CHAPTER - III

EXPERIMENTAL TECHNIQUES
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EXPERIMENTAL TECHNIQUES

3.1. INTRODUCTION

In this chapter, the various experimental techniques employed for the characterization of the grown crystals are briefly discussed. The X-ray diffractometers (single crystal and powder) have been used to find the structure and crystalline nature of the grown crystals. To identify the presence of various functional groups, the Fourier transform infrared (FT-IR) spectrometer has been used. The colourimetric estimation method is adopted to identify the amino groups in the grown crystal. The Thin Layer Chromatography (TLC) and Nuclear Magnetic resonance (NMR) techniques are used for confirmational analysis. To study the thermal and optical properties of the grown crystal, the thermal analyser and UV-Vis-spectrometers are used. Further, the micro hardness measurement has been carried out using Vicker’s microhardness tester. The photoluminescence spectrum is used for supporting SHG property of the grown crystal and from that spectrum the optical band edge is calculated. The surface morphology is understood by SEM/EDAX and etching studies. The Kurtz and Perry powder technique has been used to measure the second harmonic generation (SHG) conversion efficiency of the grown crystals.

3.2. SINGLE CRYSTAL X-RAY DIFFRACTION

3.2.1. Introduction

From the simplest to those with many thousands of atoms, the X-rays have helped to establish detailed features of the molecular structure of every kind of stable chemical species in a crystalline form. Single crystal X-ray diffraction is a non-
destructive analytical technique which provides detailed information about the internal lattice of crystalline substance, including unit cell dimensions, bond-lengths, bond-angles and details of site ordering. Directly related is single crystal refinement, where the data generated from the X-ray analysis are interpreted and refined to obtain the crystal structure.

3.2.2. Fundamental Principle

Max von Laue (1912) discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths and it has become a common technique for the study of crystal structures and atomic spacing, in recent times. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate and directed towards the sample. The interaction of the incident rays with the sample produces constructive interference as shown in Fig. 3.1. (and a diffracted ray) when the conditions satisfy Bragg’s law ($n\lambda = 2d\sin\theta$).

![Fig. 3.1: Bragg’s Diffraction Condition](image_url)
The geometry of an X-ray diffractometer is shown in Fig. 3.2. The single crystal diffractometers use either 3- or 4-circle goniometers. These circles refer to the four angles \(2\theta, \omega, \chi\) and \(\phi\) that define the relationship between the crystal lattice, the incident ray and detector. Samples are mounted on thin glass fibers which are attached to brass pins and mounted onto goniometer heads. Adjustment of the X, Y and Z orthogonal directions allows centering of the crystal within the X-ray beam.

![Fig. 3.2: Geometry of X-ray diffractometer](image)

Bragg’s law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By changing the geometry of the incident rays, the orientation of the centered crystal and the detector, all possible diffraction directions of the lattice should be attained. All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays \([1,2]\).
3.2.3. Working Principle

X-ray diffractometer consist of three basic elements, an X-ray tube, a sample holder and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and impact of the electrons with the target material. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being $K_{\alpha}$ and $K_{\beta}$. Then, $K_{\alpha}$ consists of $K_{\alpha 1}$ and $K_{\alpha 2}$. But, $K_{\alpha 1}$ has a slightly shorter wavelength and twice the intensity as $K_{\alpha 2}$. The specific wavelengths are characteristics of the target material. Filtering, by foils or crystal monochromators, is required to produce monochromatic X-rays needed for diffraction. The lines $K_{\alpha 1}$ and $K_{\alpha 2}$ are sufficiently close in wavelength such that average of the two is used. Molybdenum is the most common target material for single crystal diffraction, with Mo$K_{\alpha}$ radiation of $\lambda = 0.7107\text{Å}$. These X-rays are collimated and directed onto the sample. When the geometry of the incident X-rays impinging the sample satisfies the Bragg equation, constructive interference occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor. X-rays may also be produced using a synchrotron, which emits a much stronger beam. X-rays leave the collimator and are directed at the crystal. Rays are either transmitted through the crystal, reflected off the surface or diffracted by the crystal lattice. A beam stop is located directly opposite the collimator to block transmitted rays and prevent burn-out of the detector. Reflected rays are not picked up by the detector due to the angles involved. Diffracted rays at the correct orientation for the configuration are then collected by the detector.
Modern single crystal X-ray diffractometer as shown in Fig. 3.3, use CCD (charge-coupled device) technology to transform the X-ray photons into an electrical signal which are then sent to a computer for processing.

![Single Crystal X-ray diffractometer](image)

**Fig. 3.3: Single Crystal X-ray diffractometer**

Single crystal X-ray diffraction is most commonly used for precise determination of a unit cell, including cell dimensions and positions of atoms within the lattice. Bond-lengths and angles are directly related to the atomic positions.

### 3.3. POWDER X-RAY DIFFRAC TOMETRY

Powder X-ray diffractometry can be used as a powerful tool for the study of materials because of its ability to give a broad range of information such as crystal structure, composition and defects. It is a non-destructive technique and does not require any specific sample preparation methods. In the present study, the structural analysis of the sample is done using X-Ray Diffraction technique. RICH SIEFERT X-ray diffractometer using Cu Kα radiation with wavelength (λ=1.5418Å) is used for the
crystallographic analysis. The X-ray tube is operated at 40 kV, 30 mA with a scanning speed of 0.25 second per step. The schematic diagram of X-ray diffractometer is shown in the Fig. 3.4. The X-ray diffraction is based on the constructive interference of monochromatic X-rays caused by crystalline materials. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation collimated to concentrate and directed towards the sample. The interaction of the incident rays with the sample produces constructive interference when Bragg’s law \((n\lambda=2dsin\theta)\) conditions are satisfied. This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample.

![Fig. 3.4: Schematic diagram of X-ray diffractometer](image)

The substance is identified if its pattern coincides with one of the reference patterns [3,4]. The wave nature of X-ray means that they are diffracted by the lattice of the crystal to give a unique pattern of peaks of ‘reflections’ at different angles and intensities, just as light can be diffracted by a grating of suitably spaced lines. The diffracted beams from atoms in successive planes cancel unless they are in phase and
the condition for this is given the Bragg relationship, \( n\lambda = 2dsin\theta \), where \( \lambda \) is the wavelength of the X-ray, ‘d’ is the distance between different plane of atoms in the crystal lattice, ‘\( \theta \)’ is the angle of diffraction. The X-ray detector moves around the sample and measures the intensity of these peaks and the position of the peaks at a diffraction angle \( 2\theta \). The highest peak is defined as the 100 percent peak and the intensity of all the other peaks are measured as a percentage of the 100 percent peak.

When X-rays are fired at a crystalline sample placed in the X-ray camera, a portion is diffracted by the regular crystal structure. These diffracted X-rays produce a pattern of lighter and darker lines on a film. The pattern on the film depends on what is in the sample and by reference to standard data; this pattern can be used as a kind of ‘finger print’ to identify a wide variety of materials. Such materials include corrosion products on metals; pigments used on wall paintings and inlaid decoration in jewellery. The X-ray diffraction analysis is particularly useful in the study of museum objects because it is almost non-destructive. It requires a very small sample, much less than the size of a pinhead, to use the X-ray camera [5]. The X-ray powder diffraction of the grown crystals are recorded with RICH SIEFERT X-ray diffractometer using Cu K\(_\alpha\) radiation with wave length \( \lambda = 1.5418\text{Å} \).

3.4. FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared (FT-IR) spectroscopy is a technique which is used to analyze the chemical composition of many organic chemicals, polymers, paints, coatings, adhesives, lubricants, semiconductor materials, coolants, gases, biological samples, inorganics and minerals. FT-IR can be used to analyze a wide range of materials in bulk or thin film forms, liquids, solids, pastes, powders, fibres and other forms. FT-IR spectroscopy is a technique that provides information about the
chemical bonding or molecular structure of materials, whether organic or inorganic. It is used to identify unknown materials present in a specimen. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic of that molecule. During FT-IR analysis, a spot on the specimen is subjected to a modulated IR beam. The specimen’s transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks. The resulting FT-IR spectral pattern is then analyzed and matched with known signatures of identified materials in the FT-IR library. FT-IR spectroscopy does not require a vacuum, since neither oxygen nor nitrogen absorbs infrared rays. FT-IR analysis can be applied to minute quantities of materials, whether solid, liquid, or gas. When the library of FT-IR spectral patterns does not provide an acceptable match, individual peaks in the FT-IR plot may be used to yield partial information about the specimen. Schematic diagram of a FT-IR spectrometer is shown in the Fig. 3.5.

A parallel beam of radiation is directed from the source to the interferometer, consisting of a beam splitter and two mirrors. The beam splitter is plate of suitably transparent material coated so as to reflect 50% of the radiation falling on it. Thus half of the radiation goes to one mirror and half to the other mirror, returns from both these mirrors along the same path and is then recombined to a single beam at the beam splitter. If a monochromatic radiation is emitted by the source, the recombined beam leaving the beam splitter shows constructive or destructive interference depending on the relative path lengths between the beam splitter and the two mirrors. As the dynamic mirror is moved smoothly towards or away from the beam splitter, a detector sees radiation alternating in intensity.
The production of spectrum is a two stage process. Firstly, without the sample in the path of the beam, mirror is moved smoothly over a period of time through a distance of about 1 cm, while the detector signal is collected into the multichannel computer; the computer carries out the Fourier transformation of the stored data to produce the background spectrum. Secondly with the sample, an interferogram is recorded exactly in the same way, Fourier transformed and then ratioed against the background spectrum for plotting as a transmittance spectrum. Alternatively the sample and background spectra may each be calculated in absorbance forms and the latter simply subtracted from the former [6-8]. IR spectroscopy is classified into three regions of the electromagnetic spectrum.

a. NIR (Near IR) (12000-4000) cm\(^{-1}\)

b. MIR (Mid IR) (4000-200) cm\(^{-1}\)

c. FIR (Far IR) (200-10) cm\(^{-1}\)
The Mid IR range encompasses a vast amount of analytical applications. It provides a unique fingerprint of the molecules, which can be clearly distinguished from the absorption patterns of other vibrations. Conventionally there are two methods adopted for recording IR spectrum of the solid sample, one is the mull method and the other is KBr pellet method. The powdered sample is dispersed in the spectrometer. The KBr pellet method is more popular and has several advantages over the mull method, such as low scattering loss, higher spectral resolution, the homogeneity of the sample etc. In the present work, KBr pellet method is used to record the IR spectra of the grown crystals. The weight of the sample required for the analysis is about 5 gm. The photograph of PERKIN-ELMER spectrometer is shown in Fig. 3.6.

![Fig. 3.6: Perkin-Elmer spectrometer](image)

3.5. UV-VIS-NIR SPECTROPHOTOMETRY

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 ultra-violet-visible spectrometers consist of light source, monochromator, detector, amplifier and the recording devices. The most suitable sources of light are: Tungsten filament lamp and hydrogen-deuterium discharge lamp which cover the whole of the UV-Visible region. Tungsten filament lamp is particularly rich in red radiations i.e. radiations with wavelength 375 µm, while the deuterium discharge lamp covers the region below it. The intensity of the deuterium discharge source falls above 360 µm. The Fig. 3.7., shows the UV-Vis-NIR Spectrophotometer. One of the beams of selected monochromatic light is passed through the sample solution and the other beam of equal intensity is passed through the reference solvent. The solvent as well as the solution of the sample may be contained in cells made of a material which is transparent throughout the region under study. Glass cannot be used since it absorbs strongly in the ultra violet region. Silica cells can be used. These must be properly stored and their optical surfaces should never be handled. Quartz cells also serve the purpose best. Glass can be used satisfactorily in the visible region [10]. After the beam pass through the sample cell as well as the reference cell, the intensities of the respective transmitted beams are then compared over the whole wavelength range of the instrument. The spectrometer electronically subtracts the absorption of the solvent in the reference beam from the absorption of the solution. Hence the effects due to the absorption of light by the solvent are minimized. In this way the absorbance or the transmittance characteristic of the compound alone can be measured. The signal for the intensity of absorbance versus corresponding wavelength is automatically recorded on the graph. The spectrum is usually plotted as absorbance (A) against wavelength (λ).
When the sample absorbs light its intensity is lowered. Thus the photoelectric cells $P_1$ and $P_2$ will receive an intense beam from the reference cell and a weak beam from the sample cell. This results in the generation of pulsating or alternating currents which flow from the photoelectric cells to the electronic amplifier [9,10]. The amplifier is coupled to a small servomotor which in turn is coupled to a pen recorder. Thus it records the absorption automatically. Fig. 3.8 shows the modern PERKIN-ELMER LAMBDA-35 Spectrophotometer which is used to record both the spectra.
3.6. THERMOGRAVIMETRY

3.6.1. Thermo Gravimetric Analysis

Thermo gravimetric analysis (TGA) or thermo gravimetry (TG) provides a quantitative measurement of any weight changes associated with thermally induced transitions. It can record directly the loss in weight as a function of temperature or time for transitions that involve dehydration or decomposition. Thermogravimetric characteristic curves of a given compound or material due to the unique sequence of physical transitions and chemical reactions that occur over definite temperature ranges. The rates of these thermally induced processes are often a function of molecular structure. Changes in weight are due to the physical and chemical bonds forming and breaking at elevated temperatures. These processes may evolve volatile products or form reaction products that result in a change in weight of the sample. The usual temperature range for TG is from ambient to 1200°C in either inert or reactive atmospheres.

Samples are placed in a crucible that is positioned in a furnace on a quartz beam attached to an automatic recording balance. Fig. 3.9 shows a TG instrument that contains a suspension electro mechanical transducer. The horizontal quartz beam is maintained in the null position by the current flowing through the transducer coil of an electromagnetic balance. A pair of photosensitive diodes acts as a position sensor to determine the movement of the beam.
Any change in the weight of the sample causes a deflection of the beam which is sensed by one of the photodiodes. The beam is then restored to the original null position by a feedback current sent from the photodiodes to the coil of the balance. The current is proportional to the change in weight of the sample [11-13].

3.6.2. Differential Thermal Analysis

Differential thermal analysis measures the temperature, direction and magnitude of thermally induced transitions in a material by heating or cooling a sample and comparing its temperature to the temperature of an inert reference material under similar conditions. This difference in temperature is determined as a function of time or temperature in a controlled atmosphere and provides useful information about the temperatures, thermodynamics and kinetics of reactions. This technique is sensitive to endothermic or exothermic processes including phase transitions, dehydration, decomposition and solid state reactions. A plot of the differential temperature ($\Delta T$) versus the programmed temperature ($T$) indicates the transition temperature(s) and whether the transition is exothermic or endothermic.
It consists of balance, furnace and sample carrier. The system is first evacuated with a pump and then evacuated to $2 \times 10^{-4}$ mbar with turbomolecular drag pump through port 4. Then the system is filled with standard grade argon or oxygen gas until reaching atmospheric pressure and the top valve (port 1) is opened to allow a constant flow of purge gas through the sample chamber. A 20 ml/min and 10ml/min flow are set through ports 2 and 3 for the balance protection and sample purge. A series of aluminum oxide plates are inserted at the base of the sample carrier to prevent radiation effects on the balance and to create a homogeneous gas flow through the furnace. A platinum sample carrier and platinum crucibles are used to allow temperature measurements in the higher temperature range without radiation effects. A heating cycle is programmed into the operating computer and a control system monitors and adjusts the furnace power to control sample and reference temperatures. The base line correction generated for each set of crucibles and heating rate. The base line correction is generated by running a specific heating program with an empty sample and reference crucible. The combined TGA-DTA/DSC system is shown in Fig. 3.10. The furnace contains a block with identical and symmetrically located chambers.
Fig. 3.10: Combined TGA-DTA/DSC instrument

The sample is placed in one chamber and a reference material, such as Al$_2$O$_3$ is placed in the other chamber. A thermocouple is inserted into the center of the material in each chamber. The furnace and sample blocks are then heated by a microprocessor controlled heating element. The difference in temperature between sample and reference thermocouples, connected in series opposition, is continuously measured. After amplification by a high gain, low noise dc amplifier for microvolt level signals, the difference signals is recorded as the y-axis. The temperature of the furnace is measured by an independent thermocouple and recorded as the x-axis. Because the thermocouple is placed in direct contact with the sample, DTA provides the highest thermometric accuracy of all thermal methods. In the present investigation, SDT Q600 V20.5 Build 15 analyzer is used at a heating rate of 20°C/min. in nitrogen atmosphere.
3.6.3. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) has become the most widely used thermal analysis technique. In this technique, the sample and reference materials are subjected to a precisely programmed temperature change. When a thermal transition (a chemical or physical change that results in the emission or absorption of heat) occurs in the sample, thermal energy is added to either the sample or the reference containers in order to maintain both the sample and reference at the same temperature. Because the energy transferred is exactly equivalent in magnitude to the energy absorbed or evolved in the transition, the balancing energy yields a direct calorimetric measurement of the transition energy. Since DSC can measure directly both the temperature and enthalpy for a transition or the heat of a reaction, it is often substituted for differential thermal analysis as a means of determining these quantities except in certain high temperature applications [13].

Cross section of a typical DSC cell is shown in Fig. 3.11. A DSC cell uses a constantan (Cu-Ni) disk as the primary means of transferring heat to the sample and reference positions and also as one element of the temperature sensing thermoelectric junction. The sample and reference are placed in separate pans that sit on raised platforms on the disk. Heat is transferred to the sample and reference through the disk. The differential heat flow to the sample and reference is monitored by the chromel/constantan thermocouples formed by the junction of the constantan disk and the chromel wafer covering the underside of each platform. Chromel and alumel wires connected to the underside of the wafers form a chromel / alumel thermocouple, which is used to directly monitor the sample temperature. Constant calorimetric sensitivity is maintained by the computer software, which linearizes the cell calibration coefficient. DSC provides maximum calorimetric accuracy from -170 to 750°C. Sample sizes range from 0.1 to 100 mg.
3.7. ETCHING STUDY

3.7.1. Introduction

The process of removal of the outer layer is called etching. The material which is used to remove the layers is called etchant. The etching process gives rise to various types of geometrical feature on crystal surface [14]. The rates of attack on the crystal surfaces are also anisotropic, being different along different directions. These anisotropies usually give rise to the production of conical depressions with regular geometrical outline on crystal surfaces. They are usually known as etch pits, etch marks or etch figures.

Dissolution of a crystal is now thought to occur by the retreat of molecular steps, being reverse to that of surface. It is believed that when a perfect crystal face is exposed to solvent, dissolution usually begins by the nucleation of unit pits of one molecular depth [14,15].

The understanding of etch phenomenon is enhanced by the recognition of various types of imperfections in a crystal. The defect points are not bound with the surface as strongly as the points of the crystal. They need less energy to dissociate.
than the atoms in the regular structure. If chemical or physical change gives energy to
dissociate imperfections from the exposed surface substantially, etch pits or etch
features may be observed on it. There are the many ways for obtaining preferential
dissolution on a crystal surface. Chemical etching is predominantly used among them,
for dissolution on the crystal surface.

3.7.2. Chemical Etching

Chemical etching produces usually a few or all of the following etch features
on a crystal surface: (a) etch spirals, (b) etch pits, terraced, flat bottomed and point
bottomed, (c) linear etch rows, (d) polygonised etch figures, (e) tunnels and dendritic
etch features and (f) shallow pits, etch hillocks etc.

Frank has considered the effect of screw dislocation on crystal growth and
suggested that if a crystal grows by screw dislocation mechanism, a growth spiral
should be obtained on the surface. Spiral etch pits are observed on the surface of
many crystals including metals and semiconductors.

The chemically etched surface mostly consists of etch pits. They represent the
early stages of crystal dissolution. Etch pits are used to locate dislocation terminating
on a crystal surface. Whether all etch pits reveal dislocations or all dislocations give
rise to etch pits is a question which is not yet fully answered. The visibility of an etch
pit depends on the combination of the rate at which the steps move on the surface.
The necessary condition for revealing dislocations by etch pits is the occurrence of
cleavage counterparts of a single crystal having cleavage planes.
The chemical etching is carried out, using Leitz Metallux-II Optical Microscope and is shown in Fig. 3.12. The etching time is varied from 2 to 5 seconds. After etching by the etchant, the crystal is dried quickly with filter paper. Using the optical microscope, etch patterns (before etching and after etching) are photographed and analyzed.

3.8. HARDNESS STUDIES

3.8.1. Introduction

Hardness of a material is the resistance it offers to indentation by a much harder body. An important use of microhardness studies is the possibility of making an indirect estimate of other mechanical characteristics of a material having a specific correlation with their hardness [16].

The hardness depends not only on the properties of the materials under test but also on the condition of measurement. Microhardness tests have been applied to time components of clock and instrument mechanics, thin metal strip, foils, wires, metallic fibers, thin galvanic coatings, artificial oxide films etc. [17].
3.8.2. Methods of Hardness Test

Hardness measurement can be carried out by various methods. They are classified as follows:

1. Static indentation test
2. Dynamic indentation test
3. Scratch test
4. Rebound test
5. Abrasion test

The most popular and simplest form is the static indentation test wherein an indenter of specific geometry is pressed into the surface of a test specimen under a known load. The indenter may be ball or diamond cone or diamond pyramid. Upon removal of the indenter, a permanent impression is retained in the specimen. The hardness is calculated from the area or the depth of indentation produced. The variables are the type of the indenter or load. The indenter is made up of a very hard material to prevent its deformation by the test piece so that it can cover materials over a wide range of hardness. For this reason either a hardened steel sphere or a diamond pyramid or cone is employed. A pyramid also has the advantage that geometrically similar impressions are obtained at different loads. So naturally a pyramid indenter is preferred. In this static indentation test the indenter is pressed perpendicularly in the surface of a sample by means of an applied load. Then by measuring the cross sectional area or the depth of the indentation and knowing the applied load an empirical hardness number may be calculated [18,19].
In the dynamic indentation test, a ball or a cone (or a number of small spheres) is allowed to fall from a definite height and the hardness number is obtained from the dimensions of the indentation and the energy of impact.

The scratch test can be classified into two types:

i. Comparison test in which one material is said to be harder than another if the second material is scratched by the first.

ii. A scratch test with a diamond indenter on the surface at a steady rate and under a definite load. The hardness number is expressed in terms of the width of depth of the groove formed.

In the rebound test, an object of standard mass and dimensions is bounced from the test surface and the height of rebound is taken as the measure of hardness. In abrasion test, a specimen is loaded against a rotating disk and the rate of wear is taken as a measure of hardness.

3.8.3. Vicker’s Test

Among the various methods of hardness measurements discussed above, the most common and reliable method is the Vicker’s hardness test method. In this method, micro indentation is made on the surface of a specimen with the help of diamond indenter (Fig. 3.13). Smith and Sandland have proposed that a pyramid be substituted for a ball in order to provide geometrical similitude under different values of load. The Vicker’s pyramid indenter where opposite faces contain an angle ($\alpha = 136^\circ$) is most widely accepted pyramid indenter. A pyramid is suited for hardness tests due to the following two reasons:

i. the contact pressure for a pyramid indenter is independent of indent size and

ii. pyramid indenters are less affected by elastic release than other indenters.
The base of the Vickers pyramid is a square and the depth of indentation corresponds to $1/7^{th}$ of the indentation diagonal. Hardness is generally defined as the ratio of the load applied to the surface area of the indentation. The Vickers hardness number ($H_v$) is defined as:

$$H_v = \frac{2P \sin\left(\frac{\alpha}{2}\right)}{d^2} \text{ kg / mm}^2$$

... (3.1)

where $\alpha$ is the apex angle of the indenter ($\alpha = 136^\circ$), $P$ is the applied load in kg and ‘d’ is the diagonal length of the indentation mark in mm. Hardness values are measured from the observed size of the impression remaining after a loaded indenter has penetrated and has been removed from the surface. Thus the observed hardness behaviour in the final measurement of the residual impression is the summation of a number of effects involved in the materials response to the indentation pressure during loading.

Fig. 3.13: Schematic of Vicker’s Diamond Pyramid Indenter

The variation of $H_v$ with applied indentation test load shows that with the increase of applied indentation test load $H_v$ decreases at the low load region, reaching a saturation value at higher loads. Such phenomenon referred to as the indentation
size effect (ISE). In order to describe the ISE behavior of materials several models for the relationship between applied indentation test load and indentation diagonal length have been reported in the literature (19). The most common explanation of the ISE found in the literature is directly related to the intrinsic structural factors of the test material. According to this theory in order to analyze the ISE in the hardness testing one needs to fit the experimental data according to Meyer’s Power law [20], which correlates the applied load $P$ and the resulting indentation size $d$ with each other.

$$P = A d^n$$ \hspace{1cm} \ldots (3.2)

where $n$ is the Meyer index or work hardening exponent and $A$ is constant for a given material. These parameters are derived from the curve fitting of experimental results of indentations. Combining equation (3.1) and (3.2), we get

$$H_v = B d^{n-2}$$ \hspace{1cm} \ldots (3.3)

From this relation it is clear that for the ISE the values of $n$ should be less than 2. Note that the ISE is usually related to the deviation of the $n$ value from 2 for $n$ equal to 2 is the absence of an ISE. The Meyer’s law is simply an empirical expression to describing the relationship between indentation load and the resultant indentation size. It gives suitable results only in a narrow range of indentation loads. The Meyer’s parameters are used for the characterization of the experimental data; however it is found that the classical Meyer’s law is insufficient for the description of our experimental data. It is more suitable to use a polynomial equation for representation of experimental data. Recently a number of workers explain the indentation size effect with the proportional specimen resistance (PSR) model. According to the PSR model, there are two factors which are responsible for the decrease of microhardness with load. These are (i) the frictional force between the test specimen and the indenter facets and (ii) the elastic resistance of the test specimen.
the PSR model of Li and Bradt, microhardness can be described by two different parts: (i) the indentation load dependent part or the ISE regime and (ii) the indentation load independent part. The indentation test load $P$ is related to the indentation size $d$ as follows:

$$P = a_1 d + a_2 d^2$$  \hspace{1cm} \ldots \ (3.4)$$

Then,

$$\frac{P}{d} = a_1 + \left[ \frac{P_o}{d_o^2} \right] d$$  \hspace{1cm} \ldots \ (3.5)$$

In equation (3.5), co-efficient $a_1$ is the contribution of proportional specimen resistance to the apparent micro hardness and $a_2$ is a co-efficient related to load independent micro hardness. $P_o$ is the applied load at which micro hardness becomes load independent and $d_o$ is the corresponding diagonal length of indentation. The parameters $a_1$ and $P_o/d_o^2$ are included in equation and can be evaluated through the linear regression of $P/d$ versus $d$. A plot of $P/d$ against $d$ will give a straight line, the slope of which gives the value $P_o/d_o^2$. The load independent micro hardness value can be calculated by multiplying $P_o/d_o^2$ with the Vicker’s conversion factor 1.8544, using equation (3.6).

$$H_v = 1.8544 \times \frac{P_o}{d_o^2} \text{ kg/mm}^2$$  \hspace{1cm} \ldots \ (3.6)$$

### 3.9. PHOTOLUMINESCENCE (PL) SPECTROSCOPY

#### 3.9.1. Introduction

The process of photon emission as a consequence of the atomic excitation is called photoluminescence. Photoluminescence spectroscopy is the recording of the intensity of photoemission as a function of wavelength caused by the radioactive
recombination of electron-hole pairs or excitons following an optical excitation. In a radiative transition, the energy difference between the two states is emitted as electromagnetic radiation.

As photoluminescence spectroscopic techniques involve measuring the energy distribution of emitted photons after optical excitation, the analysis of this energy distribution is useful in determining the properties of the material, including defect species, defect concentrations and possible stimulated emission [8]. The radiative recombination of excited electron-hole pair can take place through various kinds of processes as shown in Fig. 3.14. The band-to-band de-excitation where a free electron excited in the conduction band recombines with a hole in the valence band is the most simple recombination process. Traps and donor and acceptor levels in the band energy gap, introduced by the impurities, provide alternate paths for de-excitations as shown in Fig. 3.14.

![Fig. 3.14: Radiative Recombination of Excited Electron-Hole Pair](image)

**3.9.2. Experimental Setup**

Fig. 3.15 illustrates typical PL setup. The sample to be studied is excited by laser (say He-Cd which emits 325 nm UV light) or a Xenon lamp. The beam is directed through mirrors (M1, M2 and M3) as shown in the Fig. 3.15 and focused by a
quartz lens onto the sample mounted in the optical cryostat. The luminescence emitted by the sample is allowed to pass through a quartz lens and a filter which cuts off all luminescence below a certain wavelength (say 345 nm). The cryostat is pumped to below $10^{-6}$ torr using a vacuum system and is cooled down to a temperature of 9 K. Low temperature measurements are necessary to obtain the entire spectroscopic information by minimizing thermally activated non-radiative recombination process, occurring in the material and thermal line broadening. The monochromator converts the polychromatic light into monochromatic light of individual wavelengths and are scanned across the exit slit opening.

![Fig. 3.15: Block Diagram of PL spectrometer](image)

A photomultiplier tube is used to measure the intensity of individual wavelengths. Light of individual wavelengths are focused at different horizontal portions along the exit port of the spectrograph and detected simultaneously by a CCD system and finally the intensity values are recorded in a computer. The
photograph of 1403-SPEX photoluminescence spectrometer having Argon ion laser source with wave length $\lambda$=457.9 nm operating at 2.71eV, is used in the present work is shown in Fig. 3.16.

![Photograph of PL Spectrometer](image)

**Fig. 3.16: Photograph of PL Spectrometer**

**Advantages**

1. It is a simple technique.
2. It does not need sample preparation processing.

**3.10. SCANNING ELECTRON MICROSCOPY (SEM)**

Scanning electron microscopy is a surface imaging technique widely used for the morphological study of solids using electron-beam-generated secondary electrons. The primary beam may be focused to a spot less than 50Å in diameter and upon interacting with the solid, secondary electrons are generated. These secondary electrons are collected by the detector, whose output is given to a cathode ray tube (CRT) as shown in Fig. 3.17. The CRT inputs are synchronized with x-y deflection voltages of the microscope, so that every point the beam strikes on the specimen is mapped directly onto a corresponding point on the screen, producing an image of the specimen surface [34]. One of the most common analytical attachments to the SEM is
the energy dispersive X-ray spectrometer (EDX). The high energy primary electron beam causes emission of a core electron which leaves the atom in excited state and the atom undergoes de-excitation by emitting X-rays. The emitted X-rays are characteristic of the elements from which they originated and thus help for elemental analyses of the solid under probe [20-21].

![Fig. 3.17: Schematic of Scanning Electron Microscope](image)

**3.11. ENERGY DISPERSIVE X-RAY ANALYSIS (EDX/EDAX)**

The composition of various elements in the grown crystals can be studied using the analysis technique like energy dispersive X-ray and inductively coupled plasma. Energy dispersive X-ray (EDX) analysis EDX/EDAX analysis stands for energy dispersive X-ray analysis. It is sometimes referred to also as EDS or EDAX analysis. It is a technique used for identifying the elemental composition of the specimen, on an area of interest thereof. The EDX analysis works as an integrated feature of a scanning electron microscope (SEM) and cannot be operated its own without the latter. During EDX analysis, the specimen is bombarded with an electron
beam inside the scanning electron microscope. The bombarding electrons collide with
the specimen atom’s own electrons, knocking some of them off in the process. A
position vacated by an ejected inner shell electron is eventually occupied by a higher-
energy electron from an outer shell. To be able to do so, however, the transferring
outer electron must give up some of its energy by emitting an X-ray. The amount of
energy released by the transferring electron depends on which shell it is transferring
from, as well as which shell it is transferring to. Furthermore, the atom of every
element releases X-rays with unique amounts of energy during the transferring
process.

Thus, by measuring the energy of the X-rays emitted by a specimen during
electron beam bombardment, the identity of the atom from which the X-ray is
emitted, can be established. The output of an EDAX analysis is an EDX spectrum,
which is a plot of how frequently X-ray is received for each energy level. EDX
spectrum normally displays peaks corresponding to the energy levels for which the
most X-rays had been received. Each of these peaks is unique to an atom and
therefore corresponds to a single element. The higher a peak in a spectrum, the more
concentrated the element is in the specimen. An EDX spectrum-plot identifies the
element corresponding to each of its peaks [22].

For example, a peak corresponding to the amount of energy possessed by X-rays
emitted by an electron in the L-shell going down to the K-shell is identified as a $K_{\alpha}$
peak. The peak corresponding to X-rays emitted by electrons transitions from upper
levels to the K-shell is identified as a $K_{\alpha}$, $K_{\beta}$ and $K_{\gamma}$ etc., as shown in Fig. 3.18. The
principle of SEM/EDAX and the instrument’s photograph is shown in Figs. 3.19 and
3.20 respectively. In the present work, HITACHI-S-3000H instrument is used to
examine the surface morphology.
Fig. 3.18: Illustration of EDAX

Fig. 3.19: Principle behind SEM/EDAX

Fig. 3.20: The Photograph of SEM/EDAX Instrument
3.12. THE CHROMATOGRAPHY TECHNIQUES

3.12.1. Introduction

Chromatography is the physic-chemical separation of the components of the mobile phase, e.g. gas or solution when it flows along another stationary phase e.g. liquid or solid. This method is first discovered by Michael Tsweet, a Russian biochemist in 1903. He separated chlorophyll from a mixture of plant pigments in 1906. Because of the nature of pigments in the sample, each band had a distinctive colour. Thus the name of the process is coined from the Greek words for colour “Chromo” and “to write” graphy.

The term chromatography bunches together a family of closely related extremely powerful separation methods. The separation is called chromatographic separation. The column is called the chromatographic column, and the distribution of the differently coloured components in the column is called a chromatogram. Chromatographic separation is no longer restricted to coloured constituents. It is also used for uncoloured constituents, the principle being the same. At present, not only dissolved substances but also vapors and gases are separated by chromatography. Chromatography is a separation method in biology and chemistry. It involves the interaction between mixture to be separated, a column, paper or thin layer (stationary phase), a solvent (a mobile phase). The various molecules are separated on the basis of size, shape, mass, charge, solubility and adsorption property [23,24].

3.12.2. Principle of Chromatography

The feature common to all types of chromatography is that two mutually immiscible phases are brought into contact with each other. One of these phases is stationary, while the other is mobile; the mobile phase either moves over the surface or percolates through the interstices of the stationary phase. The sample mixture,
introduced into the mobile phase undergoes repeated interactions (partition) between the stationary and mobile phases while being carried through the system by the mobile phase. Different components of the mixture interact with two phases differentially on the basis of small differences in their physico-chemical properties. Since these different rates of interaction govern the migration of the sample components through the system, each one of the components migrates at a different rate. The compound which interacts more with the mobile phase and least with the stationary phase migrates fast. The component showing least interaction with the mobile phase and strongly with the stationary phase migrates slowly (retarded). This differential movement of the components is responsible for their ultimate separation from each other. Migration of a particular component is measured in terms of RF value (retardation force or relative front) and is given as,

\[ RF = \frac{\text{Distance of the component from the origin}}{\text{Distance of the solvent from origin}} \]

3.12.3. Types of Chromatography

All the chromatographic techniques, involve the process of either absorption or partition. The chromatography technique can be classified as shown in Fig. 3.21.

---

**Fig. 3.21: Classification of Chromatography Techniques**
3.12.4. Column Chromatography

This technique is a type of solid-liquid adsorption Chromatography. The fixed phase is a solid and the mobile phase is a liquid. The basis of separation is selective adsorption of the components present in the liquid phase on the solid. A column such as the one shown in Fig. 3.22 is used. According to scale of the experiment, columns from a few mm to several cm in diameter are used. A long, thin column assures best separation in many difficult cases but large quantities of readily separable substances can be separated more rapidly using a wide column. The column is packed with an active solid such as alumina or silica gel. Apart from these two substances, charcoal, magnesia, calcium carbonate, starch, sucrose, talc and fluorisil (fluorinated silicon polymer) are also used as solid adsorbents. For a good separation, the solid adsorbent should be of uniform particle size and of high specific area, a property which can contribute to rapid equilibrium of the solute between the two phases.

![Fig. 3.22: Chromatography Column](image)

Solvent (Eluent)

Cotton
The column is to be filled with the adsorbent very carefully because irregularities such as air gaps may lead to: (i) mixing of separated zones and (ii) channels through which the solution can flow unchanged. The adsorbent is usually made into slurry with a solvent such as petroleum ether, poured into the column and allowed to settle. During the packing continual tapping of the column with a pencil or a glass rod may help form an evenly packed column. After packing the column with the adsorbent, a small liquid sample is poured on the top. The sample gets adsorbed at the top of the column. An eluting solvent is then allowed to flow through the column. This solvent carries with it the components of the mixture. Because of the selective adsorption capacity of the solid phase, the components move down the column at different rates. The progressive separation of the components into bands is shown in Fig. 3.23.

![Fig. 3.23: Separations of Components into Bands](image)

The bands may be directly observed only with coloured materials. In dealing with colourless materials, the chromatographic experiment is followed by collecting a series of fractions of eluent of constant volume, say 10 ml. The solvent is then evaporated to check whether any solute is present. The different components are obtained in different containers, though each component may be distributed among
several containers. Even though the method is tedious, this is generally adopted. With a few colourless materials, the separation can be attempted with coloured derivatives: for example, mixed aldehydes and ketones could be concerted into dinitrophenylhydrazones and then separated. Colourless samples may sometimes be made visible on the column by fluorescence excited by an ultraviolet source.

The best solvent for a particular mixture is chosen only by trial and error. A mixture of two solvents is sometimes found to be more useful than individual solvents.

3.13. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

3.13.1. Instrumentation

In contrast to the relatively low efficiency of classical liquid chromatography, modern liquid chromatography is highly efficient and usually referred to as high-performance liquid chromatography (HPLC) [25,26].

HPLC is much faster, as well as more efficient, than classical liquid chromatography; it can resolve complicated multi component samples within a few minutes. The various components that are used in a modern HPLC separation are shown in the following Fig. 3.24.

![Block diagram of HPLC](image)

*Fig. 3.24: Block diagram of HPLC*
A pump is used to force the eluent through the column and detector. A pump provides constant flow regardless of the back-pressure of the column. The flow rate can be adjusted by changing the piston stroke of the pump. Unless damping is employed, pumps cause surges in liquid flow that may result in baseline noise on the chromatograph.

At low pressures, the sample injector can be simply a syringe that injects the sample into the eluent stream through a rubber septum. At higher pressures, a sample loop with a two-way valve is used. In one valve position, the loop can be filled with sample at atmospheric pressure. Then the valve is switched, flushing the sample into the column along with the incoming eluent. Usually, a few micro liters of sample are injected.

The column is stainless steel or heavy-walled glass, built to withstand high pressures. The inside column diameter is typically 1-3 mm, and the length is up to 1 meter. The column is packed with small particles of porous silica, alumina or organic resin.

The column and detector cell are often enclosed in a constant-temperature oven. Just after the column, the carrier stream enters a detector. Currently, the most commonly used detector is an ultraviolet detector with a fixed wavelength at 254nm (or sometimes at 280 nm also). Variable-wavelength detectors covering the ultraviolet and visible spectral regions are also used. Table 3.1., gives the experimental difference between conventional chromatography and high performance liquid chromatography.
Table 3.1: Experimental difference between conventional chromatography and HPLC.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>Conventional Chromatography</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Number of plate per second</td>
<td>0.02</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Pressure</td>
<td>Negligible</td>
<td>Upto 8000</td>
</tr>
<tr>
<td>3.</td>
<td>Flow rate (mm/min)</td>
<td>5-50</td>
<td>600</td>
</tr>
<tr>
<td>4.</td>
<td>Time/Duration of the experiment</td>
<td>Hours to days</td>
<td>Minutes to hours</td>
</tr>
<tr>
<td>5.</td>
<td>Equipment</td>
<td>Simple column and accessories</td>
<td>Integrated chromatography</td>
</tr>
<tr>
<td>6.</td>
<td>Quantity chromatographed</td>
<td>µg to Kg</td>
<td>ng, µg to Kg</td>
</tr>
<tr>
<td>7.</td>
<td>Purpose</td>
<td>Predominantly comparative</td>
<td>Predominantly analytical</td>
</tr>
</tbody>
</table>

3.13.2. Applications of HPLC

The wide applicability, speed and sensitivity of HPLC has resulted in it becoming the most popular form of chromatography and all types of biological molecules have been purified using this technique.

1. A reversed phase partition HPLC is useful for the separation of polar compounds such as drugs and their metabolites, peptides, vitamins and steroids.
2. The technique is particularly widely used in clinical and pharmaceutical work, as it is possible to apply biological fluids such as serum and urine.
3. HPLC has probably the biggest impact on the separation of oligopeptides and proteins.

3.13.3. Advantages of HPLC

 רשימת
- It is extremely efficient in separation
- A wide variety of materials can be analyzed by this method.
- It requires only a few microlitres or micrograms of the analyte.
Even though, the HPLC instrument is expensive, it is mostly used for separation. Using SCIMADZU-SPINTRON HPLC-530, HPLC analysis is carried out to confirm and identify the amino acids present in the Aloe vera extract and its photograph is shown in Fig. 3.25.

Fig. 3.25: Photograph of HPLC Set up

3.14. THIN LAYER CHROMATOGRAPHY (TLC)
3.14.1. Introduction

Thin layer chromatography, abbreviated as TLC, involves the same principles as those of column chromatography. It is a form of solid-liquid adsorption chromatography. In TLC, separation takes place on a layer of finely divided solid that is fixed on a flat surface [27-29].

The same solid adsorbents used for column chromatography are employed for TLC. Silica and alumina are widely used. A thin layer plate is prepared by spreading an aqueous slurry of the finely ground solid on the clean surface of a glass or rigid plastic. A small amount of a binder (for example, plaster of paris, calcium sulphate or starch) is incorporated into the slurry to increase adhesion of the solid particles to the glass and to one another. The plate is then heated in an oven for about 30 minutes and
cooled inside the oven itself). Care is taken to avoid exposing the surface to the atmosphere. Otherwise, the plate adsorbs water in a few minutes and the solid merely becomes a support for water.

3.14.2. Plate Development

A drop of the solution to be separated is placed near one edge of the plate and its position is marked with a pencil. The plate is then placed in a container (developing chamber) with enough solvent to come to a level just below the original sample spot. The chamber is kept closed with a flat glass plate. The solvent migrates up the plate carrying with it the components of the mixture at different rates. After the solvent has reached almost the top edge of the plate. Then it is taken out and dried. Coloured materials appear as dots in the chromatogram (Fig. 3.26.).

![Thin Layer Chromatogram](image)

**Fig. 3.26: Thin Layer Chromatogram**

Under a given set of conditions (adsorbent, solvent, layer thickness) the rate of movement of a compound with respect to the rate of movement of the solvent front is a property characteristic of the compound. This property represented by the symbol RF (Retention Front) is obtained by dividing the distance traveled by the substance by the distance traveled by the solvent front from the original spot.
3.14.3. Applications of TLC

1. Since the technique is easy and can be done rapidly, it is ideal for routine analysis of mixture composition. A great advantage of the method is that only a very small quantity of the sample (approximately $10^{-9}$ g) is required.

2. TLC may be used to find out the best eluting agent for column chromatography.

3. A semi-quantitative estimate of the amount of a component present can be obtained by a comparison of the area of a spot with that of a standard.

3.15. SPECTROSCOPY: COLOURIMETRY

3.15.1. Introduction

An instrument used for measuring absorption in the visible region is called a colorimeter. Consider two solutions of potassium dichromate of concentrations 1% (solution A) and 5% (solution B) placed in a similar glass tube. The colour of the solution A, appear light yellow and that of solution B be will dark yellow. Thus, by looking simply at the intensity of the yellow colour in the two cases, we understand that solution B is more concentrated than the solution A. It means that the colour intensity is related to the concentration of the substance producing the colour. This is the basis of what is known as colourimetric analysis [30,31].

When a beam of light falls upon a homogeneous medium, a part of the incident light is reflected a part is absorbed and the remainder is transmitted so that

$$I_0 = I_r + I_a + I_t$$

where

$I_0 = $ intensity of incident light

$I_r = $ intensity of reflected light
$I_a =$ intensity of absorbed light

$I_t =$ intensity of transmitted light.

If $I_t$ is reflected, we can write,

$I_\circ = I_a + I_t$

### 3.15.2. Instrumentation and Working

Spectrophotometer consists of a light source, monochromator cuvettes and photo sensitive detector.

**Radiation source**

In the visible region, a tungsten filament is the most satisfactory. In order to get best result, it is important that the tungsten lamp should emit constant radiation energy. The sources of UV radiation are hydrogen or deuterium discharge lamp or mercury vapour lamp.

**Monochromator**

The band width is selected by the monochromator. The essential elements of a monochromator are an entrance slit, a prism or grating and an exit slit. The functions of the entrance slit are to provide a narrow source of light. The function of a prism or grating is to split the incident beam into different wavelength. The exit slit select a narrow band of dispersed spectrum for observation by the detector.

**Cuvettes: (Container) or (Optically transparent cell)**

These cells obtain the material under study which is normally dissolved in a suitable solvent. A reference cuvette optically identical is required in setting the spectrophotometer to read zero extinction. For UV region, quartz or silica cells must be used and for visible region, normal silica cells are used.
**Detector**

To detect the radiation a photocell or photomultiplier is used.

Photo cell consists of evacuated glass bulb. Inside the bulb there is a cathode coated with a light sensitive layer. A metal ring inserted near the centre of the bulb acts an anode. When radiation is incident upon the cathode photo electron are emitted. These are attracted and collected by an anode. These are then returned via the external circuit. There occurs a potential drop across the resistor $R_L$, which is proportional to the current and is taken as the measure of the light striking the surface. The output is amplified in the amplifier and delivered to the recorder. Its functioning is illustrated in the Fig. 3.27.

![Fig. 3.27: Block Diagram of a Spectrophotometer](image1)

**3.15.3. Types of Spectrophotometer**

These are two types of spectrophotometer

i. Single beam spectrophotometer

ii. Double beam spectrophotometer

**i. Single beam spectrophotometer**

In this case, first the blank and then the sample must be moved into the beam, adjustments made and readings taken.

![Fig. 3.28: Single Beam Spectrophotometer](image2)
ii. **Double beam spectrophotometer**

In this case the beam is split into two parts one passing through blank and the other passing through the sample at the time. The resultant measured absorbance is the difference between the two transmitted beams of light recorded by the detector.

![Double Beam Spectrophotometer Diagram](image)

**Fig. 3.29: Double Beam Spectrophotometer**

### 3.15.4. Colourimetric Estimation Method

Amino acids react with Ninhydrin involved in the development of permanent color. This is an oxidative de-amination reaction that elicits two hydrogen atoms from the amino acid. It is reduced and loses an oxygen atom with the formation of water molecule. Ninhydrin will react with amino group. The ninhydrin reaction has been conventionally used to detect the presence of microgram amounts of substances. If the amino acids with free alpha amino groups are treated with an excess of ninhydrin, they yield a purple colored product. Under appropriate conditions, the color intensity produced is proportional to the auxochromes. Unknown compounds may be identified by their characteristic absorption spectra in the ultraviolet, visible or infrared regions.
Unknown compounds may be identified by their characteristic absorption spectra in the ultraviolet, visible or infrared regions. Color reactions frequently can be followed by measuring spectrophotometrically the appearance of a product. A spectrophotometer/colorimeter is an instrument for measuring the absorbance of a solution by measuring the amount of light (optimum density) of a given wavelength that is transmitted by a sample. To determine the absolute concentration of a pure substance, a standard curve is constructed from the known concentrations and using that standard curve, the absorbance reading (optimum density) of the sample concentration is determined. The determination of unknown concentration from the standard curve is done by drawing a line parallel to the x-axis from the point on the y-axis that corresponds to the absorbance of the unknown. This line will be made to intersect the standard curve drawn and is extended vertically such that it meets the x-axis and the concentration of unknown is read from the x-axis.

**3.16. NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROMETRY**

The discovery of NMR spectroscopy is 1946 by Purcell and Bloch is now recognized as a very important tool for the advancement of inorganic and organic chemistry. The use of NMR in the determination of molecular structure has been a major growth point in biochemistry. The appearance of spectra is dependent on slight variations in electronic configuration. Such spectra can be expected to reflect the more or less unaltered structure and confirmation of macromolecules in solution.

The principle of NMR is based on the spins of atomic nuclei. The magnetic measurements depend upon the spin of unpaired electrons where as NMR measures magnetic effect caused by the spin of protons and neutrons. Both these nucleons have intrinsic angular momenta or spins and hence act as elementary magnet. The existence of nuclear magnetism is revealed in the hyperfine structure of the spectral lines [32-34].
3.16.1. Instrumentation and Working

The appearance (Fig. 3.30) consists of,

1. Sample holder
2. Magnet
3. Sweep generator
4. Radiation frequency oscillator
5. Radio frequency receiver and recorder.

1. The magnet

It is used to supply the principal part of the field H. The magnet may be an
electromagnet or a permanent magnet. If high resolution work is needed, there must
be a very high degree of field homogeneity between the pole pieces.

2. Magnetic field sweep

By varying the current through the coil, the effective field can be changed by a
few hundred milli gauss without any loss in field homogeneity.

3. Radio frequency source

The signal from a radio frequency oscillator or transmitter is fed into a pair of
coils mounted at right angles to the path of the field. In this manner, a plane polarized
beam of radiation has been found to be obtained. A field oscillator having a capacity
of exactly 60 MHz is normally used. For high resolution work, the frequency must be
a constant about one part in $10^8$.

4. The sample holder

The usual NMR cell consists of a 5mm glass tube (cuvette) which has a
capacity of about 0.4ml of liquid. Microtubes for small sample volumes are available.
The sample must be in the liquid or solution state for high resolution spectra.
5. The signal detector and recording systems

The coil has been used to direct the radio frequency signal produced by the resonating nuclei. The electrical signal generated into the coil must be amplified before it can be recorded.

Fig. 3.30: The NMR spectrometer

The strength of the magnetic field is altered gradually in such a way that the frequency emitted by the nuclei of the sample is equal to the frequency of the radio frequency of the oscillator. As a result of this, energy could be absorbed by the sample. The nuclei would be raised to higher energy levels. As the nuclei returns to the initial energy levels, they give out the energy previously absorbed by them to the receiver coil and therefore a signal is given out by the receiver, which is then amplified and recorded. The number of signals is an NMR spectrum gives information about the number of equivalent sets of proton/nuclei that the compound possesses. The position of these signals is indicative of the environment surrounding the proton/nuclei. For example, the position of the signals can reveal whether the proton exists near an electron withdrawing or releasing group. BRUKER PROTON NMR Spectrometer is used to understand and confirm the structure of the grown sample.
3.17. KURTZ POWDER METHOD - SHG EFFICIENCY TEST

3.17.1. Introduction

Nonlinear optics is attracting lot of attention due to its wide application in the area of laser technology, optical communication and data storage technology. This phenomenon is born as a direct consequence of the invention of lasers. The existence of harmonic light waves at the boundary of a nonlinear dielectric medium is predicted by Bloembergen and Pershan (1962). Discovery of other nonlinear optical effects is followed by the discovery of second harmonic generation in quartz [35]. The light propagated through a crystalline solid, which lacks a center of symmetry, generates light at second and higher harmonics of the applied frequency. This important nonlinear property of non-centrosymmetric crystals is called second harmonic generation (SHG) and this phenomenon and the materials in which it occurs is the subject of intense study. Kurtz and Perry proposed a basic, preliminary powder SHG method for comprehensive analysis of the second order nonlinearity [36]. Employing this technique, Kurtz surveyed a very large number of compounds.

3.17.2. Experimental Procedure

The nonlinear optical property of the grown single crystal is tested by passing the output of Nd:YAG laser light through the crystalline powder sample. The schematic diagram of the experimental setup used for SHG study is shown in Fig. 3.21. A Q-switched, Nd:YAG laser light is used to generate about 6.5mJ/pulse at a wavelength of 1064 nm.

This laser can be operated in two modes. In the single shot mode the laser emits a single 8ns pulse. In the multi shot mode the laser produces a continuous pulse of 8ns at a repetition rate of 10Hz. In the present study, a single shot mode of 8ns
laser pulse with a spot radius of 1mm is used. This experimental setup used a mirror and a 50/50 beam splitter to generate a beam with pulse energies about 6.5mJ. The input laser beam is passed through an IR reflector and then directed on the finely powdered sample packed in a capillary tube of diameter 0.154 mm.

![Fig. 3.31: Schematic of Kurtz-Perry Technique](image)

The photodiode detector and oscilloscope assembly measure the light emitted by the sample. Microcrystalline powder of urea or KDP is taken in a similar capillary tube sealed at one end for comparison. The intensity of the second harmonic output from the sample is compared with that of either urea or KDP. In the present study the grown crystals are compared with KDP crystals and the figure of merit of SHG of the crystals are estimated.
REFERENCES


