Chapter II

Experimental
CHAPTER II

Experimental

2.1 Reagents

All chemicals used in the present study are of analytical grade and they are listed below in alphabetical order:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium oxide (≤ 10 µm)</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Ammonia</td>
<td>CDH</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Chloroplatinic acid hexahydrate</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Copper(II) chloride dihydrate</td>
<td>Merck</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Cytochrome c from horse heart</td>
<td>Aldrich</td>
</tr>
<tr>
<td>4-Dimethylaminopyridine</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>Merck</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CDH</td>
</tr>
<tr>
<td>3,4-Ethylenedioxythiophene</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Glucose</td>
<td>SD’s</td>
</tr>
<tr>
<td>Graphite</td>
<td>Alfa aesar</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>CDH</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Merck</td>
</tr>
<tr>
<td>Methanol</td>
<td>SD’s</td>
</tr>
<tr>
<td>Multi-walled carbon nanotube (Outer diameter = 10-15 nm; Inner diameter = 2-6 nm; Length = 0.1-10 µM)</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Myoglobin from equine heart</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Nafion solution (5 wt %)</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>CDH</td>
</tr>
<tr>
<td>4-Nitrophenol</td>
<td>Merck</td>
</tr>
<tr>
<td>Potassium chlorate</td>
<td>Aldrich</td>
</tr>
</tbody>
</table>
Apart from the above chemicals, graphene oxide and graphene were prepared from graphite and the details are given in the respective chapters.

### 2.2 Preparation of Buffers

Phosphate buffer solutions (PBS) of different pH values were prepared using 0.1 M potassium dihydrogen phosphate and 0.1 M dipotassium hydrogen phosphate as given in Table 2.1 [1].

**Table 2.1** Preparation of 0.1 M PBS of various pH values

<table>
<thead>
<tr>
<th>pH</th>
<th>Volume of 0.1 M $\text{K}_2\text{HPO}_4$ (mL)</th>
<th>Volume of 0.1 M $\text{KH}_2\text{PO}_4$ (mL)</th>
<th>Volume of 0.1 M $\text{NaOH}$ (mL)</th>
<th>Volume of 0.1 M $\text{HCl}$ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>49.5</td>
<td>-</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>6.0</td>
<td>6.60</td>
<td>43.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.0</td>
<td>30.7</td>
<td>19.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.4</td>
<td>40.2</td>
<td>9.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.0</td>
<td>47.0</td>
<td>3.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.8</td>
<td>48.3</td>
<td>-</td>
<td>1.70</td>
<td>-</td>
</tr>
</tbody>
</table>
2.3 Electrochemical Cell and Electrodes

For normal voltammetric studies, an electrochemical cell with provision for three electrodes was used. The working electrode was a glassy carbon electrode (GCE). The GCE (3 mm diameter; CH Instruments, USA) was polished with a slurry of fine alumina powder (≤ 10 µm) to a mirror finish and then thoroughly rinsed with double distilled water. A platinum wire was used as the counter electrode. The platinum electrode was cleaned by rinsing with freshly prepared chromic acid solution and then with distilled water. It was then treated with hot nitric acid (5 %). After rinsing with distilled water, the dry electrode was heated to a dull redness over a non-luminous flame. The reference electrode was either a Ag/AgCl (CH Instruments) or a saturated calomel electrode (SCE) (pH Products, India). Amperometry measurements were made using a polished GCE with a geometric area of 0.24 cm². The electrochemical experiments were carried out under deoxygenated conditions at temperature 25 ± 1 °C. Double distilled water was used to prepare the solutions.

2.4 Instrumentation

The electrochemical measurements were made using CH instruments 700C series and PARSTAT 2273. EIM6ex ZAHNER (Kroanch, Germany) was used for electrochemical impedance spectroscopy (EIS) studies using a frequency range between 1 Hz and 1 MHz at an applied AC voltage of 0.01 V. Amperometric measurements were performed by an analytical rotator AFMSRX (PINE instruments, USA) equipped with a rotating disc electrode (RDE). The absorption spectra were recorded using a JASCO (V630) spectrophotometer. The IR data were obtained using a FT-IR spectrometer (Shimadzu 8400S). Raman spectra were recorded using Labram HR800 model spectrometer. The surface morphology was obtained using a scanning electron microscope (SEM) VEGA 3 SBU and also a high resolution scanning electron microscope (Philips, XL30-SFEG). The surface topography of the samples was obtained using an atomic force microscope (AFM) (Shimadzu 9500). Transmission electron microscopy (TEM) studies were carried out with Hitachi S-3000H instrument.
2.5 Treatment of Results

The following gives a brief outline of the principle and instrumentation of the techniques used in the present study.

2.5.1 Electrochemical Measurements

(a) Linear Sweep Voltammetry

Linear sweep voltammetry involves applying a linear potential sweep to the working electrode while monitoring simultaneously the current flowing in the circuit. A signal generator produces a voltage sweep from an initial potential to a final potential and a potentiostat applies this potential ramp to the electrode under study. The scan direction can be positive or negative and in principle, the sweep rate can possess any constant value (Eq.2.1) [2,3]:

\[
\text{Sweep rate} = \frac{dE}{dt} \tag{2.1}
\]

Oxidation or reduction of a species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced. A plot of current vs potential is called a voltammogram. It is conventional to assign the positive current to reduction and the negative current to oxidation.

(b) Cyclic Voltammetry

Cyclic voltammetry is one of the most versatile electroanalytical techniques, which gives useful information regarding the mechanism of electrochemical reactions. Cyclic voltammetry is an extension of linear sweep voltammetry in that the direction of the potential scan is reversed at the end of the first scan (the first switching potential), and the potential range is scanned again in the reverse direction. In a typical voltammetric experiment, an unstirred solution containing a supporting electrolyte and an electroactive species is taken [4]. The experiment consists of applying a triangular waveform of potential (sweep rate varying from a few millivolts to a few volts per second) to the working electrode with respect to the reference electrode. The current is concurrently measured. The peak current (I_p, in Amperes) for a reversible process is given by the Randles–Sevcik equation (Eq.2.2) [5].
Experimental

\[ I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C_o^{b} v^{1/2} \] (2.2)

where, \( n \) is the number of electrons transferred, \( A \) is the area of the electrode (cm\(^2\)), \( D \) is the diffusion coefficient (cm\(^2\) s\(^{-1}\)), \( C_o^{b} \) is the bulk concentration (mol. cm\(^{-3}\)), and \( v \) is the sweep rate (V s\(^{-1}\)).

For an irreversible process, the reverse peak will be absent. The \( E_p \) value of the forward peak will vary with the sweep rate. The forward peak current \( I_p \) is given by Eq.2.3 [4]:

\[ I_p = 3 \times 10^5 n (\alpha n)^{1/2} A C_o^{b} D^{1/2} v^{1/2} \] (2.3)

where, \( \alpha \) is the transfer coefficient and \( n \) is the number of electrons transferred in the rate determining step. For a quasi-reversible process, the corresponding reverse peak may appear but the difference in the peak potentials (\( \Delta E_p \)) will be greater than that expected for a reversible process.

An electrode coated with a monolayer of an electroactive material shows a symmetrical response in the cyclic voltammogram. The ratio of the peak currents will be unity but \( \Delta E_p \) falls to zero. The peak current \( I_p \) is given by Eq.2.4 [5]:

\[ I_p = n^2 F^2 \Gamma v / 4RT \] (2.4)

where, \( \Gamma \) is the total amount of reactant initially present on the electrode surface, \( v \) is sweep rate, \( R \) is gas constant, \( F \) is faraday constant and \( T \), the temperature.

(c) Chronoamperometry

Chronoamperometry deals with the measurement of current as a function of time. This technique involves stepping the potential of the working electrode from a value at which no faradaic reaction occurs to a potential at which the surface concentration of the electroactive species is effectively zero. A stationary working electrode and unstirred solution are used. The resulting current-time dependence is monitored. As mass transport under these conditions is only by diffusion, the current-time curve reflects the change in the concentration gradient in the vicinity of the surface. This involves a gradual expansion of the diffusion layer associated with the
depletion of the reactant and hence decreased slope of the concentration profile as time progresses. Accordingly, the current decays with time, as given by Cottrell equation (Eq. 2.5) [4]:

$$i(t) = nFAC \frac{D}{\pi t}$$  \hspace{1cm} (2.5)

where $t$ is the time lapsed after the potential is stepped. Chronoamperometry is often used for measuring the diffusion coefficient of electroactive species or the surface area of the working electrode. It can also be applied to the study of mechanisms of electrode processes.

**(d) Chronopotentiometry**

Chronopotentiometry deals with the measurement of potential as a function of time. In this technique, a current is applied to the electrode and the potential changes to a value at which the flux of the electroactive species is sufficient to supply the applied current. After a certain time, the flux of redox species to the surface cannot sustain this current and the potential changes rapidly to a new value at which another species (often solvent or electrolyte) is reduced (or oxidized). This time, termed the transition time ‘$\tau$’ follows the Sand equation (Eq. 2.6) [6]:

$$\tau^{1/2} = \pi^{1/2}nFD^{1/2}C/2I$$ \hspace{1cm} (2.6)

where $I$ is the current density.

The two primary advantages of chronopotentiometry are that the transition time ($\tau$) is directly proportional to $D$ and that it is the same regardless of the heterogeneous electrode kinetics.

**(e) Differential Pulse Voltammetry (DPV)**

Differential pulse voltammetry is an important method for measuring trace level of organic and inorganic species [6]. In this technique, a fixed-magnitude superimposed on a linear potential ramp was applied to the working electrode. Current samples are taken twice per cycle, i.e. just before the pulse application and again late in the pulse life. The difference in first and second current ($\Delta I$) is
calculated as \( i(t_2) - i(t_1) \). 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 as shown in Fig. 2.1. The differential-pulse operation results in reducing the charging background current and allows a detection limit down to \( 10^{-8} \) M.

![Excitation signal for DPV](image)

**Fig. 2.1** Excitation signal for DPV

The differential pulse voltammetry is characterized by important parameters such as pulse amplitude, pulse width and sample period. Pulse amplitude is the height of the potential pulse. Pulse width is the duration of the potential pulse. Sample period is the time at the end of the pulse during which the current is measured.

**f) Electrochemical Impedance Spectroscopy (EIS)**

Impedance spectroscopy is a technique in which a sinusoidal, small-amplitude signal is used to probe multiple electrical properties of materials. EIS has been widely used in many fields of electrochemistry e.g., electrode kinetics, double-layer studies, batteries, corrosion, solid-state electrochemistry and bioelectrochemistry [7]. EIS investigates the interfacial properties of conductive and semi-conductive surfaces. A time-dependent perturbation signal, generally represented by a sinusoidal voltage, is applied to the electrochemical cell of particular frequency as shown in Eq.2.7.

\[
E_t = E_0 \ Sin (\omega t)
\]  

(2.7)
where $E_t$ is the potential at time $t$, $E_0$ is the amplitude of the signal, $\omega = 2\pi f$ is the radial frequency and $f$ is the frequency expressed in Hertz (Hz). The current response is then measured as shown in Eq. 2.8.

$$I_t = I_0 \Sigma v(\omega \cdot \tau + \phi)$$  \hspace{1cm} (2.8)

where $I_t$ is the time-dependent current intensity, $I_0$ is the amplitude of signal, $\omega = 2\pi f$ is the radial frequency and $\phi$ is the phase angle between $E_t$ and $I_t$. The total impedance of the system ($Z$) can be defined by the ratio of the applied signal to the current response as shown in Eq. 2.9.

$$Z = \frac{E_t}{I_t} = \frac{E_0 \sin(\omega t)}{I_0 \sin(\omega t + \phi)} = Z_0 \frac{\sin(\omega t)}{\sin(\omega t + \phi)}$$  \hspace{1cm} (2.9)

The total impedance of the system, $Z$, is therefore defined by a magnitude, $Z_0$, and a phase shift, $\phi$. Eq. 2.9 allows the impedance to be represented as a vector in the complex plane. This representation is well known as the Nyquist plot (Fig. 2.2), in which the imaginary part of the impedance, $-Z_i$, is represented versus the real part, $Z_r$. Electrical equivalent circuit of EIS can be associated with the electrochemical system under study. In this way, the electrical components of the circuit (e.g., resistance and capacitance) can be directly correlated to a simple physical phenomenon. A typical electrical circuit used to fit the experimental data is the Randles equivalent circuit as represented in Fig. 2.3. Parameter $R_1$ corresponds to the solution-phase resistance. When operating in a faradaic mode (i.e. in the presence of a redox probe), $R_2$ corresponds to the charge-transfer resistance, which is inversely proportional to the rate of electron transfer. $C$ represents the double-layer capacitance and $W$ corresponds to the Warburg impedance, which results from mass-transfer limitations.

![Fig. 2.2 Nyquist Plot](image-url)
2.5.2 Absorption (UV-visible) Spectroscopy

The alternate title for this technique is electronic spectroscopy, since it involves the promotion of electrons to higher energy levels. A spectrophotometer is a device, which detects the percentage transmittance of light radiation when light of certain intensity and frequency range is passed through the sample. Thus, the instrument compares the intensity of the transmitted light with that of the incident light. The modern spectrophotometers consist of light source, monochromator, detector, amplifier, and the recording device. The most suitable sources of light are: tungsten lamp and hydrogen–deuterium discharge lamp [8].

UV-visible spectroscopy is a reliable and accurate analytical laboratory assessment procedure that allows for both qualitative and quantitative analysis of a substance. Specifically, the technique probes the electronic transitions of molecules as they absorb light in the UV-visible regions of the electromagnetic spectrum. UV-visible spectroscopy is applicable to a wide range of samples (molecules and inorganic ions or complexes in solution) in different fields such as forensic science, pharmaceuticals, food, biochemistry and analytical chemistry [9]. When sample molecules are exposed to light having energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrophotometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance vs
wavelength. The peaks in a UV-visible spectrum of organic compounds are commonly due to $n \rightarrow \pi^*$ and/or $\pi \rightarrow \pi^*$ transitions. Both the shape of the peak(s) and absorption band maxima ($\lambda_{\text{max}}$) in the spectrum give information about the structure of the compound.

Since absorbance of a sample will be proportional to the number of absorbing molecules in the spectrometer light beam (e.g. their molar concentration in the sample tube), it is necessary to correct the absorbance value for this and other operational factors if the spectra of different compounds are to be compared in a meaningful way. The corrected absorption value is called "molar absorptivity", and is particularly useful when comparing the spectra of different compounds and determining the relative strength of light absorbing functions (chromophores). Molar absorptivity ($\varepsilon$) is defined as in Eq.2.10

$$\varepsilon = \frac{A}{c \cdot l} \quad \text{(Eq.2.10)}$$

where, $A$ = absorbance, $c$ = sample concentration in mol L$^{-1}$ and $l$ = length of light path through the sample in cm.

In the present study the UV-visible spectroscopy is used to measure the absorption characteristics of the metal complex, carbon nanotubes, graphene oxide, graphene and conducting polymer composites.

### 2.5.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR Spectroscopy is based on the determination of the interaction between IR radiation and a sample that can be either a solid or liquid or gas. An FT-IR spectrometer simultaneously collects spectral data in a wide spectral range. The FT-IR spectrum is an important record, which gives sufficient information about the structure of a compound. This technique is generally employed to identify all types of organic and several types of inorganic compounds [9,10]. Absorption in the IR region is due to the changes in the vibrational and rotational levels. Usually the spectrum is obtained for solid samples. Solids may be examined as an alkali halide mixture and potassium bromide serves the purpose well. The substance under investigation should be absolutely dry as water absorbs strongly at 3710 cm$^{-1}$ and also near 1630 cm$^{-1}$. 
Impurities in a compound can be detected from the nature of the bands, which no longer remain sharp and well defined. The region from 1500-500 cm$^{-1}$ is called the ‘finger print region’ which is very useful to interpret the functional groups of organic molecules.

### 2.5.4 Raman Spectroscopy

Raman spectroscopy is a useful technique for the identification of a wide range of substances - solids, liquids, and gases [11]. It is a straightforward, non-destructive technique requiring no special sample preparation. Raman spectroscopy involves illuminating a sample with monochromatic light and using a spectrometer to examine light scattered by the sample. At the molecular level photons can interact with matter by absorption or scattering processes. Scattering may occur either elastically, or inelastically. The elastic process is termed Rayleigh scattering, while the inelastic process is termed Raman scattering. Raman scattering occurs when the system exchanges energy with the photon and the system subsequently decays to vibrational energy levels above or below that of the initial state. The frequency shift corresponding to the energy difference between the incident and scattered photon is termed the Raman shift. Depending on whether the system has lost or gained vibrational energy, the Raman shift occurs either as an up- or down-shift of the scattered photon frequency relative to that of the incident photon.

The down-shifted and up-shifted components are called respectively the Stokes and anti-Stokes lines. A plot of detected number of photons vs Raman shift from the incident laser energy gives a Raman spectrum. Different materials have different vibrational modes, and therefore characteristic Raman spectra. This makes Raman spectroscopy a useful technique for material identification. In the laser Raman spectrometer, He-Ne laser with the wavelength of 633 nm is used as the excitation source due to its highly monochromatic nature and high beam fluxes. In the visible spectral range, Raman spectrometers use notch filters to cut out the signal from a very narrow range centred on the frequency corresponding to the laser radiation. Light from the sample passes back through the microscope optics into the spectrometer. Raman shifted radiation is detected with a charge-coupled device (CCD) detector and a computer is used for data acquisition and curve fitting. These
2.5.5 Powder X-ray Diffraction

X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the crystallographic structure of materials [12]. By varying the angle $\theta$, the Bragg’s law conditions are satisfied by different d-spacing in polycrystalline materials. Based on the principle of XRD, a wealth of structural, physical and chemical information about the material investigated can be obtained. Powder XRD is a non-destructive technique widely applied for the characterization of crystalline materials. The modern XRD consists of X-ray tube, detector, graphite monochromator, filter and slit. The copper X-ray tube used in XRD has a wavelength of Cu radiation at 1.54 Å. The interactions between X-ray and the crystalline material help to identify its structure. The variation of diffraction intensities is obtained against the angle (2$\theta$) of diffraction. From the XRD data, the lattice parameter and grain size can be calculated using the following formulae (Eqs.2.11-2.13):

(a) Formula to find lattice constant for cubic structure

$$\frac{1}{d^2} = \frac{h^2 + k^2 + l^2}{a^2}$$  \hspace{1cm} (2.11)

(b) Formula to find lattice constant for hexagonal structure

$$\frac{1}{d^2} = \frac{4(h^2 + hk + k^2)}{3} + \frac{1^2}{c^2}$$  \hspace{1cm} (2.12)

where $h$, $k$, $l$ is the plane indices and $d$ is the interplanar spacing (Å)

(c) Debye-Scherrer formula to find grain size (D) from XRD data

$$D = \frac{0.94 \lambda}{\beta \cos \theta}$$ \hspace{1cm} (2.13)

where $\lambda$= 1.54Å, $\beta$ = FWHM $\times \pi/180$ in which FWHM is the full width at half maximum value.
2.5.6 Energy Dispersive X-ray Spectroscopy (EDX)

Energy dispersive X-ray spectroscopy (EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample [13]. It is one of the variants of X-Ray Fluorescence (XRF) analysis. As a type of spectroscopy, it relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analysing X-rays emitted by the matter in response to being hit with charged particles. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an element's atomic structure to be identified uniquely from each other.

To stimulate the emission of characteristic X-rays from a specimen, a high energy beam of charged particles such as electrons or protons or a beam of X-rays is focused into the sample being studied. At rest, an atom within the sample contains ground state (or unexcited) electrons in discrete energy levels or electron shells bound to the nucleus. The incident beam may excite an electron in an inner shell, ejecting it from the shell while creating an electron hole where the electron was. An electron from an outer, higher-energy shell then fills the hole and the difference in energy between the higher-energy shell and the lower energy shell may be released in the form of X-rays.

The energy of the X-rays emitted from a specimen can be measured by an energy dispersive spectrometer. As the energy of the X-rays is characteristic of the difference in energy between the two shells and of the atomic structure of the element from which they were emitted, this allows the elemental composition of the specimen to be measured. The information on the X-ray energy is sent as a voltage signal to a pulse processor, which measures the signals and passes them onto an analyzer for data display and analysis.

2.5.7 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is 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 thousands of 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. TEMs find application in cancer research, virology, materials science as well as pollution, nanotechnology and semiconductor research [13,14]. TEM is a microscopy technique in which 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 electrons transmitted through the specimen. The image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. A TEM is composed of several components, which include a vacuum system in which the electrons travel, an electron emission source for generation of the electron stream, a series of electromagnetic lenses, as well as electrostatic plates.

2.5.8 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is one of the most widely used techniques for the characterization of nanomaterials and nanostructures. The resolution of the SEM approaches a few nanometers and the instrument can be operated at magnifications that are easily adjusted from 10 to over 3,00,000 [13].

In a typical SEM, a source of electrons is focused into a beam, with a very fine spot size of 5 nm and having energy ranging from a few hundred eV to 50 KeV that is restored over the surface of the specimen by deflection coils. As the electrons strike and penetrate the surface, a number of interactions occur that result in the emission of electrons and photons from the sample and SEM images are produced by collecting the emitted electrons on a cathode ray tube. Principal images produced in the SEM are of three types: secondary electron images, backscattered electron images and elemental X-ray maps. When a high-energy primary electron interacts with an atom, it undergoes either inelastic scattering with atomic electrons or elastic scattering with the atomic nucleus. In an inelastic collision with an electron, the primary electron transfers part of its energy to the other electron. When the energy transferred is large enough, the other electron will emit from the sample. If the emitted electron has energy of less than 50 eV, it is referred to as a secondary electron. Backscattered electrons are the high energy electrons that are elastically scattered and essentially
possess the same energy as the incident or primary electrons. The probability of backscattering increases with the atomic number of the sample material. Although backscattering images cannot be used for elemental identification, useful contrast can develop between regions of the specimen. The excited atom will decay to its ground state by emitting either a characteristic X-ray photon or an Auger electron, both of which have been used for chemical characterization. Combining with chemical analytical capabilities, SEM not only provides the image of the morphology and microstructures of bulk and nanostructured materials and devices, but can also provide detailed information of chemical composition and distribution. If the samples are conducting, they are directly measured while less conducting samples are to be gold sputtered before taking it onto the specimen stage of the SEM. In recent years, high resolution scanning electron microscopy (HRSEM) is used.

HRSEM generates electron beam diameters of less than 1 nm. The powerful microscope uses Schottky-type field emission source with voltage ranging from 200 V to 30 KV. Resolutions of 1.5 nm at > 10 KV and 2.5 nm at 1 KV were achieved. The microscope provides detectors for secondary imaging and back-scattered electrons imaging. HRSEM is the advanced tool for the analysis in the areas of materials science, semiconductor and the electronics industries and life-science.

2.5.9 Atomic Force Microscopy (AFM)

An atomic force microscope (AFM) can measure the force between a sample surface and a very sharp probe tip mounted on a cantilever beam having a spring constant of about 0.1-1.0 N/m [13]. The instrument consists of a cantilever with a nanoscale tip, a laser pointing at the end of a cantilever, a mirror and a photodiode collecting the reflected laser beam, and a three dimensional positioning sample stage which is made of an array of piezoelectric strips. The images are generated by scanning the tip across the surface. The AFM measures the minute upward and downward deflections of the tip cantilever while maintaining a constant force of contact.

A variety of tip-sample interactions may be measured by an AFM, depending on the separation. At short distances, the van der Waals interactions are predominant. The van der Waals force consists of interactions of three components: permanent
dipoles, induced dipoles and electronic polarization. Long-range forces act in addition to short-range forces between the tip and sample, and become significant when the tip to sample distance increases such that the van der Waals forces become negligible. Examples of such forces include electrostatic attraction or repulsion, current induced or static-magnetic interactions, and capillary forces due to the condensation of water between the sample and tip.

Measuring the force with the cantilever in the AFM is achieved by three methods. In the first method the deflection of the cantilever is directly measured which is called contact mode method. In the second method, the cantilever is vibrated and changes in the vibration properties are measured which is called non-contact mode. In the third method, called tapping mode, in which a cantilever with attached tip is oscillated at its resonant frequency and scanned across the sample surface.

The advantage of using the AFM-based technique over the SEM is that the resolution may be much better. This is because the resolution is solely determined by the geometry of the AFM probe and thus the radius of the probe’s tip determines the resolution. Besides, the surface topography, surface roughness and fractal analysis can also be observed using AFM. Unlike in SEM, both conducting and insulating materials can be analyzed without any special sample preparation.
2.6 References


