The synthesis method and the various techniques used for the characterization of materials under investigation are discussed herein. The theory and the working of various instruments like X-Ray Diffractometer (XRD), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), Wavelength Dispersive X-ray Fluorescence Spectroscope (WD–XRF), Fourier Transform Infrared Spectroscope (FT–IR) and Thermal Analysis Instrument (TG/DTA instrument) are outlined herein. Working of the Vibrating Sample Magnetometer (VSM) is also discussed in detail. Dielectric measurements and DC conductive measurement techniques have been presented. The nonlinear optical characterization technique is also described in detail. Details of various analytical methods used to determine the effect of the prepared nanoparticles on Chlorella pyrenoidosa are discussed. These powerful characterization techniques help to bring light on many unknown information of various samples studied.
Chapter 2

2.1 Introduction

The synthesis of phase pure compounds finds prime importance in the nano regime of material science. The properties of nanoparticles depend on the size, shape, phase, crystalinity etc. of the prepared sample which directly depends on the preparation process used. Hence selection of the appropriate method for synthesis is important. After preparation of the sample, a proper characterization using the several analytical techniques is essential. Various data obtained from the appropriate analysis can be converted to various informations regarding the size, shape, structure, chemical composition etc. of the samples. This chapter provides an overview of the various methods employed for the preparation and characterization of nanoparticles.

2.2 Preparation of nano particles

Various physical and chemical methods are used for the synthesis of nanoparticles like high energy ball milling, inert gas condensation, plasma deposition, reverse micelle technique, citrate precursor technique, micro emulsion, hydrothermal reaction, sol–gel technique, co precipitation, polymer pyrolysis, liquid mix technique etc. [1 - 7]. The two main approaches that are used in all these synthesis techniques are Top Down approach and Bottom Up approach. The Top Down approach involves the process of breaking down a bulk material into nanosized particles through the process of breaking, cutting or etching. Top Down approach is applied in preparation techniques such as high energy ball milling, vapour phase condensation, laser ablation, sputtering, electro–explosion, chemical, mechanical milling etc.
In Bottom Up approach, the nanoparticles are built from the bottom that is atom–by–atom to molecule–by–molecule to cluster–by–cluster. Methods used in Bottom Up approach include, sol–gel method, microemulsion, solution combustion method, reverse miscelle synthesis, chemical precipitation synthesis etc. Of all the various physical and chemical methods employed, sol–gel technique is the simplest method for the preparation of nanoparticles of narrow size distribution and good stoichiometry [8] at low synthesis temperature.

2.2.1 Sol-gel method

Sol–gel technique is a wet chemical method used mainly for the preparation of metal oxides using metal salts and organic alcohols like ethylene glycol or xylene [9]. The first step in sol–gel technique is the preparation of sols, which are different stable solutions of metal precursor. The second step is the formation of oxide–alcohol bridged network (the gel), formed by a drastic increase in the viscosity of the solution by polycondensation or polyesterification. At the third stage, on further heating,
the gel transforms into a solid mass caused by the contraction of the gel network and expulsion of the solvent from the gel pores. In the fourth and the final step, the gel is dried, thereby the water and other volatile liquids are removed from the gel network. Normally, metal oxides are synthesized by calcination of the finely grounded powder in temperature controlled furnaces for a specific duration. The temperature may vary from 300°C to 1000°C, depending on the nature of the metal oxides to be prepared.

In this section, the synthesis of ZnFe$_2$O$_4$ nanoparticles by sol-gel technique is presented. Here AR grade zinc nitrate (Zn(NO$_3$)$_2$.6H$_2$O) and ferric nitrate (Fe(NO$_3$)$_3$.9H$_2$O) are dissolved in 100ml of ethylene glycol in their stoichiometric ratios at room temperature. The solution is then heated at 60°C to form a wet gel. This gel is then dried at 120°C to form a fluffy product through a thermally induced redox reaction. The obtained product is then grounded well and is sintered for two hours in a muffle furnace at 400°C.

2.3 Structural Characterization

Structural characteristics of the prepared samples are ascertained using various techniques to find the size, shape, phase, crystalinity, surface morphology etc. The techniques include X–ray Diffraction (XRD), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), Wavelength Dispersive X–ray Spectroscope (WD–XRF), Fourier Transform Infrared Spectroscope (FT–IR) and Thermal Analysis Instrument (TG/DTA instrument).

2.3.1 X-Ray Diffractometer (XRD)

X-ray diffractometer is one of the popular techniques used for the initial structural characterization of materials [10, 11]. The pattern obtained from XRD can be considered as the finger print of the material and can shed much light on the characteristic features of the material. X-rays are produced when
high velocity electrons are made to collide with a metal target. Copper target is used here. These X-rays are filtered, concentrated and are then directed towards the sample [12].

The basic principle behind an X-Ray Diffractometer is the Bragg’s law

$$2d \sin \theta = n \lambda$$

where $\lambda$ is the wavelength of the incident ray, $d$ is the spacing between the diffraction planes, $\theta$ is the angle of incidence of X-rays, $n$ is the order of diffraction.

![Schematic representation of an X-Ray Diffractometer](image)

**Figure 2.2:** Schematic representation of an X-Ray Diffractometer

When a beam of monochromatic X-ray is allowed to fall on a sample, the beam will be scattered in all directions by various planes of the crystal. Only those planes which obey the Bragg’s law constructively interfere to produce high intensity beams. The recorder records the intensity of the diffracted beams from the various planes and the plot of $\frac{I}{I_0}$ versus $2\theta$ is thus obtained.

When the crystallite size is reduced below a certain limit (< 100nm) the width of the diffraction peak increases. Much information can be calculated from the line broadening of the peaks. The diffraction line broadens mainly
due to three factors namely instrumental effect, crystallite size and microstrain [10, 13]. The total Full Width at Half Maximum (FWHM) of the diffraction line can be expressed as,

\[ \beta_{\text{exp}} = \beta_{\text{inst}} + \beta_{\text{size}} + \beta_{\text{strain}} \]  

\[ \text{where } \beta_{\text{exp}} \text{ is the experimentally measured FWHM, } \beta_{\text{inst}} \text{ is the measured FWHM due to the instrument, } \beta_{\text{size}} \text{ is the FWHM due to the crystallite size and } \beta_{\text{strain}} \text{ is the FWHM induced by the strain.} \]

**Instrumental Broadening**

Reasons for the instrumental broadening are the slit widths, imperfections in focusing the X–ray beam and the unresolved \( k\alpha_1 \) and \( k\alpha_2 \) peaks [13]. This instrumental broadening effect can be eliminated by running a standard stress free sample under the same conditions and subtracting the broadening effect from the line broadening obtained for the test sample.

**Crystallite Size Broadening**

Assuming that the sample is strain free and there is no instrumental broadening, the size induced broadening can be expressed as,

\[ \beta (2\theta) = \frac{0.9\lambda}{D\cos\theta} \]  

\[ \text{where } \theta \text{ is the angle of diffraction, } \lambda \text{ is the wavelength of the X-ray, } D \text{ is the average crystallite size.} \]

Thus using the Scherrer equation, we can calculate the crystallite size if we eliminate the broadening effects due to strain and instrumental parameters.
Strain Broadening

For a normal unstrained crystal lattice, the diffraction peaks will be sharp and symmetric. But when an external strain comes into action the diffraction peaks shifts their positions. The strain induced broadening is given by the Wilson formula,

$$\beta_{\text{strain}} = 4\varepsilon \tan \theta$$  \hspace{1cm} (2.4)

where $\varepsilon$ is the root mean square of micro strain, hence

$$\beta = \frac{k\lambda}{\beta \cos \theta} + 4\varepsilon \tan \theta$$  \hspace{1cm} (2.5)

where $\beta$ is the FWHM after eliminating the effect of instrumental correction. The broadening effect of grain size and strain can be separated using the Hall Williamson plot [10]. Rearranging the equation (2.5),

$$\beta \cos \theta = \frac{k\lambda}{D} + 4\varepsilon \sin \theta$$  \hspace{1cm} (2.6)

The crystallite size and lattice strain can be calculated by plotting the value of $\beta \cos \theta$ as a function of $4\sin \theta$. The slope of the plot gives the lattice strain ($\varepsilon$) and the crystallite size can be calculated from the intercept, using the equation [14].

$$D = \frac{\lambda}{\text{Intercept}}$$  \hspace{1cm} (2.7)

The various parameters that can be calculated from the XRD diffraction pattern are,

(a) Determination of interplanar spacing

Diffraction peaks occur only when constructive interference takes place, that is when the Bragg’s law $2d \sin \theta = n\lambda$ is satisfied. From the equation,
Chapter 2

\[ d = \frac{n\lambda}{2\sin\theta} \]  
\[ \text{(2.8)} \]

Where \( n \) is an integer, \( \lambda \) is the wavelength of the X-ray, \( \theta \) is the Bragg’s angle, \( d \) is the inter planer spacing.

(b) Determination of lattice parameter

Crystals contain a large number of equidistant parallel planes, ‘d’ distance apart. For a cubic lattice, the lattice constant ‘a’ can be calculated from the equation,

\[ d_{hkl} = \frac{a}{\sqrt{h^2+k^2+l^2}} \]  
\[ \text{(2.9)} \]

where \( d_{hkl} \) is the inter planar spacing of the \((h,k,l)\) planes, ‘a’ is the lattice constant.

(c) Calculation of crystallite size.

The average crystallite size of the powder sample can be calculated using the Scherrer formula [10].

\[ D = \frac{k\lambda}{\beta \cos\theta} \]  
\[ \text{(2.10)} \]

here \( D \) is the crystallite size, \( \lambda \) is the wavelength of the X–ray, \( \beta \) is the full width at half maximum, \( \theta \) is the angle of diffraction, \( k = 0.9 \) for the cubic structure.

(d) Determination of density related parameters

Density related properties are independent of external factors. These properties include measured density or apparent density, X-ray density, porosity, surface area etc.
Measured Density

Measured density or apparent density is difficult to measure or characterize, as this parameter is affected by various other factors like pressure, temperature, amount of different elements etc. The measured density, \( \rho_m \), is calculated using the equation [15].

\[
\rho_m = \frac{m}{\pi r^2 h} \quad \text{(2.11)}
\]

where \( m \) is the mass of the pellet, \( r \) is the radius and \( h \) is the height of the sample pellet.

X-ray Density

X-ray density of the prepared sample can be calculated using the equation [16].

\[
\rho_x = \frac{8M}{N \alpha^3} \quad \text{(2.12)}
\]

where \( M \) is the molecular mass of the sample, \( N \) is the Avogadro’s number and ‘\( \alpha \)’ is the lattice constant.

Porosity

The shape, size of grains, degree of storing and packing capacity determine the porosity of a sample [14]. Percentage of porosity can be calculated using the equation.

\[
P(\%) = \left(1 - \frac{\rho_m}{\rho_x}\right) \times 100 \quad \text{(2.13)}
\]

Surface Area

From the value of crystallite size ‘\( D \)’ and measured density, surface area can be calculated using the equation [15].

\[
S = \frac{6000}{D \rho_m} \quad \text{(2.14)}
\]
2.3.1.1 Rietveld Refinement Method

Rietveld refinement method is an important technique developed by Hugo M. Rietveld, used for characterization of crystalline materials [17, 18]. Here the theoretical line profile is refined using the least square approach until it matches the measured profile.

The three main parameters refined by the rietveld refinement method are peak shape function, profile parameters and structural parameters. Peak shape function includes both the sample (domain size, stress/strain, defects) and the instrument (slit size, radiation source) both of which in turn depend on $2\theta$. Profile parameters include unit cell parameters, FWHM and peak symmetry of the Bragg’s peak. The type, position and occupancy of atoms in the crystal fall under the structural parameters. A knowledge of the structure, access to suitable starting model and the XRD data are sufficient to start the Rietveld Refinement technique.

At least a hundred cycles are required to fit a structure of medium complexity. Progress and goodness of the refinement can be evaluated from the resultant profile fit and reliability factors or the R values [19].

The R factors are defined as,

$$ R_{\text{pattern}} = \frac{\sum |y_i(\text{obs}) - y_i(\text{calc})|}{\sum y_i(\text{obs})} \quad \text{------------------ (2.15)} $$

R weighted pattern as,

$$ R_{w\text{p}} = \left\{ \frac{\sum w_i (y_i(\text{obs}) - y_i(\text{calc}))^2}{\sum w_i (y_i(\text{obs}))^2} \right\}^{1/2} \quad \text{------------------ (2.16)} $$

Statistically expected R of value $R_{\text{exp}}$ as,

$$ R_{w\text{p}} = \left\{ \frac{(N-P+C)}{\sum w_i (y_i(\text{obs}))^2} \right\}^{1/2} \quad \text{------------------ (2.17)} $$
where \( N \) is the number of observations, \( P \) is the number of parameters and \( C \) the number of constraints used in the refinement.

The goodness of fit is given by,

\[
\chi^2 = \sum \left( \frac{P - \Phi}{\delta^2} \right)^2
\]

The quality of the fit is assessed from the goodness of fit \( \chi^2 \) and reliability factors \( R_p \) and \( R_{wp} \). For a good fit, the goodness of fit \( \chi^2 \) must tend to 1 and the values of \( R_p \) and \( R_{wp} \) must be close to or less than 10\% [20].

There are many refinement softwares available like Fullproof, BRASS, GSAS, Prodd etc. Broadening due to particle size and strain, fits well with Cauchy and Gaussian type functions; Pseudo Void is a Linear combination of Cauchyian and Gaussian functions and is the most reliable peak shape function and is used in the Rietveld structure refinement software. In the present study, General Structure Analysis System (GSAS) has been used. This particular software can be used to analyze both single crystal data as well as powder diffraction data.

### 2.3.2 Scanning Electron Microscope (SEM)

Decades of years back, microscopy had an important role in imaging. But its resolving power has limitations due to the numerical aperture of the lens and wavelength of the electrons. Whereas a Scanning Electron Microscope uses a high energy focused electron beam to produce images of high magnification ranging from 10x to 100000x. Scanning electron Microscopy provides information about the surface morphology. SEM consists of an electron gun at the top of a microscope. The electrons from this electron gun pass through a vacuum and are then focused by the electromagnetic fields and lenses to fall on the sample. The electrons scan the sample surface in a
raster manner [21]. When the electron beams fall on the solid surface, some of them pass through while some get scattered. The elastic scattering produces secondary electrons, Auger electrons and X-rays [22]. The secondary electrons and the back scattered electrons are used for the imaging in a SEM.

![Figure 2.3: Schematic illustration of a Scanning Electron Microscope (SEM)](image)

The secondary electrons which are sensitive to surface topography provide topographic images with greater depth of field and high magnification. The intensity of the back scattered electrons are proportional to the number of elements in the sample and provide data about the elemental constitution [23, 24].

In this work, the surface morphology of the prepared nano ferrites and the algal cells are studied using a TESCAN VEGA 3 SBH Scanning Electron Microscope.

### 2.3.3 Transmission Electron Microscope (TEM)

Transmission electron microscope is a sophisticated instrument which provides three dimensional images of high resolution, projected into a two-dimensional plane.
dimensional one. TEM and light microscope have the same basic principle except that TEM uses electrons rather than light to examine a structure. TEM provides images of resolution thousand times better than that of a microscope as the de Broglie wavelength of electrons are very small. The TEM images are black and white. The contrast in projection is the contribution of the diffracted electrons. The transmitted electrons generate the bright regions while the diffracted electrons generate the dark regions.

![Transmission Electron Microscope Diagram](image)

**Figure 2.4**: Schematic diagram of a Transmission Electron Microscope (TEM)

TEM consists of a field emission gun at the top and three sets of lenses beneath. The three different sets of lenses are the condenser lens which produces the primary beam, the objective lens which helps to focus the beam on the sample and finally the projector lens which helps in the imaging, by expanding the beam on to an imaging device like film. There are mainly two types of Transmission Electron Microscope, namely the regular TEM and the HRTEM. The difference between a normal TEM and an HRTEM is that the HRTEM produces images of high resolution in atomic scale [25].
In the present study, the TEM: Philips CM–10, operated at 100kV, is used to study the size and morphology of the prepared nanoparticles. A software, Image J, is used to plot the bar diagram of the particle size and to calculate the average particle size of the nanoparticles.

2.3.4 Wavelength Dispersive X-Ray Fluorescent Spectrometer (WD-XRF)

X-ray fluorescence analysis is a powerful nondestructive technique used for the quantitative and qualitative analysis of an element. XRF can detect elements from beryllium (Be) to Uranium (U). In this analysis technique, X-rays of sufficient energy are allowed to fall on the samples and they in turn interact with the atoms in the sample and in the process emits characteristic X-rays corresponding to the elements in the sample. A detector measures the peaks of the emitted X-rays and from this data the elements and their quantity can be calculated [26, 27].

![Figure 2.5: Schematic representation of a WD-XRF](image)

An XRF measures the wavelength, energy and intensity of the photons emitted from a sample. Rhodium is used as the standard anode material. A
Experimental and Characterization Procedures

proportional counter is used as the detector. Throughout the study, WD-XRF of make: S4 PIONEER, is used to check the purity of the sample.

2.3.5 Thermo Gravimetric Analysis and Differential Temperature Analysis (TGA/DTA)

TGA/DTA measurements are used for compositional analysis, decomposition temperature, heat of transition, stability of the prepared sample, flammability studies, transition temperature detection etc. In TGA analysis, the relation between changes in weight and changes in temperature of a sample is recorded whereas in DTA analysis, the change in temperature between a sample and a reference sample as a function of time is studied.

The basic parts of thermal gravimetric analysis equipment consist of a sensitive recording analytical balance, a furnace, a temperature controller and a program to plot mass as a function of temperature. As the temperature is increased, the various components of the sample gets decomposed leading to the evolution of volatile products which in turn changes the weight of the sample. A plot of temperature against weight loss provides the result [28, 29]. The TGA/DTA measurement, to find the thermal stability of zinc ferrite, was done using a Perkin Elmer, Diamond TG/DTA, STA 6000.

2.3.6 Fourier Transform Infrared Spectroscopy (FTIR)

An FTIR spectroscopy is used to gather information about the bonding and molecular structure of both organic and inorganic molecules. Working of an FTIR is based on the vibrational motion of the chemical bonds in the infrared region. When a material is exposed to IR radiation, the molecules in the ground state get excited to a higher vibrational state. The wavelength of the radiation absorbed depends on the energy difference between the state at rest and the excited state as well as on the characteristic structure of each molecule. A plot of
the transmission/absorption/reflectance intensity of IR radiation of the sample is plotted against its wavelength [30]. An FTIR can be used to analyze samples in various forms like thin film, liquid, solid, pastes, powders, fibers etc.

The basic component of an FTIR is the Michelson Interferometer, which consists of a beam splitter to split the IR radiation, by transmitting half the radiation and reflecting the other half of the radiation falling on it. The transmitted beam passes through the beam splitter and falls on a fixed mirror while the reflected beam falls on to a moving mirror. Both the fixed and the moving mirrors then reflect back the radiation to the beam splitter where they recombine due to interference. The IR radiation after this interaction has information about the unique characteristic of the sample. The information recorded by the detector is then decoded by the fourier transform, a well-known mathematical technique. A computer performs the fourier transform and provides a plot between absorbance/ transmittance and wave number. The peaks in the FTIR spectrum provide information about the different functional groups in the compound. IR spectra of the ferrite nanoparticles in the present study were recorded using a Thermo Nicolet Avatar 370, from 4000cm\(^{-1}\) to 400cm\(^{-1}\). Potassium Bromide (KBr) was used as the binder and all the spectra were corrected for background features arising from air.
2.3.7 UV-Vis Spectrophotometer

The absorption/reflectance spectroscopy in the UV–Vis region is known as Ultraviolet-Visible spectroscopy. The basic working principle behind a UV–Vis spectrophotometer is that it measures the intensity of a light after it passes through the sample (I) and compares it with the intensity before it is incident on a sample (I₀). The ratio of I/I₀ gives the transmittance value which is usually given as (ȠT). The absorbance A is given by the relation [31].

\[ A = -\log(\eta T) \] \hspace{1cm} \text{(2.19)}

According to the Beer–Lambert law, the absorbance of a solution is directly proportional to the concentration of the absorbing substance in the solution and the path length. If ε is the molar absorptivity constant then,

\[ A = \varepsilon c l \] \hspace{1cm} \text{(2.20)}

There are mainly two types of UV spectrophotometers: single beam spectrophotometer and double beam spectrophotometer. In a single beam spectrophotometer all the light passes through the sample and to measure the intensity of the incident light the sample must be removed. This is much cheaper than a double beam spectrophotometer as the system needs fewer parts and is less complicated. The basic parts of a double beam spectrophotometer includes a light source (tungsten light source or a deuterium arc lamp), a sample holder (a transparent cell known as cuvette with an internal width of 1cm), a diffraction grating or a prism to separate the different wavelengths of light and a detector (a photo diode, photo multiplier tube, a photo diode array etc.).
In a double beam spectrophotometer, the beam from the source is separated into single wavelengths using a monochromaters prism or diffraction grating. Each of the single wavelength beam is then split into two beams of equal intensity by a half mirrored device. One beam acts as the sample beam and passes through the sample. The other beam acts as the reference beam and passes through another cuvette containing only the solvent. The intensities of both the beams i.e. the beam passing through the sample (I) and the intensity of the reference beam (I₀) are detected by the detectors and are compared. The range of scanning of a UV-Visible spectrophotometer is normally between 200nm to 800nm.

The absorption coefficient near the band edge is given by [32].

$$\alpha = \frac{A(h\nu-E_g)^n}{h\nu}$$

(2.21)

where A is a constant, E₇ is the band gap, when αhν = 0, E₇ = hν

Tauc plot is the plot between \((ah\nu)^{(1/n)}\) against \((h\nu)\). From the Tauc plot, value of the band gap of a material can be calculated at α = 0. If the
transition is allowed direct transition, then \( n = \frac{1}{2} \), for allowed indirect transition \( n = 2 \), for forbidden direct transition \( n = \frac{3}{2} \), and for forbidden indirect transition \( n = 3 \).

The UV-Vis spectrophotometer, Varian Carry 500, is used to record the UV-Vis spectrum of the ferrite samples, whereas a HITACHI U 3900 UV–Vis spectrometer is used to measure the optical density of the algal cultures.

### 2.4 Magnetic Characterization Technique

#### 2.4.1 Vibrating Sample Magnetometer (VSM)

A Vibrating Sample Magnetometer (VSM) is used to measure the complete magnetic behavior of the material under investigation. The magnetic properties of the material at different temperatures as well as the variation in magnetic characterization as a function of temperature at different applied fields can be studied using a VSM. The basic principle behind the working of a VSM is the Faraday’s law i.e. an emf will be generated in a coil when there is a change in the flux linked with the coil [33, 34].

\[
V = -N_0 \frac{dB}{dt} \quad \text{----------------- (2.22)}
\]

Since \( B = \frac{\phi}{A} \)

\[
V = -N_0 A \frac{dB}{dt} \quad \text{----------------- (2.23)}
\]

where \( A \) is the cross sectional area, \( B \) is the magnetic flux density that is the magnetic flux that passes through the coil, \( N_0 \) is the number of turns, \( \frac{d\phi}{dt} \) is the rate of flux change.
In a VSM setup, the sample is held in a sample holder, which is placed in between the pole pieces of an electromagnet. This sample holder is connected to a transducer. The transducer converts sinusoidal AC signals into sinusoidal vertical vibrations and the sample is thus made to undergo a sinusoidal motion in a uniform magnetic field. A coil is mounded on the pole piece of the magnet to pick up the signals formed from the sample motion. The AC signal produced is proportional to the magnitude of the moment induced in the sample and is also proportional to the vibrational amplitude and frequency. The signal of a particular vibration frequency is fed back to the oscillator where it is compared with the drive signal to maintain a constant drive output. This signal is then phase adjusted and routed to a signal demodulator and acts as the reference signal. The signal from the pick-up coil is also buffered and amplified and is fed to the demodulator. The demodulator synchronously demodulates the signal from the pick-up coil with respect to the reference signal producing a DC analog signal, which depends solely on the magnitude of the magnetic moment. The output gives the relation of the magnetic moment...
Experimental and Characterization Procedures

moment (M) as a function of the field (H). If a cryogenic setup is attached to the sample, low temperature measurements can also be taken. Parameters like saturation magnetization (M_s), Coercive field (H_c), remanence (M_r) and the squareness ratio (M_r/M_s) can be calculated from the hysteresis loop of the sample. In the present investigation, a Lakeshore VSM 7410, is used to analyze the magnetic behavior of the ferrite samples.

2.5 Electrical Characterization Technique

2.5.1 DC Electrical Properties

The DC resistivity of a sample with temperature variation is studied using an electrometer (KETHLEY 6221 DC and AC current source and 2182A nano voltmeter) applying the two probe method.

The DC resistance is measured based on the Ohms law. i.e. the current through a conductor is proportional to the voltage across it,

\[ V \propto I \]  \hspace{2cm} \text{(2.24)}

**Figure 2.9: DC resistivity measurement set up**
Introducing the constant of proportionality R (Resistance)

\[ V = R \times I \]  \hspace{1cm} (2.25)

The electrical resistance R is given by the relation,

\[ R = \rho \times \frac{l}{A} \]  \hspace{1cm} (2.26)

where \( \rho \) is the resistivity, \( l \) and \( A \) are the thickness and area respectively of the pellet [35].

Conductivity \( \sigma \) can be defined as the reciprocal of resistivity and can be calculated using the equation.

\[ \sigma = \frac{1}{\rho} \]  \hspace{1cm} (2.27)

The DC resistivity of the prepared ferrites depends on the preparation technique and its composition [36 – 38]. Ferrites shows semiconducting nature i.e. DC resistivity decreases with increase in temperature [39].

\[ \rho = \rho_0 \exp \left[ \frac{E_A}{K_B T} \right] \]  \hspace{1cm} (2.28)

where \( K_B \) is the Boltzmann’s constant, \( E_A \) the activation energy and \( T \) the temperature. The activation energy can be calculated from the slope of the linear plot between \( \ln (\rho) \) and \( 1/T \) for the sample.

2.5.2 Dielectric Measurements

2.5.2.1 Dielectric Parameters

Ratio of the strength of field in vacuum to the strength of field in the medium for the same charge distribution is known as dielectric permittivity or dielectric constant (\( \varepsilon' \) or \( \varepsilon_r \)). \( \varepsilon' \) depends on various factors like grain size, orientation, temperature, frequency of the applied field and molecular structure of the material [40 – 43].
Here the dielectric measurements of the pellets of the samples are measured using a 6500B Wayner Kerr impedance analyzer.

\[ C = \frac{\varepsilon_0 \varepsilon' A}{d} \]  
\hspace{1cm} \text{------------------------ (2.29)}

where \( A \) is the area of the pellet, \( d \) is the thickness of the pellet, \( \varepsilon_0 \) is the permittivity of free space.

The sample pellet coated with silver paste on both sides acts as a parallel plate capacitor. The capacitance of the parallel plate capacitor is given by,

Real part of dielectric constant \( \varepsilon' \) is the measure of energy stored in the material from the external electric field. Imaginary part of dielectric constant \( \varepsilon'' \), which is also called loss factor, is the measure of energy dissipated as heat in the material by the external electric field.

\[ \tan \delta = \frac{1}{2\pi f R_p C_p} \]  
\hspace{1cm} \text{------------------------ (2.30)}

Where \( R_p \) is the equivalent parallel resistance and \( C_p \) is the equivalent parallel capacitance.
Chapter 2

The Cole–Cole plot can be used for the presentation of dielectric parameters, where $\varepsilon''$ is plotted against $\varepsilon'$. Cole–Cole plot represents a semi-circle for a deby type process [44].

2.5.2.2 AC Conductivity

For a parallel plate capacitor AC conductivity,

$$\sigma_{ac} = \frac{J}{E} \quad \text{---------------------} \text{(2.31)}$$

Where $J$ is the current density and $E$ is the field density; $E$ can be defined as

$$E = \frac{D}{\varepsilon} = \frac{V}{d} \quad \text{---------------------} \text{(2.32)}$$

Where $D$ is the displacement vector of the dipole charges, $\varepsilon$ is the complex permittivity of the material. $V$ is the potential difference between the two ends of the pellet of the capacitor, $d$ is the thickness of the pellet.

Current density $J = \frac{da}{dt} \quad \text{---------------------} \text{(2.33)}$

By Maxwell’s Equation, $q = \frac{Q}{A} = \frac{V}{d} \quad \text{---------------------} \text{(2.34)}$

Where $Q$ is the charge in coulombs due to a potential difference of $V$ volts between the silver coated surfaces of the pellet.

$$J = \frac{da}{dt} = \frac{d}{dt} \left( \frac{V}{d} \right) = \frac{\varepsilon}{d} \frac{dV}{dt} \quad \text{---------------------} \text{(2.35)}$$

$$J = \frac{\varepsilon}{d} V_0 j\omega \quad \text{---------------------} \text{(2.36)}$$

as $V = V_0 \exp(j\omega t)$

Substituting in equation (2.31), equation (2.32) and (2.36)

Now $\sigma_{ac} = \frac{J}{E}$
Hence $\sigma_{ac} = \varepsilon j\omega$  

(2.37)

If $\varepsilon'$ is a complex quantity, $\varepsilon = \varepsilon' - j\varepsilon''$

$$\begin{align*}
\sigma_{ac} &= (\varepsilon' - j\varepsilon'')j\omega \\
\sigma_{ac} &= \varepsilon'j\omega + \omega\varepsilon'' \\
\sigma_{ac} &= \varepsilon'j\omega + \omega\varepsilon''
\end{align*}$$

(2.38)

Considering that $\sigma_{ac}$ is a real quantity,

$$\sigma_{ac} = \omega\varepsilon''$$

(2.39)

where, $\varepsilon'' = \varepsilon'\tan\delta$

Hence, $\sigma_{ac} = 2\pi f\varepsilon'\tan\delta$

(2.40)

Thus AC conductivity can be calculated from the values of dielectric constant and $\tan\delta$ for a given frequency [45, 46].

The total temperature dependent resistivity of a ferromagnetic material is expressed as [47, 48].

$$\rho(T) = \rho_r + \rho_{ph}(T) + \rho_e(T,\omega)$$

(2.41)

here $\rho_r$ is independent of temperature and is known as the residual resistivity, which is due to the presence of impurities and lattice defects.

$$\rho_{ph}(T) = \rho_0\exp\left[\frac{E_a}{k_BT}\right]$$

(2.42)

This is contributed by the electron–phonon interaction and is related to the mobility of charge carriers. Here $E_a$ is the activation energy and $\rho_0$ is the pre exponential factor. $\rho_e(T,\omega)$ is the electron–spin wave scattering contribution related to the dielectric relaxation caused by the localized electric charge carriers. $K_B$ is the Boltzmann’s constant, $T$ is the temperature in Kelvin.
An Impedance analyzer, WAYNER KERR 6500B, was used to analyze the dependence of permittivity, loss tangent and AC conductivity on frequency, temperature and composition for all the samples.

2.6. Nonlinear Optic Measurement Technique (z-scan)

Knowledge of the nonlinear properties of materials is necessary for the design and fabrication of various limiting devices. Various methods were employed earlier for the study of nonlinear properties of materials, like nonlinear interferometry [49], degenerate four wave mixing [50], nearly degenerate three-wave mixing [51], ellipse rotation [52], and the beam distortion measurements [53]. Of these various methods, nonlinear interferometry, degenerate four wave mixing and nearly degenerate three-wave mixing are very sensitive techniques and needs relatively complex experimental setups, whereas beam distortion measurements are comparatively less sensitive but requires detailed analysis. The z–scan technique developed by Sheik–Bahae [54], is a highly sensitive single beam technique used for direct measurement of the magnitude of nonlinear absorption coefficient, as well as the sign and magnitude of nonlinear refraction [54, 55]. This technique has a sensitivity, capable of resolving $\lambda/300$ wave front distortions in $n_2$ measurements using picosecond frequency doubled Nd:YAG laser pulses [54]. This technique can be employed to measure the third order nonlinear properties of solids, liquids and liquid crystals.

The main components of a single beam z-scan setup are a laser source and a beam splitter. The beam splitter splits the incident light into two halves, where one half falls on the sample through a lens and the other half falls on a reference detector. The output light from the test sample is then passed through the aperture and falls on the detector. The transmittance of the sample is measured by the detector $D_2$ as the sample is moved along the propagation direction of a focused Gaussian beam.
There are mainly two types of z-scan techniques, namely closed aperture z-scan and open aperture z–scan. In the closed aperture z–scan technique, the transmitted light is measured by a detector after it is passed through an aperture which is placed in the far field of the focal region [54, 55]. Here the detector light is sensitive to both nonlinear absorption and nonlinear refraction. If the aperture is absent in the experimental setup and the transmitted light is measured without passing through the aperture, it is then an open aperture z-scan setup [55]. Here the transmitted light is sensitive only to nonlinear absorption. Both the closed and the open aperture z-scan graphs are normalized to linear transmittance. The effect of nonlinear absorption in closed aperture z-scan can be cancelled by dividing the closed aperture z-scan data with the open aperture z-scan data. The new set of data obtained is called divided z-scan, and it contains information only about the nonlinear refraction [55]. In our present study our concern is only about the nonlinear absorption coefficient (β), and hence we have used only the open aperture z-scan.

**Open Aperture z-scan**

z-scan measurements are highly sensitive to beam profile and sample thickness. The sample thickness must be much smaller than the diffraction length of the beam (Rayleigh’s Range Z₀)

\[ Z₀ = \frac{kω₀^2}{2} \]  

(2.43)
where \( \omega_0 = \frac{f \lambda}{D} \)

here \( \omega_0 \) is the beam waist radius, \( f \) is the focal length of the lens, \( \lambda \) is the wavelength of the source and \( D \) is the beam radius of the lens. Even a small deviation from the Gaussian profile of the beam or in the sample thickness will show large errors in the result.

Nonlinear absorption is measured by the open aperture z-scan technique. The measurements will show a minimum transmission at the focal point if nonlinear absorption due to Two Photon Absorption (TPA) is present [55]. The transmission increases with increase in the incident intensity and will have a maximum transmission at the focal point if the sample is a saturable absorber [56].

The nonlinear absorption coefficient

\[
\alpha(I) = \alpha + \beta I
\]

where \( \alpha \) is the linear absorption coefficient and \( \beta \) is the TPA coefficient.

The irradiance distribution after the passing through the sample is,

\[
I_r(z, r, t) = \frac{[I(z, r, t)e^{-\alpha l}]}{1 + q(z, r, t)}
\]

where \( q(z, r, t) = \beta l[I(z, r, t)L_{eff}] \)

\( L_{eff} \) is the effective length, \( L_{eff} = \left(\frac{1-e^{-\alpha l}}{\alpha}\right) \) where \( l \) is the length of the sample.

Total power \( p(z, t) \) is obtained by integrating eqn (2.45) from \( z \) to \( r \) and is given by,

\[
p(z, t) = p_i(t) e^{-\alpha l} \left[ \frac{\ln[1 + q_0(z, t)]}{q_0(z, t)} \right]
\]
Experimental and Characterization Procedures

where $p_1(t)$ and $q_0(z,t)$ are,

**Instantaneous input power**

$$p_1(t) = \frac{\pi I_0(t)\omega_0^2}{2}$$

$$q_0(z,t) = \frac{\beta I_0(t)\text{Leff}z_0^2}{Z^2 + z_0^2}$$

For a Gaussian temporal profile, transmission can be given by integrating the equation (2.47)

$$T(z) = \frac{1}{q_0 \sqrt{\pi}} \int_{-\infty}^{\infty} \ln \left( 1 + q_0 e^{-t^2} \right) dt \quad \text{--------------------------- (2.48)}$$

If $|q_0| < 1$, equation (2.48) can be simplified as,

$$T(z, s = 1) = \sum_{m=0}^{\infty} \frac{[-q_0(z,0)]^m}{(m+1)^{3/2}} \quad \text{--------------------------- (2.49)}$$

here $m$ is an integer,

The nonlinear absorption coefficient $\beta$ can be found out from equation (2.46), if we know the value of $q$. Value of $q$ can be easily obtained by fitting the experimental data.

z-scan technique has merits as well as demerits. The merits include simplicity of the technique, ability to interpret both the sign and magnitude of nonlinearity and also the ability to isolate refractive and absorptive parts of nonlinearity. z-scan technique has high sensitivity, which is capable of resolving a phase distortion of $\lambda/300$. But it has few disadvantages too. It requires a high quality Gaussian TEM$_{00}$ beam for measurement. The nonlinear coefficient depends on the spatial and temporal profiles, energy and power content as well as the stability of the laser source.
Chapter 2

2.7 Antialgal Study

2.7.1 Phase Contrast Microscope

The algal cells before and after their interaction with nano particles were observed and photographed by a phase contrast microscope. The working of a phase contrast microscope is based on the principle that, the phase shifts in the light when passing through a transparent specimen will produce changes in the brightness of the image.

Figure 2.12: Phase Contrast Microscope

Phase contrast microscope finds wide application in the field of biology where details of the living cells are undetectable using a normal microscope due to their negligible contrast between the structures or due to the insufficient natural pigmentation. Phase contrast microscope is also used by biologists to study the living cells and their proliferation through cell division.

In a phase contrast microscope, when light passes through a medium, it causes change in the amplitude as well as in phase change, depending upon the
refractive index of the medium. The amplitude change is visible to a human eye, as the change is caused mainly by the scattering or by the absorption of light. This scattering or absorption is wavelength independent and may give rise to colours. But special arrangements are required to make the phase change visible because of the change in medium.

The main parts of a phase contrast microscope include a condenser, which focuses the incident light on to the specimen, and a specimen table where the specimen is kept. The light focused on the specimen will be scattered by it, but some portion of the light will remain unaffected by the specimen and this will form the background light. The phase shift between the scattered light from the sample and the background light will lead to a very small intensity difference between the background and the foreground of the sample resulting in a low image contrast. This image contrast in a phase contrast microscope is increased by two arrangements; a phase shift ring and a gray filter ring. The phase shift ring will introduce a phase shift of $-90^\circ/+90^\circ$ in the background light, either by eliminating the phase difference or by making the background light $180^\circ$ out of phase with the scattered light leading to an increase in the difference of intensity between the foreground and the background lights. The gray filter ring will then again phase shift the scattered light reducing its intensity, whereas the background light will remain unaffected to a great extent and thus further increasing the contrast effect. Thus an image will be formed where the foreground will be darker than the background [57 – 59].

2.7.2 Hemocytometer

The determination of cell concentration is very important in many biological fields like microbiology, cell culture, blood analysis etc.
Chapter 2

Hemocytometer is a simple counting chamber that can be used to determine the cell density. This was invented by a French anatomist Louis–Charles Malassez in the 19th century.

A hemocytometer is a thick glass microscope slide with a grid of perpendicular lines etched in the middle. The dimension of each grid is already known so that we can calculate the number of cells in a specific volume. A cover slip, thicker than the conventional coverslip, is used to overcome the surface tension of the liquid drop. The cover slip is placed over the hemocytometer and the cell suspension is filled by the capillary action that is, by expelling it with a pipette into the V shaped well. It should be made sure that the counting chamber is not over filled by the suspension. The coating grid is then brought into focus by placing the hemocytometer under a microscope. The suspension should be diluted sufficiently so that cells do not overlap.

![Figure 2.13: Hemocytometer](image)

There are certain rules for counting the cells in a hemocytometer. Usually we count the large squares at the four corners and the one in the middle. The cells touching the right and the bottom ruling are normally left out but the cells touching the left and top ruling are counted [59].
The concentration of the cells in the original mixture is given by

\[
\frac{(\text{number of cells counted})}{(\text{proportion of chamber count})(\text{volume of square counted})} \times \frac{V}{v} \quad \text{---(2.50)}
\]

where V is the volume of the diluted sample i.e. the volume of the sample after dilution and v is the volume of the original mixture i.e. the volume of the mixture before dilution.

**2.7.3 Estimation of Photosynthesis Pigmentation**

The Strickland Parson Method [60] is used to measure the chlorophyll content of the algal cells. One ml of the algal culture is withdrawn and is filtered through a 45mm Whatman GF/C filter paper by gentle vacuum filtration. The filter paper containing the algal cells is then introduced into an acid free screw capped test tubes containing 10ml of 90% (v/v) acetone. Then the test tubes are covered with an aluminum foil to ensure protection from light and are kept in a refrigerator for one hour. The clear acetone supernatant is then analyzed using a HITACHI-U 3900–UV Vis spectrometer against 90% acetone as blank for wavelength 750nm, 665nm, 645nm, and 630nm which are the maximum absorption wavelengths of the pigment. The effect of turbidity is
corrected by subtracting the optical density of 750nm from all the other optical densities that is 665nm, 645nm and 630nm absorptions.

The chlorophyll content is then calculated using the equation [61].

\[
\text{chlorophyll } a \left( C_a \right) = 11.85 (O.D \ 665) - 1.54 (O.D \ 645) - 0.08 (O.D \ 630) \quad \text{----- (2.51)}
\]

\[
\text{chlorophyll } a \left( \frac{\mu \text{g}}{L} \right) = \frac{C_a \times v}{V} \quad \text{------------------------ (2.52)}
\]

where \( v \) is the extract volume in milli litre (ml) i.e. the volume of acetone used for extraction and \( V \) is the volume of the sample used for extraction in litre (L).

References

Experimental and Characterization Procedures


Chapter 2


Experimental and Characterization Procedures


