3. Materials and Methodology

3.1. Synthesis of super paramagnetic Fe₃O₄ nanoparticles:

Materials:

1. Ferric Chloride hexahydrate, (LOBA CHEM, AR)
2. Ferrous Chloride, tetrahydrate (LOBA CHEM, AR)
3. Ammonia solution (MERCK, AR)
4. Distilled water

Method:
Super paramagnetic Fe₃O₄ nanoparticles were prepared by co-precipitation of Fe²⁺ and Fe³⁺ with NH₄OH (Liao and Chen, 2001). 0.6 mol/L ferric chloride and 0.3 mol/L ferrous chloride with a molar ratio of 2:1 were dissolved in water. Chemical precipitation was carried out at 25°C under vigorous stirring by adding NH₄OH (17.202 ml NH₄OH in 6.25 ml water). The pH was adjusted to nearly 10. The precipitates were heated at 80°C for 30 min, and then it was washed several times with double distilled water. The sample was then sintered for 2 hours at 200°C.

Block Diagram showing synthesis of Fe₃O₄ nanoparticles.
3.2. *Synthesis of super paramagnetic ironoxide/β-Cyclodextrin nanocomposites:*

**Materials:**
1. Ferric Chloride hexahydrate, (LOBA CHEM, AR GRADE)
2. Ferrous Chloride, tetrahydrate (LOBA CHEM, AR)
3. β-Cyclodextrin (HIMEDIA, AR)
4. Ammonia solution (MERCK, AR)
5. Distilled water

**Method:**
Fe$_3$O$_4$ / β-CD nanocomposites were prepared by reversed phase water-in-oil microemulsion method. 4g β-CD was dissolved in 25 ml double distilled water. 0.6 mol/L ferric chloride and 0.3 mol/L ferrous chloride with a molar ratio of 2:1 were dissolved in water and this solution was added to above solution. Temperature was maintained at 25°C to carry out precipitation under vigorous stirring by adding NH$_4$OH (17.202 ml NH$_4$OH in 6.25 ml water) to adjust pH value to 10. The precipitates were heated at 50°C for 30 min, and then it was washed several times with double distilled water.

*Fig. 3.2(a):* The separation process of β-CD modified Fe$_3$O$_4$ nanoparticles from suspension under an external magnetic field.
3.3. Characterization techniques

3.3.1. X-ray diffraction analysis (XRD)

The crystallinity and size of nanomaterials were determined by using X-ray diffractometer (Bruker Advance) using CuKα radiation. XRD pattern provides information about crystalline phase of the materials as well as the crystallite size. When the required parameters met, the X-rays that get scattered from a crystalline solid interfere constructively, and produce a diffracted beam of light. The d-spacing is calculated using Bragg’s law (Humphreys, 2013).

\[ n\lambda = 2d \sin \theta \]

(1)

Where \( \lambda \) = wavelength of X-ray, \( d \) = inter planar spacing, \( \theta \) = diffraction angle \( n = 0, 1, 2, 3, \) etc.

The average particle size of synthesized materials was obtained from XRD measurement value of FWHM using Debye-Scherrer’s formula.

\[ \text{Scherrer’s equation (D)} = \frac{K\lambda}{\beta \cos \theta} \]

(2)

Where \( K \) is constant (0.9), \( \lambda \) is the wavelength (\( \lambda = 1.54\AA \)), \( \beta \) is full width at the half maximum intensity (FWHM) and \( \theta \) is the half diffraction angle.

The lattice constants for spinel cubic structure are calculated by using the formula

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

The XRD instrument used was PANalytical XPERT PRO X-Ray Diffractometer installed at SAIF lab, Panjab University Chandigarh.

3.3.2. Fourier transforms infrared spectroscopy (FTIR)

FTIR analysis was carried in the wave number range 400 cm\(^{-1}\) to 4000 cm\(^{-1}\) using Infrared spectrophotometer (Perkin Elmer Spectrum 400). The FTIR spectrum
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was obtained using Nicolet 5700 FTIR spectrophotometer installed in SAIF Lab Punjab University, Chandigarh.

3.3.3. Scanning electron microscopy (SEM)

The scanning electron microscopy (Scanning Electron Microscope: LEO 435 VP) studies were made on scanning electron microscope with an accelerating voltage of 20 KV to determine the morphology of particles. It is a technique which images a sample by scanning it using a high-energy electron beam. The electrons then interact with atoms of the sample, thus producing signals which reveal information about the samples' composition, surface topography and other properties such as electrical conductivity. The SEM instrument used was LEO 435 VP installed in IIT ROORKEE, Uttrakhand.

3.3.4. Energy dispersive X-ray analysis (EDX)

EDX is an analytical technique used for the elemental analysis of a sample which utilizes X-rays that are emitted from the specimen when bombarded by the electron beam.

3.3.5. BET studies

The Brunauer–Emmett–Teller (BET) surface areas of SPION and SPION/β-CD were determined using a NOVA 2200e Quantachrome set up over a relative pressure range of 0.05–0.90 using nitrogen as a purge gas.

3.3.6. Transmission electron microscopy (TEM)

TEM produces a high resolution, black and white image from the interaction that takes place between prepared samples and energetic electrons in the vacuum chamber. TEM provide topographical, morphological, compositional and crystalline information. TEM images are able to yield information of surface features, shape, size and structure.
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3.3.7. Optical Studies

The Fe₃O₄ nanoparticles and Fe₃O₄ / β-CD nanocomposites were also characterized using UV- double beam spectrophotometer.

Scanning range for the sample was 200-800nm. Base line correction of the spectrophotometer was carried out by using distilled water as a reference. The UV-Vis absorption spectra of the entire sample were obtained and numerical data were recorded.

The UV-Vis spectral data is used for the determination of optical the band gap (i.e. the difference (between the conduction band energy and the valence band) in case of semi-conductor metal oxide nanoparticles by using the Tauc relation (Seoudi et al., 2012).

\[
\alpha h\nu = A (h\nu - E_g)^n
\]  

(3)
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Where, \( \alpha \) is the absorption coefficient and \( d \) is the thickness of the sample, \( E_g \) is the energy band gap

\[ n \left( \frac{1}{2}, 1, 2 \right) \] is a constant is dependent on the degree of transition,

\[ n = \frac{1}{2} \] For direct band gap semiconductors as \( \text{Fe}_3\text{O}_4 \) nanoparticles

\( h \nu \) is incident photon energy.

The band gap is then evaluated by plotting \( h \nu \) versus \((\alpha h \nu)^2 \) and extrapolating the tangent on the X-axis (Tauc Plots). A suspension of nanomaterials 5mg of sample in 10mL of distilled water was prepared and spectra were recorded.

PL spectrum of SPION/ \( \beta \)-CD nanocomposite was also recorded.

3.4. Magnetic Properties

3.4.1. Vibrating Sample Magnetometry (VSM)

Vibrating sample magnetometer is a device used to measure the magnetic moment and hysteresis loop of nano and bulk materials. A vibrating sample magnetometer (VSM) operates on Faraday's Law of Induction, which states that a changing magnetic field will produce an electric field.

When a magnetic sample is placed in the constant magnetic field, then this field will magnetize the domains in the direction of the magnetic field. The value of the magnetization depends upon the strength of the applied magnetic field.

![Fig. 3.4.1. Vibrating Sample Magnetometer (‘Microsense’EV7) installed at H.P.U Shimla.](image)
3.4.2. Mossbauer spectroscopy

Mossbauer spectrometry provides unique measurements of electronic, magnetic, and structural properties within materials. A Mossbauer spectrum is an intensity of $\gamma$-ray absorption versus energy for a specific resonant nucleus such as $^{57}$Fe or $^{119}$Sn. Mossbauer spectroscopy probes tiny changes in the energy levels of an atomic nucleus in response to its environment. Mossbauer spectroscopy provides information about the oxidation state of Fe and its chemical environment with $^{57}$Co source. Parameters like isomer shift, quadrupolar splitting, magnetic hyperfine coupling constant and line width are obtained. Mossbauer spectra give quantitative information on “hyperfine interactions,” which are small energies from the interaction between the nucleus and its neighbouring electrons. The three important hyperfine interactions originate from the electron density at the nucleus (the isomer shift), the gradient of the electric field (the nuclear quadrupole splitting), and the unpaired electron density at the nucleus (the hyperfine magnetic field).

Fig. 3.4.2. Canberra S-100 Mossbauer spectrometer

3.5. Oil Removal

The oil removal tests were performed according to the analytical procedure established in our laboratory (Souza et al., 2010; Souza et al., 2012; Oliveira et al., 2012). N100 Lubricating oil was used as a model oil to test the adsorption behavior of Fe$_3$O$_4$/β-CD nanocomposites. The oil-absorbent capacity of the Fe$_3$O$_4$/β-CD
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nanocomposites was determined by weight measurements. The process of removal of lubricating oil from water surface was as follow:

Firstly the weight of magnetic nanocomposites was measured \( m_1 \), then 50 mL of water is poured into a beaker and the total mass was determined, in next step a known mass of the oil \( m_2 \) was sprinkled on the water and the magnetic nanocomposites were added into the beaker (containing the water and the oil). After 5 min, the nanocomposites absorbed with oil were then separated from the water surface using a magnet bar approached the mixture, the oil and nanocomposites are magnetically removed and finally the mass of the oil residue was determined \( m_3 \) and the oil separation capacity \( q \) of the nanocomposites was calculated according to Eq. (4).

\[
\frac{(m_2 - m_3)}{m_1}
\]  

(4)

Weighing was carried out with the help of analytical balances and all adsorption experiments were conducted at 25°C.

The oil was removed from the surfaces of the nanocomposites by ultrasonically washing in ethanol for 5 min. After being dried in an oven, the nanocomposites could be reused to separate the water and oil mixture.

3.6. Removal of Malachite Green

The photocatalytic activity of nanoparticles and nanocomposites was studied by degradation of Malachite Green (MG) in natural sunlight. Malachite green (MG) (Color Index No. 42000), also called basic green 4 having IUPAC name 4-[(4-dimethylaminophenyl)-phenyl-methyl]-N,Ndimethylaniline with chemical formula \( C_{23}H_{25}N_2Cl \), is a green crystal powder with a metallic luster, highly soluble in water and ethanol with blue-green solutions (Sarmah and Kumar, 2011).

\[1 \times 10^{-4} \text{ M solution of Malachite Green (Merck, AR) } \lambda_{\text{max}} = 620 \text{ nm was prepared in distilled water. To 200 ml of dye solution 50 mg of sample was added to it. The solution was kept in dark for 1 hour to establish adsorption and desorption equilibrium. The solution was then kept in a double wall cylinder with water circulation, placed on a magnetic stirrer. The absorbance was recorded using a Double]
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beam UV-VISible spectrophotometer (Systronics 2202). The absorbance was recorded after different interval of times up to 3 hours of illumination by natural sunlight at different wavelengths.

The degradation percentage of dye is obtained using relation,

\[
\% \text{Degradation} = \frac{C_0 - C_t}{C_0} \times 100
\]

(5)

Where \(C_0\) is initial concentration of dye before illumination, \(C_t\) is concentration of dye at time \(t\)

It was also determined that whether the degradation of dye followed pseudo first order kinetics by plotting \(\log (C_0/C_t)\) vs. time according to relation (Bai et al., 2011),

\[
\ln(C_0/C_t) = k_{app} t
\]

(6)

Where \(k_{app}\) is the apparent rate constant, \(C_0\) is the concentrations of dye before illumination and \(C_t\) is the concentration of dyes at time \(t\). The apparent rate constant is determined from the slope of the plot. The linear correlation of the plots of \(\ln (C_0/C_t)\) versus time suggests a pseudo first-order reaction.

The effect of pH on degradation was also studied.

3.7. Removal of Bisphenol A

The photocatalytic activity of nanoparticles and nanocomposites was also evaluated by degradation of Bisphenol A in natural sunlight. Bisphenol A having IUPAC name bis (4-hydroxy phenyl) dimethylmethane (BPA) with chemical formula \((\text{CH}_3)_2\text{C(C}_6\text{H}_4\text{OH})_2\), is a colorless solid with a metallic luster, soluble in organic solvents, but poorly soluble in water

For experimental procedure, \(\text{Fe}_3\text{O}_4/\beta\text{-CD}\) nanocomposites (100 mg) were dispersed into 200 ml of BPA aqueous solution with the concentration of 20 mg L\(^{-1}\) and kept in dark for 1 hour. After equilibrium, the degradation was initiated by adding \(\text{H}_2\text{O}_2\) to the above solution. The solution was then kept in a double wall cylinder with water circulation, placed on a magnetic stirrer. The absorbance was recorded using a Double beam UV-VISible spectrophotometer (Systronics 2202). The absorbance was recorded after different interval of times up to 3 hours of illumination by natural sunlight at different wavelengths.
The degradation percentage of dye is obtained using relation given in equation no.(5) and it was also determined that whether the degradation of dye followed pseudo first order kinetics by plotting Log (C₀/Cₜ) vs. time according to relation given in equation no. (6). The apparent rate constant is determined from the slope of the plot. The linear correlation of the plots of ln (C₀/Cₜ) versus time suggests a pseudo first-order reaction.

After 30 minutes of photodegradation GC–MS analysis was carried out with a Thermo Scientific TSQ 8000 Gas Chromatograph – Mass spectrometer. Separation was performed with a capillary column (5% diphenyl/95% dimethylsiloxane). TOC was determined by total organic carbon analyzer (TOC 5000 analyzer, Shimadzu, Japan) after filtration through 0.45 lm membrane filter. Percent TOC removal was used for quantitative characterization of BPA degradation. The chemical oxygen demand (COD) was quantified by the closed reflux method using potassium dichromate as the oxidant under acidic condition. The unreacted oxidant was determined by titrating with ferrous ammonium sulphate using feroin indicator.