 1000 Raman spectrophotometer (Japan) employing a 514.5 nm laser beam was used to characterize the samples.

2.3.5 X-Ray Diffraction Analysis

The amorphous and crystalline status of surfaces was investigated with a Bruker powder X-ray diffractometer (Bruker, AXS-D8). Cu Kα (λ= 0.154 nm) radiation was used for excitation and placed at a distance of 20 mm. The scanning speed is 0.02° s⁻¹.
2.3.6 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was carried out on samples to determine if any weight loss (indicating oxidation or decomposition) occurred over the elevated temperature range. Measurements were made in an air atmosphere using a TA instrument (UK) model SDT Q600 via heating under air at 20°C/min.

2.4 Microscopy

2.4.1 Scanning Electron Microscopy

The scanning electron microscopy (SEM) is perhaps the most routinely utilized instrument for the characterization of nanomaterials. SEM makes use of electrons emitted from a tungsten filament in high vaccum [180].

![Schematic diagram of SEM setup.](image)

Figure 2.6 Schematic diagram of SEM setup.
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The primary electron beam produced is focused onto the sample with various electric field lenses and raster scanned across the sample. Once this primary beam hits the sample, electrons are ejected and produce secondary electron beam. This secondary electron beam is then accelerated towards the detector or a phosphor screen. The image produced can be photographed and or detected in terms of energy loss. This is then plotted with the co-ordinate from the raster scan and an image is produced. The general setup of SEM equipment is shown in Figure 2.6. SEM has been the most commonly used tool to characterize the morphology and topology of different samples.

The morphology of the samples was characterized using a JSM-6390 (JEOL, Tokyo, Japan) & TESCAN (VEDA3) scanning electron microscope at an accelerating voltage 20 kV and 15 kV, respectively. Samples were mounted on 100 nm gold coated silicon chips.

2.4.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electron transmitted through the specimen. The technique provides useful information regarding morphology, crystallography, particle size distribution and its elemental composition. Operation of the TEM is very similar to the SEM and must be done in high vacuum (Figure 2.8 - setup of instruments). An electron beam is produced by a tungsten filament and is focused onto the sample in a similar fashion as the SEM. However, the beam passes through and or is scattered by the sample placed on a special grid. The electron beam then is focused again onto a fluorescent plate.
and or a CCD camera for image capture. To image finer details using TEM, a high voltage is required and typically 100 kV is needed to obtain images in the nanometer range. There are few shortcomings with TEM, an obvious problem is the sample preparation, due to the need to be performed in vacuum.

![Schematic diagram of TEM setup.](image)

Other disadvantages are the particles need to be in dilute concentration before casting on the sample grid, as thick layers do not produce clear images. Further, if the particles are non-conducting, at high magnification the sample will “charge up”, due to the bombardment of electrons and cause the image to move, creating a skewed effect when the image is captured. Although there might be disadvantages to this technique, TEM still remains one of the most powerful characterization techniques used to
visualise nanomaterials, as the resoulution achieved is beyond any other techniques available apart from scanning probe microscope (SPM) [180].

A HITACHI (Technai G20) transmission electron microscope (TEM) was employed to characterize the Au nanoparticles. The colloidal were Au nanoparticles dispersed in ethanol and dropped on micro grids copper coated on a carbon support film, then dried under air and characterized by TEM.

2.4.3 Atomic Force Microscopy

Atomic force microscopy does not use any light or electrons to observe the nanostructures on a surface, but rather monitors the forces between the tip and a surface as they are placed close to each other (Figure 2.10) [180]. It was developed in 1986 by Binning, Quate and Gerber [181], and since then has had a major impact on science and technology.

Figure 2.8 Schematic of an AFM probe raster-scan over a sample surface.
To acquire an image, a probe (usually silicon on a cantilever) is brought close to a surface and due to the forces between the surface and the probe the cantilever is deflected to or away from the sample. The extent of deflection can be modelled using Hooke’s law. A laser and photo diode monitor the degree of deflection exhibited by the cantilever. The probe is then passed over the surface and the deflection measured as the probe raster scans the surface. This technique has very high resolution even down to the atomic scale, but this depends on a number of factors such as the radius curvature of the probe and the raster scan rate of the measurement.

AFM is not only used for the characterization of modified substrate and also used for chemical and biosensor applications [182-186]. In this thesis, PARK (XE-100, Korea) AFM was employed to characterize PEDOT, PEDOT-AuNP and PEDOT-Au-S-ssDNA thin films on 100 nm gold coated silicon chips.

2.5 Electrochemical Techniques

2.5.1 Cyclic Voltammetry

Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many areas of chemistry. It is widely used for the study of redox process understanding reaction intermediates and obtaining stability of reaction products. This involves applying an external triangular voltage to the electrochemical cell, sweeping through a potential range and reversing the direction of the sweep in a cyclic fashion (Figure 2.12). The resulting current \( I \) is monitored as a function of applied potential \( E \) to give the \( I-E \) curve which in this kind of experimental is called a cyclic voltammogram.
Figure 2.9 Wave form excitation signal for cyclic voltammetry.

A typical cyclic voltammogram for a reversible redox process is given in Figure 2.13. The important parameters in a cyclic voltammogram are the peak potential \( E_{pa}, E_{pc} \) and Peak current \( I_{pa}, I_{pc} \) of the anodic and cathodic peaks respectively. If the electron transfer process is fast when compared to other process (such as diffusion), the reaction is said to be electrochemically reversible and the peak separation is

\[
\Delta E_p = E_{pa} - E_{pc} = 2.303 \frac{RT}{nF}
\]

Thus, for a reversible redox reaction at 25°C with \( n \) electrons \( \Delta E_p \) should be 0.0592/n V or about 60 mV for one electron. In practice this value is difficult to attain because of factors such as cell resistance. Irreversibility due to a slow electron
transfer rate results in $\Delta E_p > 0.0592/n \text{ V}$, greater, say, than 70 mV for a one-electron reaction. The formal reduction potential ($E^0$) for a reversible couple is given by

$$E^0 = \frac{(E_{pa} + E_{pc})}{2}$$

Figure 2.10 A typical cyclic voltammogram with an oxidation peak at $E_{pa}$ with a maximum anodic current ($I_{pa}$) in the forward scan, and a corresponding reduction peak at $E_{pc}$ with a maximum cathodic current ($I_{pc}$) in the reverse scan.

For a reversible reaction, the concentration is related to peak current by the Randles-Sevick expression (at 25 °C)

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_0 \nu^{1/2}$$

where $n$ is the number of electron transferred in the reaction, $I_p$ is the peak current, $A$ is the electrode area, $D$ is the diffusion coefficient, $C_0$ is the concentration and $\nu$ is the scan rate.
In this thesis, cyclic voltammetry has been used to investigate the redox properties of CPs. It has also been used to investigate the DNA hybridization event in the presence of redox probe such as [Fe(CN)$_6$]$^{3/-4-}$ and [Ru(NH$_3$)$_6$]$^{3+}$.

2.5.2 Electrochemical Impedance Spectroscopy

Impedance measure the electrical impedance of an interface in AC steady state with constant DC bias conditions. Most often this is accomplished by imposing a small sinusoidal voltage at a particular frequency and measuring the resulting current; the process can be repeated at different frequencies. Taking the current-voltage ratio at each frequency yields an impedance spectrum. This approach, known as Electrochemical impedance spectroscopy (EIS), has been used to study a variety of electrochemical phenomena over a wide frequency range [187]. This analysis provides quantitative information about the conductance, the electric coefficient, properties of the interface of a system, and the dynamic change due to absorption or charge-transfer phenomena [188]. Data obtained by EIS is usually expressed graphically in a Nyquist plot or Bode plot, as shown in Figure 2.14.

![Figure 2.11 Impedance data present in: (A) a Nyquist plot, and (B) a Bode plot.](image)
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Electrochemical impedance spectroscopy has shown to give useful information about barrier properties and is sensitive to the interfacial electron transfer processes, as revealed in the characterization of biomolecular functionalized electrodes and biorecognition event at electrode surfaces. Therefore it has been widely used in detection of DNA hybridization [189-193]. Both Faradic and non-Faradic impedance spectroscopy has been applied for the study of DNA hybridization, either by studying the diffusion of redox probes in electrolyte [194,195] or by recording the impedance changes of DNA layers directly [196,197]. Non-Faradic impedance reveals conformational changes and relaxation process upon DNA hybridization [198]. Other hand, Faradic impedance analysis is an attractive approach an increase of negative charge density associated with phosphate backbone of DNA [199]. In chapters 4 and 5 of this thesis, Faradic EIS has been employed to analyze changes in interfacial properties of modified electrodes with PPy-PANI-AuNP and PPy-PEDOT-AgNP with the inclusion of DNA binding on the surface. The detailed experimental parameters have been given in chapters 4 and 5.

2.5.3 Chronocoulometry

Chronocoulometry (CC) involves measurement of the charge (coulombs) vs. time (chrono) to an applied potential step waveform. CC has the same wave form as the potential step-which is one of the simplest potential wave form (Figure 2.15), the potential is changed instantaneously from the initial potential (E_{rest}) to the first step potential (E_{step}), and it is held at this value for a present step time. This is a single potential step experiment. The E_{rest} is held at a potential where no Faradaic reaction takes places so that no significant current flows, whereas E_{step} is set at the redox potential of the compound of interest. In a double potential step experiment, the E_{step}
is changed to the second step potential ($E_{\text{step}}$) after the first present time, and it is then held at $E_{\text{step}}$ for another present time. The charge is monitored as a function of time (Figure 2.16). CC is useful for measuring electrode surface areas, diffusion coefficients, the time window of an electrochemical cell, adsorption of electroactive species, and the mechanisms and rate constants for chemical reactions coupled to electron transfer reactions [200-203].

![Potential wave for chronocoulometry](image)

Figure 2.12 Potential wave for chronocoulometry.

A chronocoulometry plot is used in this thesis to DNA hybridization detection with different target DNA (complementary, non-complementary, single and double base mismatched). It has also been used to quantify the amount of probe DNA on the PEDOT-AuNP composite modified glassy carbon electrode using the Cottrell equation [204,205]. The DNA hybridization and quantification of probe DNA using chronocoloumery will be explained in detail in chapter 6.
2.5.4 Differential Pulse Voltammetry

Pulse voltammetric techniques are aimed at lowering the detection limit of voltammetric measurements and it have been introduced by Barker and Jenkin [206]. Differential pulse voltammetry (DPV) is comparable to normal pulse voltammetry in that the potential is scanned with a series of pulses. However, it differs from normal pulse voltammetry (NPV) because each potential pulse is fixed, of small amplitude (10 to 100 mV) and is superimposed on a slowly changing base potential (Figure 2.16). Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse. These sampling points are selected to allow for the decay of the non-Faradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential (Figure 2.14).
In DPV technique, measured current is only a product of the Faradaic process, with the capacitive charging current eliminated. Hence, this technique has been widely used in DNA hybridization sensor to discriminate the different target DNA in the presence of electroactive indicators [207-210].

Figure 2.14 Potential wave forms for differential pulse voltammetry.

Figure 2.15 A typical differential pulse voltammogram.
A differential pulse voltammogram has been used to DNA hybridization detection with different target DNA (complementary, non-complementary, single and double base mismatched). The DNA hybridization detection using DPV will be explained in detail in chapter 3.

2.5.5 Chronoamperometry

Chronoamperometry (CA) is an electrochemical technique in which the potential of the working electrode is stepped from one potential ($E_i$) to another potential ($E_f$), and the resulting current at the electrode (caused by the potential step) is monitored as a function of time. A typical chronoamperogram is given in Figure 2.18. A chronoamperometry experiment is performed in a three electrode cell (Figure 2.4) using a CH instrument electrochemical workstation (Model 6005D). The detailed experiment parameters are given in chapter 4.

![Figure 2.16 A typical chronoamperogram](image-url)
2.6 Conductivity Measurement

Electrical conductivity is a fundamental property of CPs; many applications of CPs are based on their remarkable ability to conduct electric current. The accurate measurement of conductivity is an important step to understand particular CP and their properties.

Figure 2.17 Schematic top view configuration of a four point probe for electrical conductivity measurements.

The room temperature conductivities of as prepared CP is measured using a Keithley four-probe conductivity meter (Nanovoltmeter 2482 & Sourcemeter 2400, USA). For PPy-PANi, PPy-PEDOT, PPy-PEDOT-AgNPs, PEDOT and PEDOT-AuNP composites, the samples were first pressed into pellets (1 cm dia., 1.5 mm thick) under 4 ton pressures for 10 minutes and the conductivities of the CP pellets are then measured. For PEDOT films, the conductivities are measured directly on the
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surface of the films (measured on the side that faced the electrolyte during electropolymerization).

A schematic diagram of the four point probe is shown in Figure 2.1. The CP samples are placed on the base and the probe head was fully lowered. The probe tips made contact with the samples by pressing the probe head into the sample surface. Measurements are performed by sourcing a constant current, dependent on the resistivity of the material between the outer electrodes and the potential drop across the two inner electrodes is measured. The resistivity was calculated from the following equation

\[ \rho = \frac{V}{I} \times 1.256 \quad \text{............... (2.1)} \]

\[ \text{Conductivity} = \frac{1}{\rho} \quad \text{...... (2.2)} \]

Where \( \rho \) = volume resistivity (\( \Omega \) cm), \( V \) = potential measured in volts and \( I \) = applied current in amperes.

2.7 pH measurement

The pH of all solutions is measured using a Susima pH meter (India), which is calibrated using standard pH 4.0 (± 0.05), pH 7.0 (± 0.05) and pH 9.2 (± 0.05) buffer solutions. The pH meter had an accuracy of ±1 units.