Chapter 2

Synthesis and Characterization technique

This chapter briefly describes the experimental procedures for the synthesis of sophorolipid, isolation, purification and the characterization equipment’s used in the research that is represented in the thesis. Basic concepts and principles have been discussed herein.
2.1 Sophorolipid biosynthesis

2.1.1 Materials

Media composition for the synthesis of sophorolipid such as malt extract, glucose, yeast extract and peptone used in this study were purchased from Hi-media, India; Oleic acid was purchased from Sigma-Aldrich (USA). Sodium sulphate was purchased from Merck, India. Organic solvents such as ethyl acetate and n-hexane used were of analytical grade and were purchased from Rankem India.

2.1.2 Microorganism and maintenance

Micro-organism, a yeast Candida bombicola (ATCC 22214) was used for synthesis of sophorolipid (SL). It was obtained from American type culture collection, USA. Candida bombicola was grown for 48 h at 28 °C on agar slants containing: malt extract, 0.3 %; glucose, 2.0 %; yeast extract, 0.3 %; peptone, 0.5 % and agar, 2.0 %. C. bombicola was sub-cultured monthly and maintained at 4 °C in a refrigerator.

2.1.3 Inoculum development

Before the production of sophorolipid, inoculum was developed by transferring a loop-ful C. bombicola cells from slants to MGYP (malt extract, 0.3%; glucose, 2.0%; yeast extract, 0.3%; peptone, 0.5%) broth medium and incubated for 24 h at 28 °C with 180 rpm orbital shaking (Figure 2.1).

Figure 2.1 Incubator shakers for sophorolipid synthesis (Image from Ref. 2)
2.1.4 Synthesis of Sophorolipid

Production of sophorolipid was performed in 1000 ml Erlenmeyer flasks containing 200 ml MGYP medium. The fermentative production of SLs was initiated by transferring the inoculum (10%, v/v) into 200 ml of medium followed by incubation at 28 °C with 180 rpm orbital shaking. MGYP medium was supplemented with 1 ml Oleic acid dispersed in equal volume of ethanol. The fermented broths were examined for the production of SL at regular intervals (12, 24, 48, 72 and 96).³

Sophorolipid of oleic acid was prepared by resting cell method. In the first step adequate cell mass was harvested by growing the *Candida bombicola* (ATCC 22214) in MGYP medium. Then the cells were redispersed in production medium containing 10% glucose. This production medium is supplemented with hydrophobic secondary carbon source i.e. oleic acid in absolute alcohol. Incubation of yeast biomass with glucose and fatty acid at 28 °C and 180 rpm on shaker for 96 to 120 hrs, a brown and viscous crude SL could be seen at the flask bottom (Figure 2.2).

![Figure 2.2 Typical oily crude sophorolipid from Candida bombicola.](image)

After incubation period, cells were separated from the broth by centrifugation at 5000 rpm, 10 °C for 20 min. Extracted of SL from supernatant was done with ethyl acetate. To the ethyl acetate phase, anhydrous sodium sulphate was added for removal of residual water, filtered and then ethyl acetate was removed under vacuum. The unconverted fatty acid was removed by washing with n-hexane.
Extraction of the sophorolipids was only possible with solvent extraction. Sophorolipid can be synthesized by other lipophilic carbon materials substrates with carbon chain length C 14-18. Increased interest in lactonic sophorolipid lies in its structure which is single structural form the diacetylated lactone. This form of the sophorolipids has biological importance because of high potential application in cancer treatment.

2.1.5 Isolation of sophorolipid

After incubation of 72 hrs it was observed that SL brownish viscous layer production begin at the bottom of the culture broth which was continuously increased till 96 hrs. After 99 hrs, culture medium was centrifuged at 5000 rpm for 10 min. The supernatant was separated and extracted twice with equal volumes of ethyl acetate. To make sure the isolation of SLs, the cells were also washed with ethyl acetate to remove the glycolipids and fatty acid stick to cells. Then ethyl acetate layer obtained from both the supernatant extraction and washing of cells were collected and dried over anhydrous sodium sulphate and solvent was removed by rotary-evaporation at 45°C. The brownish viscous product was obtained, which was washed thrice with n-hexane to remove the un-reacted fatty acid and was dried under vacuum. The final compound was stored at 4 °C till further use.

2.1.6 Qualitative analysis by thin layer chromatography (TLC)

Thin layer chromatography (TLC) is the simplest, quickest and cheapest then various analytical techniques extensively used for the qualitative analysis of compounds. Commercially available standard silica gel coated on aluminum plates (Kiesel-gel 60F254, Merck, Darmstadt, Germany, silica layer thickness 2 mm) were used for the analysis of SL mixture. Glass micro-capillary is used for the spot application of samples. Many solvent combinations were tried for the separation of compounds. Ultra-visible absorbing compounds can be visualized under detection at 254 nm of UV light. The ethyl acetate extract of SL mixture was used for the determination of number of components present in the mixture by means of thin layer chromatography. Developing system used was CHCl₃:CH₃OH in the ratio of 95:5, v/v. Poly molybdic acid (PMA) solution was used as charring agent for detection of SL components at TLC plates. Spots were visualized by charring with Poly molybdic acid (PMA) solution.
2.1.7 Purification of sophorolipid

2.1.7.1 High performance liquid chromatography (HPLC)

Chromatography involves the column resolution of a small batch of flowing, multi-component fluid into separate fluid volumes of (practically) pure solute solutions. There are different types of chromatography, based on the column and method used, such as molecular-sieve (or gel-filtration or gel-permeation) chromatography, ion-exchange chromatography and affinity chromatography. For example, in analyzing mixtures of sugars such as maltose and glucose, the different affinities of these sugars for the primary amino groups on the surface of the support material in a commercially prepared carbohydrate column is used to separate the sugars in an HPLC (High Performance Liquid Chromatography) apparatus (Figure 2.3). The different sugars emerge from the column at different times, and they are then detected and quantified separately using a refractive index detector. Separations based on HPLC are useful in resolving mixtures of related components before their individual quantification. The data was collected on the HPLC system- Delta 600 series from Waters Corporation and 425 nm wavelengths was used for detection. The elution was carried out with gradient solvent systems with a flow rate of 1.0 mL/min at ambient temperature. The compounds were analyzed using HP Chem-Station software.5

Figure 2.3 High Pressure Liquid Chromatography (Image from Ref. 5)
Sophorolipid mixture obtained from fermentative production was dissolved in acetonitrile and analyzed by reversed-phase high performance liquid chromatography (HPLC) with a Waters 2487 separation module (Waters Co. Milford, Massachusetts) using analytical symmetry C18, 5 μm column (250 × 4.6 mm2). The gradient solvent elution profile used was as follows: water: acetonitrile (95:5, v/v) holding for 10 min; to a final composition of water: acetonitrile (5:95, v/v) with a linear gradient over 50 min and holding for 10 min. The flow rate was 0.5 ml/min. The peaks were detected at 220 nm wavelength by absorbance detector. Fractions from different peaks were collected and mixed separately in many runs.

2.1.8 Pulsed Laser Synthesis Method

Laser irradiation is a novel and unique technique to synthesize nanostructures from liquid/solid precursors. In this method high energy laser pulses are allowed to interact with the precursor samples present either in solid or liquid medium for certain time period. It is a very unique method for the synthesis of nanoparticles because of heat or photochemical mechanisms which result in molecular level changes. Several transient reactions can be manipulated with the laser irradiation by adding some external reagents which can construct totally new compounds after initial photochemical mechanism.⁶

![Figure 2.4 Pulsed UV Laser (KrF excimer)](image-url)
Laser irradiation is a similar technique like molecular beam epitaxy (MBE), sputter deposition (CVD) and chemical vapor deposition (CVD). Basic setup of laser irradiation is quite simple compare to other deposition techniques while physical phenomena of target and laser are quite complex (Figure 2.4). All three different types of sophorolipids crude, acidic and lactonic form of sophorolipid were dispersed in water and pulse laser irradiated at different energy intensity. Various concentration of sophorolipid has been applied for laser irradiation experiment for the synthesis of fluorescent mesostructures. Samples were collected at different time interval for complete process time. At various concentration of sophorolipid we have found some undefined morphology but the final parameter for fluorescent mesostructures from sophorolipid were 1; sophorolipid concentration 3.5 mg/ml and 2; laser pulsed excimer laser with $\lambda=248$ nm, pulse width 20 ns, energy density~166 mJ/cm$^2$.

2.2 General Characterization technique

2.2.1 Mass spectra analysis

Matrix assisted laser desorption/ionisation (MALDI) is applicable to analyse soft bio-derived materials such as biopolymers, DNA, protein, peptides and carbohydrate. It could also use for large molecules analysis such as polymers, dendrimres and other macromolecules (Figure 2.5).
These molecules tend to be fragile and fragment when ionized by usual ionization process. It resembles to electrospray ionization technique which used for soft materials analysis. Mass spectrometry techniques have vast application in the field of structural determination of biomolecules. Positive and negative ESI mass spectra were obtained with an API QSTAR PULSAR hybrid MS/MS quadrupole TOF system (Applied bio-systems).

MALDI is a three step technique; sample mix with matrix and drop on metal plate. Then laser irradiation of the sample which trigger ablation and desorption of sample and matrix. Finally the sample is ionized by being deprotonated or protonated in ablated hot plum of gases which accelerated into mass spectrometer to analyze it. The matrix molecules are crystalline in nature. There are three generally used matrix are 2, 5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA) and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid). Preparation of matrix is done by mixed it in purified water and a solvent usually acetonitrile (ACN) or trifluoroacetic acid (TFA).

Acidic and lactonic form of sophorolipid were analyzed by ESI-MS analysis and for this 10 μl of SL (stock solution of 2-3 mg dissolved in acetonitrile) was injected into the mass spectrometer for analysis.

2.2.2 NMR (nuclear magnetic resonance) analysis

NMR spectroscopy is study of differences in the magnetic properties of the various magnetic nuclei present in a molecule and to deduce in large measure what is the positions of these nuclei within the molecule. The different kinds of environment and the different kinds of atoms present in a molecule could be deduced as well as the number of atoms present can be known.

The nucleus of the hydrogen atom (the proton) possesses both electric charge and mechanical spin; thus, it generates a magnetic field (Figure 2.6). Upon the effect of an external magnetic field, the proton aligns either with the field (lower energy state) or opposed to the field (higher energy state), in accordance with the quantum restrictions. The spinning proton acting like a spinning magnet precesses around the axis of the applied external magnetic field. The spinning frequency of the nucleus does not change but the speed of the precession does.
The precessional frequency, \( \nu \), is directly proportional to the strength of the external magnetic field (\( B_0 \)).

\[
\nu \propto B_0
\]

The exact frequency is given by

\[
\nu = \left( \gamma B_0 \right) \div (2\pi)
\]

Where, \( \gamma \) is the magnetic-gyric ratio, being the ratio between the nuclear magnetic moment \( \mu \), and the nuclear angular momentum, \( I \); \( \gamma \) is also called the gyro magnetic ratio.

*Figure 2.6 Nuclear Magnetic Resonance Spectroscopy (Image from Ref. 8)*

If the precessing nuclei are irradiated with a beam of radiofrequency, the low-energy nuclei may absorb this energy and move to a higher state. The precessing proton will only absorb energy from the radiofrequency source. If the precessing frequency is identical with frequency of the radiofrequency beam; the nucleus and the radiofrequency are then in resonance, hence the term nuclear magnetic resonance. The only nucleus that exhibit the NMR phenomenon are those for which the spin quantum number \( I \) is greater than 0. The precessional frequency of all protons in the same external applied field is not same and the precise value for any one proton depends on a number of factors. Because these shifts in the frequency depend on the chemical environment, it is called the chemical shift. The factors influencing the chemical shifts
in a NMR spectrum are: van der Waals deshielding, electro negativity (shielding and deshielding), and anisotropic effects. Structural confirmation of purified form of SL was done using NMR technique. $^1$H and $^{13}$C NMR spectra were recorded using Avance Bruker-200 MHz spectrometer. Chemical shifts are given in parts per million downfield from 0.00 ppm using tetra-methyl silane (TMS) as the internal reference. The $^1$H NMR spectra of SL(A) was taken in CD$_3$OD (deuterated methanol) and SL(L) and curcumin in CDCl$_3$ (deuterated chloroform). The following abbreviations are used to present the spectral data: $s$ = singlet, $m$ = multiplet, $d$ = doublet.

### 2.2.3 UV Visible Absorption Spectroscopy

UV-Vis spectroscopy is a form of electronic spectroscopy. Reflected and transmitted light from the sample is measured and information about the electronic transitions is possible through the interpretation of the absorption signals. The perceived colour of the chemicals involved is affected by the absorption or reflectance in the visible region. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This spectroscopy is complementary to fluorescence spectroscopy. Fluorescence deals with transitions from the excited state to the ground state and absorption measures the reverse transitions. When the incident photon energy exceeds the band gap energy of the material in semiconductors, absorption occurs and signal is recorded by the spectrometer whereas in metals resonant absorption takes place as the surface free electrons vibrate coherently with the incident frequency.

![UV Visible spectrophotometer](Ref: en.wikipedia.org)

*Figure 2.7 UV Visible spectrophotometer (Ref: en.wikipedia.org)*
This spectrometer can operate in two modes (i) reflection mode and (ii) transmission. In reflectance mode, opaque thin films and NPs which are not dispersible in solvents are analysed. While in transmission mode usually thin films and colloidal NPs, well-dispersed in solvent are used. In UV-Visible absorption spectroscopy, light from a source is transmitted through a monochromator to obtain the light of desired wavelength. The emerging light is then passed through the sample under study and then into a detector (Figure 2.7). Typically, a chopper splits the light from the source to two equivalent beams; one beam passes through the sample and the other through the reference. The photodiode detector alternates between measuring the sample beam and the reference beam.

In many cases the double beam instruments have two detectors, where the sample and reference beams are measured at the same time. Sometimes though in some instruments, the two beams are recombined in a single optical path via the use chopper which blocks one beam at a time.

### 2.2.4 Photoluminescence

Spontaneous emission of light on optical excitation of a material is called photoluminescence (PL). In order to probe the sample’s discrete electronic states accurately, it is required to scan the required excitation energy and intensity appropriately. When a light of sufficient energy is incident on a material, electronic transitions arise due to absorption of photons within the allowed energy states of the material. Eventually, these excitations relax and the electrons return to the ground state. If radiative relaxation occurs, the emitted light is called PL. This light can be collected and analysed to yield a wealth of information about the photo-excited material. The PL spectrum provides information on transition energies, which can be further used to determine electronic energy levels, defects and impurity states in the sample. The PL intensity also gives a measure of the relative rates of radiative and non-radiative recombination.\(^\text{10}\) PL can be categorized into two: fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, which is characterized as the fluorescence lifetime. The process of phosphorescence occurs in a manner similar to fluorescence, but with
a much longer excited state lifetime. Hence Phosphorescence is a delayed emission process. Figure 2.8 shows photograph of the photoluminescence set-up.

![Image of PL spectrophotometer](Ref: www.fi.tartu.ee)

PL is a simple, versatile, and non-destructive measurement technique. Two essential features describe the PL signal: peak energy and intensity. The excitation energy and optical intensity can be carefully selected in order to yield additional accurate information on the energy levels available to electrons in the material. The PL signal often depends on the density of photo-excited electrons and the intensity of the incident beam. The intensity of the PL signal depends on the rate of radiative and non-radiative events, which depends in turn on the density of non-radiative interface.

### 2.2.5 Fourier Transform Infrared Spectroscopy

Every compound having covalent bonds absorbs various frequencies of electromagnetic radiation in the infrared region of electromagnetic spectrum. The region lies in between visible (longer and microwaves wavelengths. Similar to other spectroscopy techniques, on absorption of the radiation, molecules are excited to a higher energy state. This absorption process occurs corresponds to energy changes in the order of 8 to 40 kilo J/mol. As there is a very essential requirement for a molecule to show IR spectrum, all the bonds in the molecule are not IR active. Only those bonds, which have a dipole moment that changes as a function of time, are capable of absorbing IR radiation. Symmetric/Homo-nuclear diatomic molecules such as H₂, O₂,
Cl₂ etc., do not absorb IR radiation as do not have permanent dipole moment. On the other hand hetero-nuclear diatomic molecules such as CO, HCl, NO do possess permanent dipole moment and hence are IR active. IR spectra occur in the frequency range of 500 - 4000 cm⁻¹.

Two types of IR spectrometers are in common use (i) Dispersive and (ii) Fourier transform (FT) infrared spectrometer. These instruments give nearly similar spectra for a sample under study of 4000 - 400 cm⁻¹. Only the FTIR spectrometers provide the spectrum much more quickly than the dispersive IR spectrometer. Schematic of FTIR spectrometer is shown in Figure 2.9.

2.2.6 X-ray Diffraction

X-ray diffraction (XRD) is a widely used, quick, non-destructive technique helpful in determination and identification of structure in crystalline materials. The technique has been conventionally applied for phase identification and study. A large variety of materials such as metals, minerals, catalyst and inorganic compounds can be characterized by XRD. It is one of the most applicable and efficient general techniques of science.¹²-¹³

Figure 2.10 shows the schematics of X-ray diffractometer. Diffraction in general occurs only when the wave motion wavelength is of the same order of magnitude as
the repeat distance between scattering centres. This condition of diffraction is nothing but Bragg’s law and is given as,

\[ n\lambda = 2ds\sin\Theta \]

Where, \( d \) = inter-planer spacing, \( \Theta \) = diffraction angle, \( \lambda \) = wavelength of X-ray, \( n \) = order of diffraction

![XRD machine schematic](Ref: www.ehs.columbia.edu)

In powder XRD method the crystal to be examined is reduced to a fine powder and placed in a beam of a monochromatic X-rays. Each particle of the powder is the tiny crystal, or assemblage of smaller crystals, oriented at random with respect to incident beam. Some of the crystals will be correctly oriented so that their (100) planes, for example, can reflect the incident beam. Other crystals will be correctly oriented for (110) reflections and so on. The result is that every set of lattice planes will be capable of reflection. This is the principle of a powder diffractometer. Ideally, according to Bragg’s law, for the particular \( d \) value, the constructive interference of X-rays should occur only at particular \( \Theta \) value i.e. Bragg’s angle and for all other angles there should be destructive interference and intensity of diffracted beam will be minimum there.

**Identification of Phases:** From the \( d \)-spacings, phases can be identified in a film using the standard JCPDS powder diffraction file and the reflections can be indexed
with Miller indices. However, if the size of the diffracting tiny crystal is small, there is no complete destructive interference at Θ±dΘ, which broadens the peak corresponding to diffracted beam in proportion to the size of the tiny crystal. This can be used to calculate the particle size.

The relation for the same is given by Debye Scherrer formula as,

$$t = \frac{0.9\lambda}{\beta \cos \theta}$$

Where, t = particle size, Θ = diffraction angle, λ = wavelength of X-rays and β line broadening at Full Width at Half Maxima (FWHM). Further, the powder diffractometer can also be used for X-ray diffraction from thin films. Epitaxial or polycrystalline (may or may not be oriented) thin films can be considered as single crystal or powder (crystals or assembly of crystals spread on substrate) respectively. Hence, a typical epitaxial or oriented film may not show all corresponding reflections and show only few reflections for example say, a c-axis oriented film will show only (hkl) for which h and k indices are zero and l is non-zero. However, these hidden peaks can be detected by small angle X-ray diffraction technique.

### 2.2.6 Scanning Electron Microscopy

It uses a beam of electrons focused to a diameter spot of approximately 1nm in diameter on the surface of the specimen and scanned back and forth across the surface. The surface topography of a specimen is revealed either by the reflected (backscattered) electrons generated or by electrons ejected from the specimen as the incident electrons decelerate secondary electrons.

A visual image, corresponding to the signal produced by the interaction between the beam spot and the specimen at each point along each scan line, is simultaneously built up on the face of a cathode ray tube similar to the way that a television picture is generated. The best spatial resolution currently achieved is of the order of 1nm. The scanning electron microscope (SEM) is a very useful instrument to get information about topography, morphology and composition information of materials. A typical schematic of a SEM is shown in Fig. 2.11. It is a type of electron microscope capable of producing high resolution images of a sample surface. Due to the manner in which
the image is created, SEM images have a characteristic three-dimensional appearance and are useful for judging the surface morphology of the sample.

![Figure 2.11 Schematic of Scanning Electron Microscopy (Ref: www.purdue.edu)](image)

The SEM has an ability to image a comparatively large area of a specimen and also to image bulk materials. Topology of the powder samples in the present study was carried out using a Leica Stereoscan – 440 Scanning Electron Microscope.

### 2.2.7 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is an imaging technique whereby a beam of electrons is focused onto a specimen causing an enlarged version to appear on a fluorescent screen or a layer of photographic film, or to be detected by a CCD camera. TEM operates on the same basic principles as the light microscope but uses electrons instead of light. The line diagram of a typical TEM column is shown in Figure 2.12. The column consists of a source of electrons, electrodes for electron acceleration, electromagnetic focusing and deflecting lenses and the electron detection system such as a CCD array. By using electron energy of several hundred kilovolts the de Broglie wavelength associated with the electron can be reduced to a small fraction of
nanometer and hence atomic resolution imaging becomes feasible. Virtually, TEM is useful for determining size, shape and arrangement of the particles which make up the specimen.

![Schematic of Transmission Electron Microscopy](Ref: www.nobelprize.org)

**Figure 2.12 Schematic of Transmission Electron Microscopy (Ref: www.nobelprize.org)**

Moreover, it is highly useful for the determination of the lattice planes and the detection of atomic-scale defects localized in areas of few nano-meters in diameter with the help of selected area electron diffraction (SAED) technique. The d-spacing between lattice planes of crystalline materials can be calculated from a SAED pattern using the relationship:

$$dr = \lambda L$$

Where, L is the distance between the specimen and the photographic plate, \(\lambda L\) is known as the camera constant and r is the radius of diffracted rings. It is easy to measure r directly from the photographic plate, and \(\lambda L\) can be established from the instrument by calibrating it with a standard material (usually Ag), and hence one can easily get d values. Since, each d value corresponds to a specific lattice plane for a specific crystal structure; description of the crystal structure of a crystalline specimen
can be obtained from SAED pattern. In some cases SAED pattern is more helpful as compared to XRD, due to the limited detection limit of XRD instrument. Also, the XRD generally gives global information.

### 2.2.8 X-ray Photoelectron Spectroscopy

X-ray photoelectron Spectroscopy (XPS) probes the binding energies of core electrons in an atom. Although such electrons play little part in chemical bonding, different chemical environments can induce small changes in their binding energies; this is because the formation of bonds changes the distribution of electrons in the system and hence by modifying the nuclear shielding, produces changes in the effective nuclear charge of the bound atoms.

XPS is also rarely called as electron spectroscopy for chemical analysis (ESCA). Since only the photoelectrons from the atoms near the surface escape the information obtained is typically from the surface layer of 2-5 nm with a typical sampling area of 1 cm². The actual depth varies with the materials and electron energy. This technique mainly gives information about the elemental composition of the surface of the materials and the information about the chemical state of elements. Usually Al and Mg source is used for producing X-rays to excite photoelectrons from the core levels of atoms in a specimen (Figure 2.13). When an atom or molecule is subjected to higher energy radiations, photons in the radiations colloid with and eject electrons from atoms, leaving behind ions. 

![XPS instrument](Ref: www.ine.kit.edu)

**Figure 2.13 XPS instrument (Ref: www.ine.kit.edu)**
Ejected electrons depart with different velocities and photoelectron spectroscopy measures the velocity distribution of the released electrons. Each electron is held in place by nucleus with a characteristic binding energy. The energy of the photon is imparted to the electron and, if this energy is greater than the B.E., the electron will leave the atom and carry with it an excess energy – thus it will have certain K.E. (and velocity). Clearly the total energy must conserve:

\[ h \nu = \text{binding energy} + \text{Work Function} + \text{kinetic energy} \]

\[ \text{Binding Energy} = h \nu - \text{Kinetic Energy} \]

Since the excitation energy is known and the kinetic energy is measured, the binding energies of electrons in the atom under examination can be determined. Main components of XPS are (i) X-ray source, (ii) Sample holder, (iii) electron energy analyser. The (ii) and (iii) component must be in UHV. The X-ray source is a simple X-ray tube with double anodes (typically Al and Mg) incident radiation energy can be switched from one to the other. In both, XPS, the kinetic energy of the ejected electrons is measured using a hemispherical analyser.

Monochromatic X-ray or UV radiation falls on the sample and ejected electrons pass between a pair of electrically charged hemispherical plates which act as an energy filter, allowing electrons of only a particular kinetic energy to pass through – the pass energy, \( E_{\text{pass}} \). The resulting electron current, measured by an electron multiplier, indicates the number of electrons ejected from the surface with that kinetic energy. \( E_{\text{pass}} \) can be systematically varied by changing the retarding voltage (VR) applied to the analyser. XPS measurements of different samples were carried out on a VG MicroTech ESCA 3000 instrument at Center for Materials Characterizations (CMC), National Chemical Laboratory Pune. The core level binding energies (BE) were corrected with the carbon binding energy of 285 eV.

**References**


