CHAPTER III
CHARACTERIZATION TECHNIQUES

The physical techniques and methods used to analyze and study solids are often quite different from those used in other branches of chemistry. Thus, there is much less emphasis on spectroscopic methods and more emphasis on a variety of diffraction, mainly single crystal X-ray diffraction and microscopic techniques like electron microscope. In addition, other techniques such as thermal analysis, dielectric studies, optical property, microbial and microhardness measurements may give valuable information in certain cases.

3.1 Elemental analysis

Elemental analysis is an experimental technique used to determine the amount (typically weight percent) of various elements present in a compound. The most common type of elemental analysis is CHN analysis which calculates carbon, hydrogen and nitrogen weight percentages in a compound. The weight percent of oxygen is easily calculated by subtracting the weight percentage composition of all the elements from 100. The elemental analysis of a compound is particularly useful in determining the empirical formula of the compound.

3.2 UV-visible spectroscopy

There are many different spectroscopic techniques but all of them work on the same basic principle. Under certain conditions, materials are capable of absorbing or emitting electromagnetic radiation.

Spectroscopic measurements on solids complement well with the results obtained from X-ray diffraction since spectroscopy gives information on local order whereas diffraction is concerned primarily with long range order.

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length and the concentration of the absorbing species.
Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule.

UV-vis spectroscopy is the measurement of the wavelength and intensity of absorption of near-ultraviolet and visible light by a sample. UV-vis spectroscopy is usually applied to molecules and inorganic ions or complexes in solution. The UV-vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law.

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in ultraviolet or visible region to excite these electrons to higher energy anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer is the wavelength of light it absorbs. Optical clarity of crystals can be determined indirectly by dissolving them in a suitable solvent and subjecting to visible spectroscopy.

3.3 FTIR spectroscopy

Infrared spectroscopy deals with the study of vibrational spectra of molecules. An infrared absorption spectrum originates from molecular vibrations (vibration of chemical bonds) which cause a change in the dipole moment of the molecule. Molecules of complex nature can also be exposed to infrared radiation to observe their spectra. Fourier Transform Infrared (FTIR) absorption spectra for the crystals are recorded using sophisticated instruments. This technique is based on the blending of a Michelson interferometer with a sensitive infrared detector and a computer. Among the spectroscopic techniques infrared spectrum is characteristic of the entire molecule. The characteristic bands in the spectrum permit the chemist to obtain useful structural information by simple inspection and reference to generalized charts of characteristic group frequencies.

In general for a polyatomic molecule, we may deduce the number of vibrational degrees of freedom, by subtracting the number of translational and rotational degree of freedom from the total number of degrees of freedom possessed
by the molecule. Besides the fundamental vibrations, overtones, combination and difference bands also appear in the spectrum. Deformation modes produce absorption in the lower frequency regions compared to the fundamental stretching modes. The uniqueness of infrared absorption arises largely from those bands which are characteristic of the entire molecule [1]. The vibrational frequencies, their relative intensities and the shape of the infrared bands are used in the qualitative characterization of the molecule. FTIR spectrometers provide high resolution, total wavelength coverage, higher accuracy in the frequency and intensity measurements.

3.4 Raman spectroscopy

Raman spectroscopy is a spectroscopic technique used to study vibrational, rotational and other low-frequency modes in a system. It relies on inelastic scattering or Raman scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in the energy of the laser photons being shifted to up or down. The shift in energy gives information about the vibrational modes in the system. Infrared spectroscopy yields similar, but complementary, information.

Typically, a sample is illuminated with a laser beam. Light from the illuminated spot is collected with a lens and sent through a monochromator. Wavelengths close to the laser line due to elastic Rayleigh scattering are filtered out while the rest of the collected light is dispersed onto a detector.

3.4.1 Polarized Raman spectroscopy

Polarized Raman spectroscopy probes information about molecular orientation and symmetry of the bond vibrations, in addition to the general chemical identification which ‘normal’ Raman provides. The spectral information arising from this analysis gives insight into molecular orientation and vibrational symmetry. The measurement is made by inserting a polarizer in the beam path between the sample and the spectrometer, allowing the Raman polarization to be selected by the user. The polarization of the laser beam can also be kept in its normal state, rotated by 90° or ‘scrambled’ to remove any polarization by inserting polarizing optics between the laser and the sample. Polarization measurements provide useful information about
molecular shape and the orientation of molecules in ordered materials, such as crystals, polymers and liquid crystals.

Study of the technique is useful in teaching the connections between group theory, symmetry, Raman activity and peaks in the corresponding Raman spectra. It allows the user to obtain valuable information relating to the molecular shape, for example in synthetic chemistry or polymorph analysis. It is often used to understand macromolecular orientation in crystal lattices, liquid crystals or polymer samples.

3.5 NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy deals with the interaction of radio frequency waves to induce transitions between magnetic energy levels of nuclei of a molecule. The magnetic energy levels are created by keeping the nuclei in a magnetic field. In the absence of magnetic field, the spin states of nuclei are degenerate and energy level transition is not possible. Presence of magnetic field is essential so that radio frequency radiation can cause transitions between these energy levels. Elements that have non-zero nuclear spin can only exhibit NMR spectrum. Under the influence of magnetic field, magnetic levels split into two groups depending on whether the nuclear spins are aligned parallel or anti parallel in the applied magnetic field. NMR technique enables us to study the shape and structure of a molecule. In particular, it reveals the different chemical environments of the various kinds of protons present in a molecule and ascertains the structure of molecule. The types of NMR include $^1\text{H}$, $^{13}\text{C}$ and $^{31}\text{P}$ depending on the nucleus we investigate.

3.6 X-ray diffraction method

An understanding of the structure of materials has become essential in the world of novel materials. Any property of a material like mechanical, chemical or electrical depends strongly on its internal structure. Hence, it has become easier to design a material to suit any application by appropriate modification of the internal structure. However, such an intelligent design of materials needs a complete assessment of structure-property correlation.

X-ray diffraction technique plays a vital role in coordination chemistry and determination of bond lengths, bond angles, residual stress, solvent curves for solid solutions, preferential orientation, grain size, particle size, temperature factor etc.
3.6.1 Powder X-ray diffraction method

X-ray powder diffraction is a non-destructive instrumental technique that is widely applied for the characterization of various macro, micro and nano crystalline materials including inorganic, organics, drugs, minerals, zeolites, catalysts, metals and ceramics as well as other materials like crystalline polymers. A powder sample of a crystal should contain an infinite number of randomly oriented crystallites and thus a large number of them corresponding to different Miller indices will be in a position to satisfy the diffraction conditions. The interaction of X-rays and crystals is described by Bragg’s law \( n\lambda = 2d_{\text{hkl}} \sin\theta \), where \( n = 1, 2, 3 \ldots \) is number of diffraction, \( \lambda \) is the wavelength of the X-ray used, \( d \) is the distance between the lattice planes and \( \theta \) is the angle of incidence.

Every crystal plane is thus capable of diffraction. Each lattice plane is treated as a mirror reflecting the X-rays back at the same angle \( \theta \) that they strike the mirror. As a result, the focus of directions making forms angle \( 2\theta_{\text{hkl}} \) with a given direction forms a cone as shown in Figure 3.1.

![Figure 3.1](image)

**Figure 3.1** Scattering of X-rays into cones by a powder sample

The diffraction pattern is a spectrum of real space periodicities in a crystal. The powder diffraction pattern of a sample can be compared with those listed in the powder diffraction file which contains entries for approximately more than 30,000.

3.6.2 Single crystal X-ray diffraction method

Single crystal X-ray diffraction is a very important tool to characterize the structure of a new compound. Today, a wide range of experimental methods are available for the evaluation of structure of materials with high accuracy and precision.
The main uses of single crystal methods are to determine unit cells and space groups and if there is sufficient interest, to measure the intensities of reflections and carry out a full crystal structure determination. In the rotating crystal method, a single crystal is mounted with an axis normal to a monochromatic X-ray beam. A cylindrical film is placed around it and the crystal is rotated about the chosen axis. As the crystal rotates, sets of lattice planes will at some point make the correct Bragg angle for the monochromatic incident beam and at that point a diffracted beam will be formed. The reflected beams are located on the surface of imaginary cones. A data set containing several thousand reflections can be measured in a period of few days. Equivalent reflections are then merged and corrections are made for Lorentz and polarization effects. The resulting list of $F_{hkl}$ values provides the basis for the determination of the structure.

After the collection of all X-ray data, the structure is solved using computer programmed methods. Once the structure is solved, then it is again refined to a reasonable extent using computer softwares. The single crystal methods are used in the determination of unit cell and space group, crystal structure, electron distribution, atom size, bonding and finally in crystal defects and disorder.

3.7 Thermal analysis

Thermal analysis is a group of techniques in which some physical parameters of the system are determined or recorded as a function of temperature. Various techniques of thermal analysis are thermogravimetry (TG), derivative thermogravimetry (DTG), differential thermal analysis (DTA), differential scanning calorimetry (DSC), etc. It is important to note that a single thermal analysis method does not provide complete information about a given system. It is common to complement all DTA or DSC data with thermogravimetry (TG).

3.7.1 Thermogravimetric analysis (TGA)

In a thermogravimetric analysis the mass of a sample in a controlled atmosphere is recorded continuously as a function temperature or time as the temperature of the sample is increased linearly with time. A plot of mass or mass percent as a function of time or temperature is called a thermogram. A schematic and typical single step reaction is shown in Figure 3.2.
The weight loss, $\Delta W$ is fundamental properties of the sample and can be used for quantitative calculations as well as qualitative compositional change.

![Diagram of weight loss](image)

**Figure 3.2** Schematic representations for a single step decomposition reaction.

### 3.7.2 Differential thermal analysis (DTA)

Differential thermal analysis is a technique in which the temperature of a sample is compared with that of an inert reference material during a programmed change of temperature. The temperature of the sample and reference should be the same until some thermal event, such as melting, decomposition or change in crystal structure occurs in the sample. In such cases the sample temperature either lags behind (if the change is endothermic) or leads (if the change is exothermic) the temperature.

The DTA results are presented as $\Delta T$ against temperature as shown in Figure 3.3. A horizontal base line corresponds to $\Delta T = 0$ and superposed on this is a sharp peak due to the thermal event in the sample.

The size of the $\Delta T$ peak may be amplified so that events with very small enthalpy changes may be detected. Representing DTA in the form of a figure is therefore a much more sensitive and accurate way of presenting data and is the normal method for presenting DTA results. With modern, automatic thermal analysis equipments TGA, DTA and DSC thermograms are recorded using the same instruments.
3.7.3 Differential scanning calorimetry (DSC)

DSC involves heating of the sample and the reference substance by continuously increasing temperature (usually at a rate of 10°C/min), but heat is simultaneously added either to the sample or to the reference as necessary so as to maintain the two at identical temperatures. The added heat either to sample or reference compensates for the heat lost or gained respectively as a consequence of endothermic or exothermic reaction occurring in the sample. The heat energy supplied to the sample or reference is continuously recorded as function of temperature. DSC finds widespread use in calculating enthalpy of transition such as enthalpy of melting, enthalpy of crystallization, enthalpy of fusion, etc of polymeric materials. Moreover, DSC curves can be used to determine the purity of drug samples.

3.8 Nonlinear optical (NLO) properties

Nonlinear optics is the branch of optics that describes the behaviour of light in nonlinear media, that is, media in which the dielectric polarization responds nonlinearily to the electric field of the light [2]. This nonlinearity is typically only observed at very high light intensities such as those provided by pulsed lasers. In nonlinear optics, the superposition principle no longer holds.
3.8.1 Nonlinear materials

In recent years, many significant achievements have occurred in the field of nonlinear optical because of the development of laser technology and new nonlinear optical materials of both inorganic and organic types. The phenomenon of SHG in inorganic materials, first discovered in 1961, led to the development of recent NLO materials such as potassium dihydrogen phosphate (KDP) cadmium sulphide (CdS). These are appropriate infrared NLO materials, although they suffer from laser damage thresholds (~10MV cm⁻²) and poor optical transparency.

3.8.2 Detection of nonlinear optical activity

Nonlinear optics (NLO) phenomenon is a material phenomenon and the process occurs within a nonlinear medium, usually a crystal [3]. This phenomenon results due to the interaction of intense electromagnetic radiation with matter producing magnified fields that are different from the input field in frequency, phase or amplitude [4]. At relatively low intensities, the optical properties of crystals are quite independent of the intensity of illuminate. However, if the illumination is made sufficiently intense, the optical properties begin to depend on the intensity and other characteristics of the light. The light radiation may then interact within as well as with the crystals. This is the realm of nonlinear optics. The high intensities necessary to observe these effects can thus be obtained by using the output from a coherent light source, which was possible only after the invention of lasers [5,6].

3.8.3 Kurtz-Perry powder technique

It is highly desirable to have some technique of screening crystals to determine whether they possess nonlinear activity or not. Such a preliminary test should enable us to carry out the activity without the requirement of perfectly oriented samples. Kurtz and Perry [7] proposed a powder SHG method for comprehensive analysis of the second order nonlinearity. Employing this technique Kurtz [8] tested a very large number of compounds.

In this technique, the sample is packed as a polycrystalline powder into a cell sandwiched between two glass slides. The crystal powder must be so finely ground that the average size of the particle is much less than the coherence length for the second harmonic generation; otherwise there would be no signal. Typical value of
coherence length is ~ 2µm. The sample is then subjected to a Q switched Nd:YAG laser emitting 1.064 µm wave length and 10 ns laser pulses with spot radius of 1mm to assess the SHG intensity.

3.9 Microhardness testing

Hardness is resistance of material to plastic deformation caused by indentation. Sometimes hardness refers to resistance of material to scratching or abrasion. In some cases relatively quick and simple hardness test may substitute tensile test. Hardness may be measured from a small sample of material without destroying it. There are hardness methods, allowing to measure hardness onsite.

The term microhardness test usually refers to static indentations made with loads not exceeding 1 kg. The indenter is either the Vickers diamond pyramid or the Knoop elongated diamond pyramid. The procedure for testing is very similar to that of the standard Vickers hardness test, except that it is done on a microscopic scale with higher precision instruments. The surface being tested generally requires a metallographic finish; the smaller the load used, the higher the surface finish required. Precision microscopes are used to measure the indentations.

A precision diamond indenter is impressed into the material at loads from 15 to 1000 g. The impression length, measured microscopically and the test load are used to calculate the hardness value. The Vickers Diamond Pyramid hardness number is the applied load (kg) divided by the surface area of the indentation (mm²)

\[ H_V = \frac{2F \sin 136/2}{d^2} \]

\[ H_V = 1.854 \frac{F}{d^2} \text{ approximately} \]

where F is load in kg, d is arithmetic mean of the two diagonals \(d^1\) and \(d^2\) in mm, \(H_V\) is Vickers hardness. The Vickers Diamond Pyramid indenter is ground in the form of a squared pyramid with an angle of 136° between faces. The depth of indentation is about 1/7 of the diagonal length. When calculating the Vickers Diamond Pyramid hardness number, both diagonals of the indentation are measured and the mean of these values is used in the above formula with the load used to determine the value of \(H_V\).
3.10 Dielectric studies

A dielectric is an electric insulator that can be polarized by an applied electric field. When a dielectric is placed in an electric field, electric charges do not flow through the material, as in a conductor, but only slightly shift from their average equilibrium positions causing dielectric polarization. Because of dielectric polarization, positive charges are displaced toward the field and negative charges shift in the opposite direction. This creates an internal electric field which reduces the overall field within the dielectric itself [9].

The dielectric constant of an insulating material is numerically the ratio of the capacitance of a capacitor containing that material to the capacitance of the same electrode system with vacuum replacing the insulation as the dielectric medium. All materials have a dielectric constant greater than 1. The dielectric constant of any given material varies with temperature and for polymers a rapid increase begins near their glass transition temperature. Dielectric constants also vary as a function of frequency and this aspect will be important when you look at high frequency designs. Most materials used for capacitors have substantially higher dielectric constants than polymers, sometimes many tens of thousands. However, this is often achieved at the expense of stability.

Dielectric loss results from the heating effect on the dielectric material between the conductors. Power from the source is used in heating the dielectric. The heat produced is dissipated into the surrounding medium. When there is no potential difference between two conductors, the atoms in the dielectric material between them are normal and the orbits of the electrons are circular.

3.11 Antimicrobial studies

Microbes are microscopic life forms, usually too small to be seen by the naked eye. Although many microbes are single-celled, there are also numerous multi-cellular organisms. Microorganisms are very diverse and include bacteria, fungi and algae. Microorganisms live in all parts on the earth where there is liquid water, including hot springs, on the ocean floor, high in the atmosphere and deep inside rocks within the Earth's crust. Certain microbes have adapted to withstand unusual environmental conditions, including extreme pressure, temperature, acidity and radiation. These
microscopic organisms are found in plants and animals as well as in the human body.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi and or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbiostatic).

3.11.1 Antibacterial activity study

Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. However, prolonged use of certain antibiotics can decrease the number of gut flora, which can have a negative impact on health. Some recommend that, during or after prolonged antibiotic use, one should consume probiotics and eat reasonably to replace destroyed gut flora.

3.11.2 Antifungal activity study

Fungi are multi-celled organisms with structures analogous but not identical to plants. They can be found in air, in soil, on plants and in water. Thousands, possibly millions, of different types of fungi exist on Earth. The common types are mushrooms, yeast, mold and mildew. Some live in the human body, usually causing illness. Fungal diseases are called mycoses. Mycoses can affect human skin, nails, body hair and internal organs such as lungs and body systems such as the nervous system. Aspergillus fumigatus, for instance, can cause aspergillosis, a fungal infection in the respiratory system.

Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. An antifungal drug is medication used to treat fungal infections such as athlete’s foot, ring worm, candidiasis, serious systemic infections such as cryptococcal meningitis and others.
References