CHAPTER III

CHARACTERISATION OF IRON AND SILVER NANOPARTICLES

3.1 INTRODUCTION

The structural studies of materials always play a prominent role as their physical properties depend very much on their structure. The knowledge about the structure of the materials very is important to understand and predict the nature of the materials. The microcrystalline structure of nanomaterials is found to have a high influence on the optical, electrical, mechanical and other properties.

The iron and silver nanoparticles are known for their tremendous applications in the field of therapeutics and diagnosis. The behavior of nanoparticles is mostly under the control of physiochemical properties and their limits such as size, surface area and surface activity. The high surface-to-volume ratio together by size effects (quantum effects) of nanoparticles introduces huge size assisted phenomena such as chemical, optical, electronic, magnetic and mechanical properties.

There is an ample evidence to show that the particle size and surface chemistry can take control of nanoparticle character dramatically in biological systems based on its acid and base conditions. Hence there is a growing consensus on the necessity of proper and accurate characterisation of silver and gold nanoparticles. For the characterisation of synthesized iron and silver nanoparticles, the following techniques were used.

1. X-ray Diffraction studies (XRD)
2. Scanning Electron Microscope (SEM)
3. Transmission Electron Microscopy (TEM)
4. Energy Dispersive analysis of X-rays (EDAX)
5. Raman Analysis
6. Ultraviolet–visible Spectroscopy (UV analysis)

This chapter deals with the structural, compositional and optical studies of iron and silver nanoparticles prepared by chemical precipitation technique.

3.2 X-RAY DIFFRACTION

X-ray diffraction is one of the most powerful methods for investigation of the internal structure of material. This technique is used uniquely to identify the crystalline phases present in materials and study the structural properties of these phases. The techniques based on X-ray diffraction have dominated the field mainly because of their simplicity, reliability, quantitative and non-destructive nature. The x-ray diffraction technique is non-contact and non-destructive, which makes it ideal for the in situ studies.

The relative ease and convenience, large diffraction angle, representation of the average crystalline lattice throughout the film and simultaneous display of diffraction pattern from the film makes the XRD method a successful analytical technique for the study of thin films.

Bragg’s Law

Diffraction of coherent radiation by the three dimensional array of atoms in a crystal is governed by Bragg’s law (Figure 3.1), which states that the incident beam is reflected by the set of lattice planes (hkl)

Figure 3.1 Schematic diagram for determining Bragg's law
In crystal, the atoms are arranged in different set of parallel planes. When x-ray impinges on a crystal, constituent atoms can diffract the x-rays to form interference pattern. Condition for constructive interference is

\[ 2d_{hkl} \sin \theta = n \lambda \]  

(3.1)

where, ‘\( \theta \)’ is glancing angle of incident x-ray, ‘\( d_{hkl} \)’ is distance between parallel planes from which reflection occurs and ‘n’ is an integer corresponding to the order. Constructive interference occurs only if the incident waves are coherent with definite phase and they must scatter elastically, so that secondary waves will interfere with one another to produce resultant intensity, which is more than the sum total of the individuals. Otherwise destructive interference will occur which results in decrease in intensity.

**X-Ray Techniques**

In X-ray works, one can choose between white and monochromatic radiation. With white radiation “Laue Pattern” is obtained and the lattice planes are easily recognizable. The Laue technique is mainly used to determine the orientation of single crystal. It is a convenient tool because a pattern can be recorded in Polaroid film within a few minutes. X-ray techniques based on monochromatic radiations are generally more important because the ‘d’ spacing can be calculated from the observed diffraction angles. An important feature of X-ray diffractometer is its ability to focus into a sharp diffraction line with the radiation, which is Bragg-reflected from an extended specimen area. This considerably improves the sensitivity and the signal to noise ratio. Focusing is achieved by making the specimen as a part of the circumference of a circle, so called focusing circle, so that all the beams diffracted in different areas by the same family of (hkl) planes, cross over again and are detected on this circle. A design of Bragg – Brentano diffractometer (Figure 3.2) and details regarding formation of X-ray diffraction pattern and focusing method are shown in (Figure 3.3). It is worthwhile to note that for X-ray diffraction a film can remain on its substrate and allow 'nondestructive testing' of the film-substrate system, the‘d’ spacing of films attached to the substrate usually contain contribution of homogeneous strains caused either by intrinsic stresses in the film or by differential thermal expansion if the temperature of the
fabrication and observation are different. One of the main application of X-ray diffractometer technique in thin films is to make use of their good resolution of ‘d’ spacing to investigate strains in thin films Maissel and Glang (1983).

Figure 3.2 Bragg – Brentano focusing on a powder diffractometer

Figure 3.3 Schematic diagram giving details regarding formation of X-ray diffraction pattern and focusing method.

Structural parameters

From the x-ray diffraction profiles, the interplanar spacing \( d_{hkl} \) can be calculated using the Bragg’s relation

\[
d_{hkl} = \frac{n\lambda}{2\sin\theta}
\]  

(3.2)
The crystallite size \( D \) can be calculated from the full width at half maximum (FWHM) using the Scherer’s formula,

\[
D = \frac{K\lambda}{\beta \cos \theta}
\]

(3.3)

where the constant ‘k’ is the shape factor \( \approx 0.94 \), ‘\( \lambda \)’ is the wavelength of the x-rays used (1.541 Å for CuK\( \alpha \)), ‘\( \theta \)’ is the Bragg’s angle and ‘\( \beta \)’ is the FWHM.

From the XRD profiles, the interplanar spacing \( d_{hkl} \) is calculated using the Bragg’s relation

\[
d_{hkl} = \frac{n\lambda}{2\sin \theta}
\]

(3.4)

where ‘\( \theta \)’ is the Bragg’s angle and ‘\( n \)’ is the order of diffraction.

In the present work, the structural properties of the prepared nanoparticles have been studied using PAN analytical x-ray diffractometer with copper K\( \alpha \) (\( \lambda = 1.541 \) Å) radiation and its microstructural parameters have been calculated.

### 3.3 RAMAN SPECTROSCOPY

Raman spectroscopy is a form of vibrational spectroscopy, much like infrared (IR) spectroscopy. A change in the dipole moment of a molecule takes place in IR bands, whereas a change in the polarizability of the molecules takes place in Raman bands. In many cases, the transitions that are allowed in Raman are forbidden in IR; hence, these techniques are often complementary.
Figure 3.4, is the schematic diagram of Raman spectroscopy which describes the process of scattered light. Raman spectra of the prepared samples over the range of 100-1200 cm\(^{-1}\) were collected in back scattering geometry using an Argon ion laser as an excitation source using a Jobi-Yuon Lab Ram Spectroscopy. The laser light was focused on the sample using an objective of an inverted metallographic optical microscope with an analyzed spot size of \(\sim 2\mu\). The scattered light was analyzed using 560 nm focal length grating with a spectral range of 270–1300 nm employing a cooled photomultiplier for photon counting and a cooled CCD detector for spectral acquisition. The optical microscope was also provided with closed loop high resolution x and y scanners for spectral mapping of the sample. A low power laser was used to avoid sample heating.

3.4 SCANNING ELECTRON MICROSCOPE

The Scanning Electron Microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a faster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. The types of signals produced by a SEM include secondary electrons; back scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence) and transmitted electrons.

In a typical SEM (Figure 3.5), an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Other types of electron emitters include lanthanum hexaboride (LaB\(_6\)) cathodes, which can be used in a standard tungsten filament SEM, if the vacuum system is upgraded and Field Emission Guns (FEG), that may be of the cold cathode type using tungsten single crystal emitters or the thermally-assisted Schottky type, using emitters of zirconium oxide. Different specialized detectors are required for their detection and are not usually present on a single machine. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. In the most common or standard detection mode, Secondary Electron Imaging or SEI, the SEM can
produce very high-resolution images of a sample surface, revealing details about 1 to 5 nm in size. Due to the way these images are created, SEM micrographs have a very large depth of field, yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. When the primary electron beam interacts with the sample, the electrons lose energy by repeated random scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 µm into the surface. The size of the interaction volume depends on the electron's landing energy, the atomic number of the specimen and the specimen's density. The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current. The image may be captured as photo from a high resolution cathode ray tube, but in modern machines it is digitally captured and displayed on a computer monitor and saved to a computer's hard disk. Magnification in a SEM can be controlled over a range of about 5 orders of magnitude from × 25 or less to × 250,000 or more. In a SEM, as in scanning probe microscopy, magnification results from the ratio of the dimensions of the raster on the specimen and the raster on the display device. Magnification is therefore controlled by the current supplied to the x, y scanning coils, and not by objective lens power.

Figure 3.5 Schematic diagrams of Scanning Electron Microscopic arrangements
In the present study, SEM images were recorded for the samples using a JEOL JSMS 800-V and JEOL Model JSM - 6390LV scanning electron microscope.

3.5 ENERGY DISPERSIVE ANALYSIS OF X-RAYS (EDAX)

Energy dispersive X-ray spectrometer helps to detect the compositional variation in the specimen. In this technique, an impinging electron or an X-ray photon removes a core level electron of a surface atom. The excited atom decays to a lower energy state through an electron rearrangement in which an additional electron from a higher level is knocked out leaving the atom in a doubly ionized state. The energy difference between the two states is given to the ejected electron, which will have a kinetic energy characteristic of the atom. During EDAX 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.

In order to produce characteristic X-rays from an element it is necessary to apply a suitable critical excitation potential ($E_g = 17.5$ KeV for K radiation for Molybdenum). With an accelerating voltage of $30$ kV, sufficiently intense K radiation can be excited in atoms with mass number $Z$ upto $Z = 40$. For higher atomic numbers L and M radiation can be excited. Once created, the characteristic X-rays are analyzed. This can be done in two methods namely,

- Energy Dispersion (ED)
- Wavelength Dispersion (WD)

In the ED analysis system, a lithium drifted silicon detector is placed close to the sample and receives from it the whole wavelength spectrum of x-rays. The detector discriminates the various energies falling on it and the results are fed to a multi-channel analyzer, which displays the number of quanta corresponding to any particular x-ray energy. Energy dispersive system can simultaneously accept wavelengths from any element.
The resolution of a solid-state detector is about 150 eV. Comparing with WD, the ED is fast and convenient in scanning. The electron probe is focused on the spot of an electron to provide a point analysis. The whole range of X-rays can be collected and analyzed to allow identification of the element in the region of question. The EDAX pattern displays the different peaks corresponding to the elements present simultaneously. From this, it is also possible to determine the concentration of the elements present in the region.

In the present investigation, the presence of the elements is identified using energy dispersive X-ray analysis. Figure 3.6 shows the identification of elements in an EDAX spectrum based on the energy content of the X-rays emitted by their electrons as these electrons transfer from a higher energy shell to a lower energy level. The EDAX spectrum is just a plot of how frequently an X-ray is received for each energy level. An EDAX 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 EDAX spectrum plot not only identifies the element corresponding to each of its peaks, but also the type of X-ray to which it corresponds to. 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 M-shell electrons going to the K-shell is identified as a K-Beta peak.

In the present investigation, present of elements have been identified using energy dispersive X-ray analysis system JEOL Model JED – 2300 and JEOL JSMS 800-V attached to the scanning electron microscope.

3.6 TRANSMISSION ELECTRON MICROSCOPE (TEM)

In a Transmission Electron Microscope (TEM), the electrons from a source such as an electron gun enter the sample and they are scattered as they pass through it, focused by an objective lens, amplified by a magnifying (projector) lens, and finally produce the desired image. Electrons interact much more strongly with matter than do X-rays or neutrons. Much thinner films or particles exhibit too little scattering to provide useful images, and in thick films or materials multiple scattering events dominate, making the image blurred and difficult to interpret. Thick specimens can be studied by detecting backscattered electrons. A transmission electron microscope can form images by the use of the Selected Area Electron Diffraction (SAED) aperture located between the objective and projector lenses. The main part of the electron beam transmitted by the sample consists of electrons that have not undergone any scattering. The beam also contains electrons that have lost energy through inelastic scattering with no deviation of their paths, and electrons that have been reflected by various hkl crystallographic planes. To produce what is called a bright-field image, the aperture is inserted so that it allows only the main un-deviated transmitted electron beam to pass. The bright-field image is observed at the detector or viewing screen.

In the present study to gain further inside in to the microstructure, TEM images were recorded using a Philips TECNAI F20 microscope.

3.7 OPTICAL ABSORPTION AND BAND GAP

Optical photons incident on any material may be reflected, transmitted or absorbed.
During the absorption process one or more of the following process takes place.

1. Photoionization of semiconductor atom: this is excitation of valence electrons to the conduction band. The energy required is greater than or equal to the band gap.
2. Photoionization of impurity atoms: this leads to the transition of electron from the donor level to the conduction band or from the valence band to the acceptor level.
3. Excitation of electrons from the valence band to an imperfection level.
5. Excitation of free carriers to a higher energy state in the same band.
6. Excitation or absorption of phonons.
7. Excitation of crystal vibrations.

Of all these absorption processes the first three directly give rise to photoconductivity. The optical absorption and transmittance studies are useful for the identification of band gap, impurity states, refractive index, extinction coefficient etc. The optical absorption spectra of semiconductors generally exhibit a sharp rise at a certain value of the incident photon energy, which can be attributed to the excitation of electrons from the valence band to the conduction band. The absorption coefficient ($\alpha$) can be estimated from the optical transmittance spectra using the relation,

$$\alpha = \frac{2.303\log(1/T)}{t}$$

where ‘T’ is the transmittance value at a particular wavelength and ‘t’ is the thickness of the sample. The study of absorption coefficient ($\alpha$) of a semiconductor in the fundamental region and near the fundamental edge provides valuable information about inter band transitions and consequently about energy band structure of the material. The absorption of radiation gives rise to transition of electrons from valence band to conduction band. There are two types of transitions (i) direct transition, (ii) indirect transition.
The necessary condition for a direct transition to take place is that in the excitation processes no change in the k value (momentum vector) of the electron should occur. This occurs when the conduction band minimum and valence band maximum occurs at the same value of k. The transition in which a change in crystal momentum occurs is known as indirect transition. The minimum of the conduction band and the maximum of valence band occur at different values of k for an indirect transition. In this case absorption of both photon and phonon or the absorption of a photon and emission of a phonon takes place. The transition probability is given by the relation

\[(\alpha \nu)^p = A (\nu - E_g)\]  \hspace{1cm} (3.6)

where ‘A’ is a constant, ‘E\(_g\)’ is the energy gap, ‘\(\nu\)’ is the frequency of incident radiation and ‘\(h\)’ is Planck’s constant. The exponent ‘p’ is the number which characterizes the transition process,

- P = 2 for direct allowed transitions
- P = 2/3 for direct forbidden transitions
- P = 1/2 for indirect allowed transitions and
- P = 1/3 for indirect forbidden transitions.

The electronic transitions between the valence band and conduction band can be direct or indirect. In both cases, it can be allowed as permitted by transition probability (P) or forbidden where no such probability exists. The band gap is determined by plotting \((\alpha \nu)\) against photon energy \(\nu\). For suitable value of ‘p’ the graph is a straight line and the value of the band gap (\(E_g\)) is obtained by extrapolating the linear portion of the graph to intercept the photon energy axis. The optical method provides a very simple way of finding the band gap as compared to the electrical method using the thermal excitation which is less reliable because of the fact that the effective mass of electron and holes also influence most of the electrical properties.

In the present investigation, the optical absorption spectra of the samples were recorded using spectrophotometer. The optical absorption spectra has been recorded
from 190nm to 2500 nm wavelength using a UV-VIS-NIR spectrophotometer (JASCO V-570) at room temperature using unpolarised lights from deuterium and tungsten lamps which are used at near normal incidence. This spectrophotometer is a double beam system with single monochromator with the wavelength range of 190 nm to 2500 nm and accuracy of ± 1.5 nm. This spectrometer has PbS photocell detector, selectable anywhere in a wavelength range. Since the spectrophotometer is interfaced with computer the recorded spectrum is obtained directly from the computer.

3.8 LITERATURE SURVEY

3.8.1 Literature Survey for Iron Nanoparticles

Alina Mihaela Prodan et al., (2013) have studied the XRD patterns of the iron oxide particles. The 2θ values of the peaks are compared with the standard data for γ-Fe₂O₃. Diffraction peaks at (220), (311), (400), (422), (511) and (440) are readily recognized from the XRD patterns in the sample. The XRD peaks of iron oxide nanoparticles have been indexed to the spinel cubic lattice type with a lattice of 0.835nm. This value of the lattice parameter is in agreement with the values of the standard data (JCPDS no. 4-755). The refinement of XRD indicated that no other phase except the maghemite are detectable. The XRD showed a finite broadening of the diffraction lines. The particle size calculated using the Scherer formula is found to be around 7.7nm. TEM image clearly showed that the product is entirely composed of iron oxide nanoparticles with spherical morphology and with very uniform size distribution. The average grain size of the mono-disperse nanoparticles is 7±0.2nm. The selected area electron diffraction (SAED) pattern has been recorded from an area containing a large number of nanoparticles and a high-resolution TEM picture. The rings in the SAED pattern have been indexed as the (220), (311), (400), (422), (511) and (440) reflections of the cubic maghemite.

Ameer Azam et al., (2012) have analyzed the XRD patterns of the ZnO, CuO, and Fe₂O₃ nanoparticles annealed at 400°C. The peak positions of the samples exhibit the hexagonal, monoclinic, and rhombohedral structures of ZnO, CuO, and
Fe₂O₃, which were confirmed from the International Centre for Diffraction Data card numbers 80-0075, 80-1916, and 85-0987, respectively. Furthermore, no impurity peaks were observed in the XRD patterns, as all of the three metal oxides showed single-phase sample formation and the crystallite sizes were calculated to be 18 nm, 22 nm, and 26.1 nm for ZnO, CuO, and Fe₂O₃ nanoparticles, respectively. Average particle sizes obtained from TEM images were found to be 19.89 ± 1.43 nm, 29.11 ± 1.61 nm, and 35.16 ± 1.47 nm for ZnO, CuO, and Fe₂O₃ nanoparticles, respectively. The average particle sizes determined by TEM images were very close to the crystallite size calculated from XRD results. Thus, the TEM results correlate well with XRD results. FTIR spectra were recorded in solid phase using the KBr pellet technique in the region of 3500–400 cm⁻¹. FTIR spectra of all three metal oxide (ZnO, CuO, and Fe₂O₃) nanoparticles exhibited vibrations in the region 400–600 cm⁻¹, which can be attributed to the vibrations of M–O (M = Zn, Cu, and Fe) which confirms the formation of ZnO, CuO and Fe₂O₃ nanoparticles. A weak band at around 2300 cm⁻¹ may be attributed to the vibrations of atmospheric CO₂. In the case of Fe₂O₃, the bands appearing at 1632 cm⁻¹ can be attributed to the angular deformation of water δH−OH, while the band appearing at 3436 cm⁻¹ can be assigned to the O−H stretching of water.

Selvarani and Prema (2013) showed that the XRD pattern of synthesized metal nanoparticles and it shows that they are in amorphous stage and in tetragonal system. The XRD pattern clearly showed the crystalline nature of nanoparticles. The diffraction peaks at 2θ value of 38.51°, and 44.80° correspond to the (111) and (110) reflection planes of copper and iron respectively. The diffraction intensities were compared with the standard JCPDS files. Crystallite size of the nanoparticles was calculated from the line broadening of X-ray diffraction peak according to the Debye-Scherer suggested that the resultant nanoparticles should have a very small crystallite size and its size is found to be 44.87 nm (Fe⁰). The SEM image of the synthesized nanoparticles of zero valent iron nanoparticles shows that the particles are spherical in shape. The EDS micrograph explains the surface atomic distribution and chemical composition of nanoparticles. Quantitative measuring results obtained from EDS analysis reflect that 97.07% and 72.11% atom particles were of copper
and iron respectively which confirm the purity of copper and iron metals. The other weaker signals of K, C and O are owing to used precursor salts for the synthesis of nanoparticles.

Narendra Chandrasekar et al., (2013) have synthesized iron particles and the spectra showed a peak at 328 nm typical to the peak of zero-valent iron nanoparticles. The size and shape of the synthesized iron oxide nanoparticles was determined using FESEM. These can be due to the denaturation and aggregation during the sputtering process. As the temperature rises, the chitosan polymer aggregates and forms micron sized aggregates. This can be avoided by use of the calcination process where the polymer is completely evaporated and only the iron particles remain. This will eliminate the possibility of encapsulation of the drug into the matrix of the polymer.

Saba et al., (2012) have discussed the SEM image of synthesized zero-valent Iron Nanoparticles (NZVI) indicating that the synthesized NZVI particles are almost spherical. They are evenly distributed spherical particles approximately 2µm in size, and under higher magnification, confirm the spherical shape and the size range of each particle. On the spherical particles there were threads-like or tube-like structures clearly visible. These structures increased the available surface area of reaction. The X-ray diffraction patterns of the as-prepared NZVI samples are amorphous, as no diffraction peaks appear. The spectrum shows two major diffraction intensity peaks at 2θ = 36.08° and 41.01°. The peaks were identified to originate from the (1 1 1) and (2 0 0) planes of FeO respectively (JCPDS no: 772355). The X – ray could be indexed to the Fm$\bar{3}$m (225) face group (Face – centered) cubic structure, with cell parameter a = 4.309 Å. The information of the particle size was obtained from the full width at half maximum (FWHM) of the diffracted beam results in an average crystalline size of 31.1nm ± 0.5. As the annealing time or temperature increases, the crystalline size increases.

Shraddha Shah et al., (2014) have validated Zero valent iron synthesized by chemical reduction method by using UV-Visible spectroscopic analysis and the
absorbance vs wave length (\(\lambda\)) had been established. The characteristic peaks of zero
valent iron nanoparticles were observed at 292.5 nm. The peak at 20 of 44.250
indicates the presence of \(\text{Fe}^0\) nanoparticles. FTIR techniques provide information
about the vibrational state of adsorbed molecule and hence the nature of surface
complexes. The band at 3,381.80 cm\(^{-1}\) indicates the OH stretching vibration, which
suggests the formation of ferrioxy-hydroxide (FeOOH) layer on \(\text{Fe}^0\) nanoparticles.
The analysis of SEM image of synthesized zero valet iron showed clear image of
\(\text{Fe}0\) nanoparticles which are almost spherical in size and the size of most of the
nanoparticles ranges from 67.48 nm to 113.9 nm and mean particle size of 90.69 nm.
However the percentage of nanoparticles beyond 100 nm is very small.

Nhiem Tran et al., (2010) have discussed the TEM images of the
synthesized PVA-coated Iron Oxide nanoparticles and showed that the size of the
nanoparticles was 9 nm ± 4 nm. The nanoparticles formed necklace-like chains with
a typical length of approximately 100–200 nm. A similar formation was reported in
an earlier study, in which IO nanoparticles were believed to precipitate along the
polymer chain of PVA. The measured average zeta potential was −19 mV. This low
value suggested that the nanoparticle solution was stable mostly because of steric
repulsion but not electrostatic repulsion. The XRD pattern confirmed that the final
product was a mixture of \(\text{Fe}_3\text{O}_4\) and \(\gamma\)-\(\text{Fe}_2\text{O}_3\). The existence of \(\gamma\)-\(\text{Fe}_2\text{O}_3\) was common
because of the oxidation of \(\text{Fe}_3\text{O}_4\) during synthesis.

3.8.2 Literature Survey for Silver Nanoparticles

Maribel et al., (2009) monitored the formation of the silver nanoparticles
using UV-Vis absorption spectroscopy. The UV-Vis spectroscopy revealed the
formation of silver nanoparticles by exhibiting the typical surface plasmon
absorption maxima at 418–420 nm from the UV–Vis spectrum. Comparison of
theoretical (Mie light scattering theory) and experimental results showed that the
diameter of silver nanoparticles in colloidal solution is about 60 nm. We have used
Energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD), transmission
electron microscopy (TEM) and, UV–Vis spectroscopy to is used in the present
study to characterize the nanoparticles obtained. The energy-dispersive spectroscopy
(EDX) of the nanoparticles dispersion confirmed the presence of elemental silver signal and no peaks of other impurity were detected. The average size and morphology of silver nanoparticles were determined by Transmission Electron Microscopy (TEM). TEM photographs indicate that the nanopowders consist of well dispersed agglomerates of grains with a narrow size distribution (40 and 60 nm), whereas the radius of the individual particles are between 10 and 20 nm. The synthesized nanoparticles have been structurally characterized by X-ray diffraction and transmission high-energy electron diffraction (HEED). The peaks in the XRD pattern are in good agreement with the standard values of the face-centered-cubic form of metallic silver (ICCD-JCPDS card no. 4-0787) and no peaks of other impurity crystalline phases were detected.

Anu Kumar, et al., (2013) synthesized silver nanoparticles and analyzed them by UV-Visible spectroscopy, Scanning Electron Microscopy. The SEM analysis has shown that size of silver nanoparticles synthesized from the leaves extract of M.nigra was 200 nm and seems to be spherical in morphology. Morphology of chemically synthesized silver nanoparticles is nearly spherical and of size ranges from 300-500 nm. The average particle size analyzed from SEM analysis was observed to be 350 nm.

Karunakar et al., (2013) used UV spectral analysis and observed maximum absorption spectra within the range of 450nm-470nm which stands as a strong proof to report them as silver nanoparticles. The FTIR analysis was carried out to find out the capping material present in the particles which enabled the synthesis. This analysis gave strong peaks indicating prominent absorption at 3354 cm$^{-1}$, 2125cm$^{-1}$, 1638 cm$^{-1}$, 1370 cm$^{-1}$, 1213 cm$^{-1}$ for leaf extract mediated silver nanoparticles extract mediated silver nanoparticles. The leaf and root representative spectra’s of nanoparticles peaks located at about the band at 3354 cm$^{-1}$ corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2125cm$^{-1}$ corresponds to O-H stretch COOH. The assignment at 1638 cm$^{-1}$ corresponds to N-H bend primary amines. FTIR analysis reveals that the carbonyl group from amino acid residues and proteins has stronger ability to bind metal indicating that the proteins could possibly

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form metal nanoparticles. This indicates that the biological molecules could possibly have the functions group which facilitates the formation of Ag nanoparticles in the aqueous mediums. From the TEM image of silver nanoparticles, it is evident that the morphology and size of silver nanoparticles is spherical which is in agreement with the shape of SPR band in the UV-vis spectrum.

Sohail Yasin et al., (2013) have analyzed the synthesized sample on XRD using a diffractometer equipped for CuKα radiation (λ = 1.5418˚A) in the 2θ range of 0-85˚ with a step size of 0.04˚ and a scanning rate of 5.0/˚min. The comparison of XRD spectrum results with standards confirmed the crystalline nature of silver nanoparticles formed peaks at 2θ values corresponding to 111, 200, 220 and 311 of Bragg reflections for silver metal. The crystallite size of Ag-NPs can be calculated by using Debye-Scherer’s formula. The average size of synthesized Ag-NPs was found to be 13 ± 3.5 nm from XRD data. The structural peaks present in XRD pattern and crystalline size suggests that the bio-synthesized Ag-NPs by bamboo leaves are crystalline in nature. From the TEM images it is evident that the morphology of Ag-NPs are nearly spherical and some non-spherical in nature having size less than 100 nm. It is known that spherical as well as non-spherical (triangle or hexagonal) nanoparticles exhibit better physical properties if they are produced small in size, as the antibacterial properties of silver nanoparticles are size dependent.

Kaushik Roy et al., (2013) discussed the UV-Vis absorption spectrum of ‘Green’ silver nanoparticles in the presence of Grape fruit extract. The SP band in silver nanoparticles solution was found to be close to 450 nm throughout the observation period as the nanoparticles were dispersed in the solution without the possibility for aggregation in UV-Vis spectrum. The high OD of the solution suggests a high conversion of Ag⁺ to Ag⁰ as nanoparticle. Under TEM, the silver nanoparticles synthesized by grape fruit extract were observed to have an average mean size of 18-20 nm corroborating well the DLS pattern. The particles appeared to be spherical in shape with weak crystalline structure.
Florence Okafor et al., (2013) did the analysis of UV-Visible spectroscopy data showed that an appearance of surface plasmon resonance peak (SPR) at the 417-430 nm wavelength range, which corresponds to Ag nanoparticles production. Ag nanoparticles absorb radiation intensely at a wavelength of 400 nm due to the transition of electrons. The exact mechanism of the extracellular biosynthesis of metal nanoparticles in not well understood. It was hypothesized that NADH coenzyme was working as an electron shuttle to neutralize Ag\(^+\) ion. The Ag-NPs was characterized by TEM and AFM which shows particles with spherical shape surrounded by biological molecules, which prevent Ag nanoparticles from aggregation and the average size of Ag nanoparticles is in the 3-9 nm range.

Tanmay and Ramachandraraao (2009) carried out XRD studies for phase identification of the samples prepared. The silver nanoparticles synthesized in gelatin medium show broader diffraction peaks than the particles synthesized in PVA or in aqueous medium. On the whole, XRD results reflect the association of organic molecules with the inorganic phases and their role in maintaining a state of mono-dispersion thus preventing the crystals from growing freely. UV studies were carried out to confirm the reduction of silver nitrate to silver nanoparticles upon the addition of glucose solution. The silver nanoparticles can be identified from the peaks obtained around 400 nm, which is the signature for the silver nanoparticle formation, apart from the color change. Slight shift in the position of the peak can be seen for silver nanoparticles synthesized in different media. This could also be due to the size dependence of the interaction of silver particles with optical radiation resulting in a peak shift. The particles of silver show similar diversities in their morphologies when synthesized in different media. The particles which were synthesized in aqueous media show mostly spherical particles about 20–25 nm in diameter. Aggregates of the particles mostly appear to arise from flocculation.

Rebecca Thombre et al., (2013) have synthesized nanoparticles using SEM, XRD, FTIR, EDS and UV Vis spectroscopy analysis. The reduction of silver ions to nano-silver was monitored and confirmed using UV spectra. After color change was obtained a small aliquot of sample was diluted with distilled water and subjected to
UV analysis. The characteristic peak value for silver nanoparticles is between 400-570nm. The spectrum of the sample was obtained for a wavelength range from 270nm to 570nm. The λ max of the nanoparticles was observed at 470nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles. The silver nanoparticles oscillate when exposed to electromagnetic radiation and this oscillation gives a typical peak value. The SEM image of the nanoparticles represents the topography of the particles has suggesting the presence of roughly spherical silver. The incidence of X-rays on the powdered nanoparticles gives a particular pattern which helps to characterize the nanoparticles as shown in the XRD graph using Scherrer formula. The size of silver nanoparticles synthesized by green synthesis was found to be around 20-50 nm. The FTIR image clearly indicates the presence of proteins.

3.9 RESULT AND DISCUSSION

3.9.1 Results and Discussion for Iron Nanoparticles

3.9.1.1 XRD Analysis

X-ray diffraction provides information about the arrangement and the spacing of atoms in crystalline materials.

Figure 3.7 shows the X-ray diffraction pattern of Fe. The diffraction peak at 20 (degrees) value of 43.63° is indexed as the (110) plane of Fe and corresponds to the body centered cubic structure of Fe with lattice constant a = 2.8541Å, and this is in good agreement with those on the standard card (JCPDS card No. 87-0721). The sharpness of the diffraction peaks suggests that the product is well crystallized. No additional peaks belonging to other iron phase was observed, which indicates good crystallinity and high purity of the prepared Fe nanoparticles.
The crystallite size of Fe is calculated using Scherrer’s equation

\[ D = \frac{K\lambda}{\beta \cos \theta} \]

where, D is the grain size, K is a constant taken to be 0.94, \( \lambda \) is the wavelength of the x-ray radiation, \( \beta \) is the full width at half maximum and \( \theta \) is the angle of diffraction.

The lattice constant (a), d-spacing (d) and crystalline size (D) of the Fe samples are given in the Table 3.1

### Table 3.1 Grain size, d-spacing and lattice parameter of Fe Nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>D (nm)</th>
<th>d (m)</th>
<th>a (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>39.06</td>
<td>2.018x10^{-10}</td>
<td>2.85</td>
</tr>
</tbody>
</table>

#### 3.9.1.2 Raman Analysis

Raman spectra are used to identify the iron oxide phase present in the Fe nanoparticles. Figure 3.8 shows the typical Raman spectrum of the prepared Fe nanoparticles. Vemula Madhavi et al., (2013) have reported the Raman spectrum of Fe nanoparticles relate with work. The Raman spectrum of a Fe nanoparticle has four Raman active bands. The four allowed modes of Fe nanoparticles appear at 208 cm\(^{-1}\), 266 cm\(^{-1}\), 377 cm\(^{-1}\) and 581 cm\(^{-1}\).
3.9.1.3 SEM Analysis

Morphology studies of the synthesized nanoparticles were carried out by using scanning electron microscopy (SEM). Figure 3.9 shows the scanning electron microscope (SEM) image of Fe. The SEM image clearly shows the formation of Fe nanoclusters. It indicates that the particles are very small in size. The surface is observed to be free of cracks or voids.

![Figure 3.9 SEM image of as prepared Fe nanoparticles](image)

3.9.1.4 Compositional Analysis

Energy dispersive X-ray analysis was used to determine the composition of the nanoparticles. Figure 3.10 shows the EDAX pattern of Fe nanoparticles. The
The figure shows a very weak signal from the C along with strong peaks corresponding to Fe. Thus the analysis indicates that the obtained Fe nanoparticles are quite pure.

![EDAX Spectra of Fe nanoparticles](image)

**Figure 3.10 EDAX Spectra of Fe nanoparticles**

### 3.9.1.5 TEM Analysis

Figure 3.11 shows the transmission electron microscope (TEM) image of Fe nanoparticles. It can be clearly seen that the Fe particles have got agglomerated. Using the particle number and particle diameter of the particle in the TEM image the particle size has been calculated. The average particle size of Fe is found to be 41 nm (±0.1nm).

![Transmission electron microscope image of as prepared Fe nanoparticles](image)

**Figure 3.11 Transmission electron microscope image of as prepared Fe nanoparticles**
3.9.1.6 UV-Visible Spectroscopy Analysis

![UV–Visible Spectroscopy Analysis Graph]

Figure 3.12 UV–Vis absorption spectra of Fe nanoparticles

The absorption spectrum of as prepared Fe nanoparticles is shown in Figure 3.12. Absorption band at about 326 nm was observed in the spectra. Narendhar Chandrasekar et al., (2013) have reported the UV-Vis absorption spectroscopy of iron nanoparticles relate with present work. The peak present at 326 nm exists at the range which is typical to the peak of as prepared Fe nanoparticles.

3.9.2 Results and Discussion for Silver Nanoparticles

3.9.2.1 XRD Analysis

Figure 3.13 shows the X-ray diffraction pattern of Ag. The diffraction peaks at 2θ (degrees) values of 38.26°, 44.41°, 64.47° and 77.57° can be attributed to the (111), (200), (220) and (311) crystallographic planes, of the face-centred cubic (FCC) structure of Ag nanocrystals respectively (JCPDS Card No. 89-3722).
The crystallite size of Ag is calculated using Scherrer’s equation

\[ D = \frac{K\lambda}{\beta \cos \theta} \]

where, ‘D’ is the grain size, ‘K’ is a constant taken to be 0.94, ‘\( \lambda \)’ is the wavelength of the x-ray radiation, ‘\( \beta \)’ is the full width at half maximum and ‘\( \theta \)’ is the angle of diffraction.

The lattice constant (a), d-spacing (d) and crystallite size (D) of the Ag samples are presented in the Table 3.2

<table>
<thead>
<tr>
<th>Sample</th>
<th>D (nm)</th>
<th>d(m)</th>
<th>a(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>38.18</td>
<td>2.35x10(^{-10})</td>
<td>4.0755</td>
</tr>
</tbody>
</table>

3.9.2.2 Raman Analysis

Raman spectroscopy is a powerful tool to investigate the structural properties of nanoparticles. Figure 3.14 shows the Raman spectrum of the as prepared silver particles. Jean-Christophe Valmalette et al., (2014) have reported the Raman spectrum of Ag nanoparticles which is close relative to this work. The Raman
spectrum of Ag nanoparticle reveals Raman active bands. The allowed mode of Ag nanoparticles appears at 1051 cm\(^{-1}\).

![Raman spectra of as prepared Ag nanoparticles](image1.png)

**Figure 3.14** Raman spectra of as prepared Ag nanoparticles

### 3.9.2.3 SEM Analysis

Figure 3.15 shows the SEM image of the prepared silver nanoparticles. The SEM image reveals that the silver nanoparticles are of uniform size and have got agglomerated.

![SEM image of as prepared Ag nanoparticles](image2.png)

**Figure 3.15** SEM image of as prepared Ag nanoparticles
3.9.2.4 Compositional Analysis

Energy dispersive X-ray analysis was used to determine the composition of the Ag nanoparticles. Figure 3.16 shows the EDAX pattern of Ag nanoparticles.

![Figure 3.16 EDAX spectra for Ag nanoparticles](image)

The figure shows a very weak signal for C along with strong peaks corresponding to Ag. Thus the analysis indicates that the obtained Ag nanoparticles are quite pure.

3.9.2.5 TEM Analysis

Figure 3.17 shows the TEM image of as-prepared silver nanoparticles. The image shows that silver particles have got agglomerated. The average particle size of Ag is found to be nearly 36 nm.

![Figure 3.17 Transmission electron microscope images of as prepared silver nanoparticles](image)
3.9.2.6 UV-Visible Spectroscopy Analysis

The absorption spectrum of as prepared silver nanoparticles is shown in Figure 3.18. Absorption band at about 458 nm has been observed in the spectra. Maribel et al., (2009) have reported the UV-Vis absorption spectra of silver nanoparticles. The peak at 458 nm is observed at the range typical to the peak of as prepared silver nanoparticles.

![UV-Visible Spectroscopy Analysis of Silver Nanoparticles](image)

Figure 3.18 UV–Vis absorption spectroscopy analysis of silver nanoparticles

3.10 Conclusion

Fe nanoparticles have been prepared by a simple chemical method. X-ray diffraction pattern reveals that Fe nanoparticles exhibit body centred cubic structure. The average particle size of the prepared Fe nanoparticles is found to be 39.06 nm. The Raman spectrum of Fe nanoparticles has been studied and four active Raman bands have been observed.

The surface morphology of the prepared Fe nanoparticles has been studied using scanning electron microscope. Compositional analysis of the prepared nanoparticles has been carried out by EDAX analysis. The TEM studies show that the particle size of Fe nanoparticles is found to be 41 nm. The absorption spectrum of the as prepared Fe nanoparticles has been studied using UV-Visible spectroscopy.
The prepared Fe nanoparticles will be used to study the improvement of hemoglobin content in biological system (pig model).

Ag nanoparticles have been prepared by a simple chemical method. X-ray diffraction pattern reveals that Ag nanoparticles exhibit face-centred cubic structure respectively. The average particle size of the nanoparticles is found to be 38.18 nm. The Raman spectrum of Ag nanoparticles has been studied and the allowed mode of Ag nanoparticles appears at 1051\text{cm}^{-1}.

The surface morphology of the prepared Ag nanoparticles has been studied using scanning electron microscope. Compositional analysis of the prepared nanoparticles has been confirmed by EDAX analysis. The TEM studies show that the average particle size of Ag nanoparticles is 36 nm. The prepared Ag nanoparticles will be used to study the wound healing performance in biological system (rabbit model).