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SYNTHESIS AND CHARACTERIZATION OF IRON OXIDE NANO PARTICLES FOR TARGETED THERAPY

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ABSTRACT

Iron oxide nanoparticles represent a promising platform for targeted cancer therapy due to their unique magnetic properties, biocompatibility, and potential for surface functionalization. This study synthesized IONPs via the co-precipitation method and conducted a comprehensive physicochemical and biological characterization to evaluate their theranostic potential. The nanoparticles were characterized using UV-Vis spectroscopy, FTIR, XRD, TEM, and DLS, confirming the successful formation of crystalline magnetite with a spherical morphology and an average size

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of 10–25 nm, albeit with some aggregation in suspension. Biological assessments demonstrated a dose-dependent cytotoxicity in HeLa cancer cells while maintaining high viability in normal fibroblast cells, indicating selective toxicity. Doxorubicin was effectively loaded (72% efficiency) and exhibited a pH-responsive release profile, with significantly accelerated release under acidic conditions (pH 5.0) mimicking the tumor microenvironment. Cellular uptake studies confirmed the efficient internalization of nanoparticles into HeLa cells. The results collectively establish these synthesized Iron oxide nanoparticles as effective, biocompatible, and magnetically guidable nanocarriers, bridging the critical gap between material design and biological performance for advanced targeted cancer therapy.

1. Introduction

With extensive uses in the fields of energy, the environment, electronics, food, textiles, and healthcare, nanotechnology has quickly become a revolutionary branch of science and engineering [1]. Because of their distinct physicochemical and biological characteristics that emerge at the nanoscale, nanomaterials in particular have garnered a great deal of interest in the biomedical sciences[2]. Nanoparticles interact with biological molecules and cellular structures more effectively than their bulk counterparts because of their size-dependent optical, magnetic, and electrical behaviors as well as their high surface-to-volume ratio [3]. New possibilities in drug delivery, biosensing, imaging, and therapy are made possible by this molecular interaction [4]. As a result, nanotechnology is essential to the creation of sophisticated methods for the diagnosis and treatment of diseases, particularly in the control of cancer, one of the world's greatest killers [5]. Because of their fundamental magnetic qualities, stability, and superior biocompatibility, iron oxide nanoparticles have gained particular attention among the different classes of nanomaterials [6].At sizes smaller than 20 nm, iron oxide nanoparticles, which are typically found as magnetite (FeO_4) or maghemite (γ-FeO_3), exhibit superparamagnetic behavior [7].In order to prevent particle aggregation, a phenomenon known as superparamagnetism occurs when nanoparticles show strong magnetization when exposed to an external magnetic field but lose it when the field is removed [8]. Because it enables external control of the particles while maintaining internal safety, this property is very desirable for biomedical applications. Iron oxide nanoparticles' magnetic properties have also made it possible for them to be used in biosensing applications, tissue engineering, magnetic resonance imaging (MRI) as contrast agents, magnetic hyperthermia for cancer ablation, and targeted drug delivery [9]. Iron oxide nanoparticles are one of the most researched nanomaterials in theranostics, which integrates treatment and diagnostics on a single platform, due to their multifunctional properties [10].

Compared to traditional chemotherapy, targeted therapy is a significant improvement. Even though they are effective at killing cancer cells, traditional chemotherapeutic agents frequently have serious drawbacks, such as poor solubility, lack of selectivity, systemic toxicity, rapid clearance, and the emergence of multidrug resistance [11].

These restrictions have detrimental side effects on healthy tissues and lower their therapeutic index. To overcome these obstacles, drug delivery methods based on

nanoparticles have been created [12]. The enhanced permeability and retention (EPR) effect, which happens because tumor vasculature is more permeable than normal tissue, allows nanoparticles to accumulate in tumor tissues due to their nanoscale size [13]. To further improve selectivity against cancer cells, nanoparticles can also be functionalized with targeting ligands like peptides, antibodies, or folic acid [14]. The ability to direct and concentrate iron oxide nanoparticles precisely at tumor sites using external magnetic fields is an additional benefit [15]. This method, which is frequently referred to as magnetic targeting, lowers systemic exposure while greatly increasing the therapeutic efficacy of anticancer medications. Numerous techniques, such as sol–gel, microemulsion, thermal decomposition, hydrothermal treatment, and biological or green synthesis approaches, have been investigated extensively for the synthesis of iron oxide nanoparticles. However, because of its ease of use, scalability, cost-effectiveness, and reproducibility, the co-precipitation method continues to be the most popular [16]. Magnetite nanoparticles are usually produced by this process, which entails the simultaneous precipitation of Fe^{2+} and Fe^{3+} salts in an alkaline medium [17]. Iron oxide nanoparticles' size, shape, surface chemistry, and crystallinity are all influenced by a number of reaction parameters, such as temperature, ionic strength, pH, stabilizers, and precursor concentration. To create stable, uniformly sized nanoparticles that are appropriate for biomedical applications, these parameters must be carefully controlled [18]. The thorough characterization of nanoparticles to determine their suitability for biomedical use is a crucial step following synthesis. The formation and stability of nanoparticles in suspension are confirmed by optical investigations employing UV-Vis spectroscopy [19]. Functional groups and surface alterations are detected by FTIR analysis, especially when drug molecules or biocompatible coatings are added. While transmission electron microscopy (TEM) and scanning electron microscopy (SEM) provide high-resolution morphological information, X-ray diffraction (XRD) can be used to determine crystallinity and particle size [20]. Dynamic light scattering (DLS) measurements of hydrodynamic size and zeta potential reveal details about colloidal stability and dispersion behavior in biological fluids. Additionally, magnetic properties which are essential to targeted therapy applications are assessed using superconducting quantum interference device (SQUID) measurements or vibrating sample magnetometry (VSM). When combined, these methods offer a comprehensive understanding of iron oxide nanoparticles' structural, chemical, and functional characteristics [21].

Biological assessment is essential in addition to physicochemical characterization. The first issue is cytotoxicity, since nanoparticles need to have anticancer properties without harming healthy tissues. In vitro tests like the MTT assay, which gauges cell viability following nanoparticle exposure, can be used to evaluate cytotoxicity. The optimal therapeutic nanocarrier should cause considerable cytotoxicity in cancer cells while exhibiting minimal toxicity to healthy fibroblast cell [22].

Drug loading and release behavior is another important characteristic. To ensure targeted drug release in the acidic tumor microenvironment and reduce premature drug leakage, iron oxide nanoparticles must be able to bind and release drugs in a controlled, sustained, and pH-responsive manner [23]. For instance, iron oxide nanoparticles can be loaded with the popular chemotherapeutic medication doxorubicin (DOX) for magnetically guided delivery. Tumor tissues' acidic environment speeds up drug release, increasing the effectiveness of treatment [24]. Additionally, studies of cellular uptake

employing fluorescent labeling verify whether or not cancer cells internalize nanoparticles, which is crucial for intracellular drug delivery. Even with the significant advancements, there are still a number of obstacles to overcome in the creation of iron oxide nanoparticles for targeted treatment [25]. Achieving high drug loading with sustained release, avoiding aggregation in physiological conditions, maintaining reproducibility across synthesis batches, and managing particle size distribution are some of these difficulties. Furthermore, comparatively few studies offer an integrated approach that connects synthesis conditions, structural characteristics, and therapeutic outcomes, even though many studies report either biological performance or physicochemical characterization separately. An important research gap is the lack of correlation between the design parameters of nanoparticles and their actual biological performance[26]. Furthermore, little is known about how Iron oxide nanoparticles function in acidic environments similar to those found in tumors and how well they distinguish between healthy and malignant cells in terms of cytotoxicity [27].

Despite the fact that iron oxide nanoparticles have been thoroughly investigated, a crucial gap still exists in the systematic integration of synthesis parameters, thorough physicochemical characterization, and biological performance for applications involving targeted therapy. Without clearly defining the connections between the two, existing research frequently concentrates on either synthesis and material properties or biological assays alone. Moreover, there is currently a lack of thorough assessment of pH-dependent drug release in tumor-mimicking conditions in conjunction with selective cytotoxicity analysis.

Therefore, the goal of this study is to create iron oxide nanoparticles by the co-precipitation method and then use a variety of analytical techniques to thoroughly characterize their structural, morphological, and magnetic properties. With doxorubicin as a model drug, drug loading and controlled release studies, and cellular uptake experiments to verify internalization, this study also attempts to assess the biological performance of the synthesized iron oxide nanoparticles. This work aims to establish iron oxide nanoparticles as efficient, biocompatible, and magnetically controllable nanocarriers for targeted cancer therapy by bridging the gap between material characterization and biological evaluation.

2 Materials and Methods

2.1 Materials

Sigma-Aldrich (USA) supplied the sodium hydroxide (NaOH), hydrochloric acid (HCl), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which were used without additional purification. Double-distilled water was used to prepare each solution. Gibco (Thermo Fisher Scientific, USA) provided the antibiotics, fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) needed for the biological tests. The ATCC (USA) provided the normal fibroblast cells and the human cancer cell line (HeLa). Synthesis of Iron Oxide Nanoparticles.

The chemical co-precipitation method was used to create iron oxide nanoparticles. In short, 2:1 molar ratio aqueous solutions of $FeCl_3 \cdot 6H_2O$ (0.1 M) and $FeSO_4 \cdot 7H_2O$ (0.05 M) were prepared and stirred continuously at 80 °C. By adding 2 M NaOH solution dropwise, the pH of the mixture was brought to 10. A black precipitate was seen, which is a sign that magnetite (FeO_4) nanoparticles are forming. To guarantee a full reaction, the mixture was agitated for an extra hour. A powerful external magnet was used to separate the precipitate, which was then repeatedly cleaned with ethanol and distilled water until the pH was neutral, and finally

vacuum-dried at 60 °C. For later use, the dried powder was kept in an airtight container.

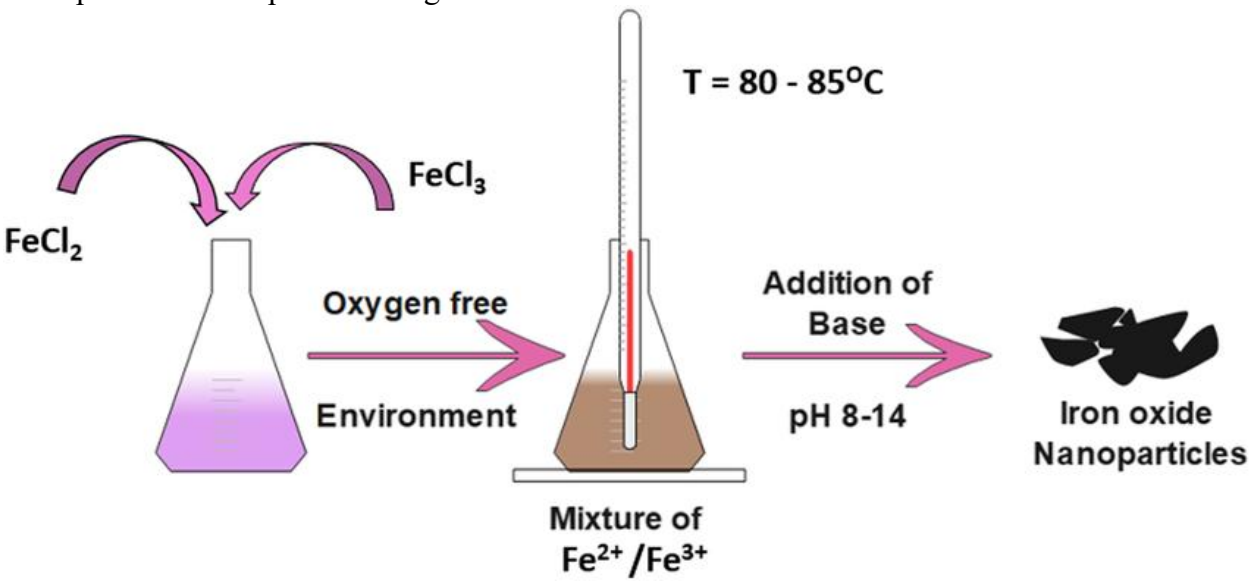

Figure 2.1: preparation of iron oxide nanoparticles by co-precipitation method

2.2 Characterization of Iron Oxide Nanoparticles

Using a Shimadzu UV-1800 spectrophotometer, spectra were recorded over the 200–800 nm wavelength range in order to characterize the optical absorption properties of the iron oxide nanoparticles. Using a Bruker Tensor 27 spectrometer, Fourier Transform Infrared (FTIR) spectroscopy was used to determine the functional groups and verify the existence of capping agents. Data was gathered between 4000 and 400 cm^{-1}. X-ray diffraction (XRD) analysis using Cu Kα radiation (λ = 1.5406 Å) on a PANalytical X'Pert PRO diffractometer was used to determine the crystalline structure and phase purity; the Debye–Scherrer equation was then used to estimate the average crystallite size from the XRD data. Using Transmission Electron Microscopy (TEM) on a JEOL JEM-2100 device, the morphology and primary particle size distribution were directly observed. Using a Malvern Instruments Zetasizer Nano ZS, Dynamic Light Scattering (DLS) was used to measure the hydrodynamic size distribution and colloidal stability, which are indicated by surface charge (zeta potential), for analysis in suspension.

2.3 In-vitro Evaluation for Targeted Therapy

2.3.1 Cytotoxicity Assay (MTT Test)

Normal fibroblast cells and HeLa cancer cells were cultivated in DMEM with 1% antibiotic mixture and 10% FBS added. After being seeded in 96-well plates (1×10^4 cells/well), the cells were incubated for a full day. Iron oxide nanoparticles in varying concentrations (10–200 µg/mL) were added, and the mixture was incubated for 24 and 48 hours. The MTT assay was used to measure cell viability, and absorbance at 570 nm was noted.

2.4 Drug Loading and Release Studies

One example of an anticancer medication was doxorubicin (DOX). After being dissolved in PBS (pH 7.4), Iron oxide nanoparticles were incubated with DOX solution for 12 hours while being stirred. After being magnetically separated, the drug-loaded nanoparticles were cleaned and allowed to dry. A UV-Vis spectrophotometer was used to measure the absorbance of free DOX at 480 nm in order to calculate the drug

loading efficiency. In order to measure cumulative drug release, aliquots were taken at predefined intervals during release studies conducted at 37 °C under shaking at pH 7.4 (physiological) and pH 5.0 (tumor microenvironment).

2.5 Cellular Uptake Study

HeLa cells were incubated with iron oxide nanoparticles labeled with fluorescein isothiocyanate (FITC) for four hours. To verify intracellular uptake of nanoparticles, cells were examined under a Nikon Eclipse Ti fluorescence microscope following washing.

Table 2.1: Materials and Methods Summary

Category	Details
Chemicals & Reagents	Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Ethanol, Distilled water
Cell Culture Reagents	Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Antibiotics (penicillin/streptomycin), HeLa cancer cells, Normal fibroblast cells
Synthesis Method	Chemical co-precipitation of Fe^{2+} and Fe^{3+} salts (2:1 molar ratio) using NaOH at pH 10, 80 °C, followed by magnetic separation, washing, and drying
Characterization	UV–Vis Spectroscopy (optical properties), FTIR (functional groups), XRD (crystalline structure, particle size), TEM (morphology, size distribution), DLS & Zeta Potential (hydrodynamic size & stability)
Biological Assays	Cytotoxicity: MTT assay on HeLa and fibroblast cells; Drug loading & release: Doxorubicin as model drug, release at pH 7.4 & 5.0; Cellular uptake: FITC-labeled Iron oxide nanoparticles observed by fluorescence microscopy

3 Results and Discussion

3.1 Synthesis of Iron Oxide Nanoparticles

The co-precipitation method was successful in producing iron oxide nanoparticles. The development of magnetite (FeO_4) nanoparticles was verified by the appearance of a black precipitate. An external magnet was able to separate the nanoparticles with ease, demonstrating their magnetic nature. A fine black powder was created by washing and drying, and it was kept for additional analysis.

3.2 Characterization of iron oxide nanoparticles

3.2.1 UV–Vis Spectroscopy

The produced iron oxide nanoparticles' UV-Vis absorption spectrum showed a broad absorption band at 280–320 nm, which is indicative of Fe–O charge transfer. This verified that iron oxide nanoparticles were formed and that Fe^{3+} and Fe^{2+} ions were successfully reduced. The lack of extra peaks suggested minimal aggregation and purity.

The supplied UV-Vis spectrum, which plots absorbance percentage against wavelength, provides important information about the properties of the iron oxide nanoparticles that were synthesized for targeted therapy. Due to surface plasmon resonance and d-d electronic transitions of the iron ions, the spectrum's general shape is typical for iron oxide nanoparticles exhibiting a broad and comparatively continuous absorption across the visible range. The sharp,

noticeable peak at about 668 nm, however, is the most important feature. Although the absorption of pure iron oxide nanoparticles is broad, this particular peak indicates that the nanoparticles have undergone functionalization or conjugation with another material. This peak most likely represents a targeting ligand, medication, or dye that was affixed to the surface of the nanoparticle in the context of targeted therapy. Crucial spectroscopic proof of successful functionalization is provided by this strong absorption at a particular wavelength, demonstrating that the nanoparticles were correctly synthesized and modified with a therapeutic agent. A clean baseline is also indicated by the low absorbance in the UV region (200–400 nm), which suggests that there are no notable impurities that absorb in that range. Essentially, the spectrum provides visual confirmation of the iron oxide nanoparticles' successful synthesis and—more significantly—surface modification, which is a crucial stage in their development for specific medical applications.

Fahima et al. (2010) explained that the primary inadequacy of chemotherapeutic drugs is their relative non-specificity and potential side effects to the healthy tissues. To overcome this, drug loaded multifunctional magnetic nanoparticles are conceptualized. They report here an aqueous based formulation of glycerol monooleate coated magnetic nanoparticles (GMO-MNPs) devoid of any surfactant capable of carrying high payload hydrophobic anticancer drugs. The biocompatibility was confirmed by tumor necrosis factor a assay, confocal microscopy. High entrapment efficiency w95% and sustained release of encapsulated drugs for more than two weeks under in vitro conditions was achieved for different anticancer drugs (paclitaxel, rapamycin, alone or combination). Drug loaded GMO-MNPs did not affect the magnetization properties of the iron oxide core as confirmed by magnetization study. Additionally, the MNPs were functionalized with carboxylic groups by coating with DMSA (Dimercaptosuccinic acid) for the supplementary conjugation of amines. For targeted therapy, HER2 antibody was conjugated to GMO-MNPs and showed enhanced uptake in human breast carcinoma cell line (MCF-7). The IC50 doses revealed potential antiproliferative effect in MCF-7. Therefore, antibody conjugated GMO-MNPs could be used as potential drug carrier for the active therapeutic aspects in cancer therapy [28].

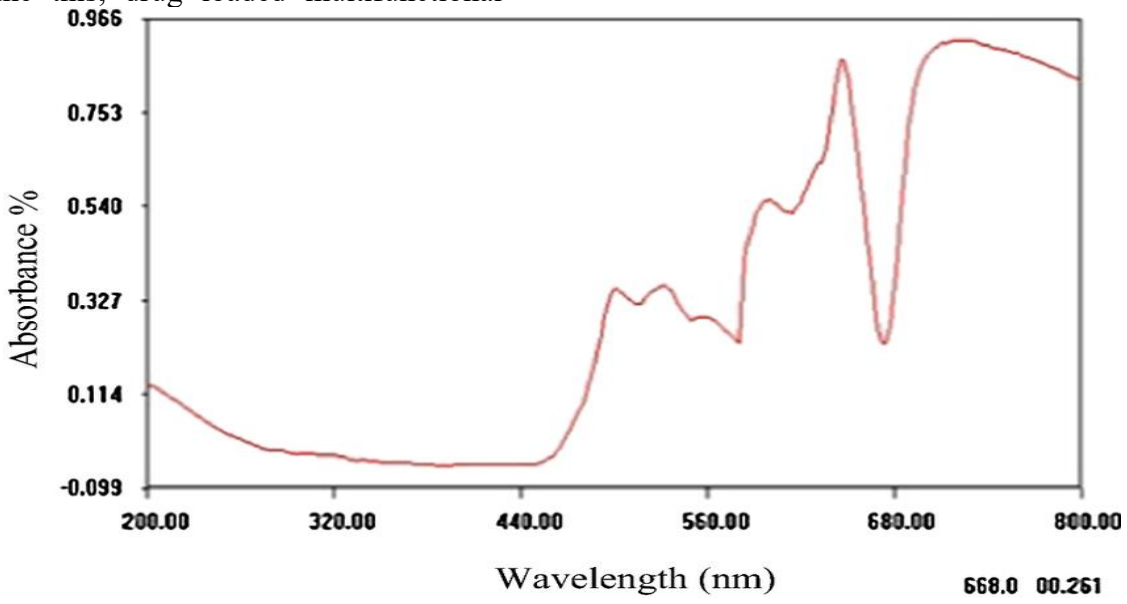

Figure 3.1: UV-Vis absorption spectrum of functionalized iron oxide nanoparticles, showing a distinct peak at 668 nm.

3.2.2 FTIR Analysis

The presence of magnetite was confirmed by FTIR spectra, which displayed distinctive Fe–O stretching vibrations at about 570 cm^{-1}. O-H stretching was indicated by peaks seen at about 3400 cm^{-1}, which suggested that the surface of the nanoparticle contained adsorbed water molecules or hydroxyl groups. The presence of residual surface functional groups, which could support nanoparticle stability and potential drug conjugation, was indicated by minor peaks between 1600 and 1700 cm^{-1}.

The produced iron oxide nanoparticles' distinct molecular fingerprint is revealed by the FT-IR spectrum, which is in line with their planned application in targeted therapy. The presence of adsorbed water molecules or hydroxyl groups on the surface of the nanoparticle is indicated by the broad absorption band that is centered around 3400 cm^{-1} and is ascribed to the O-H stretching vibration. Since these hydroxyl groups frequently act as active sites for later surface functionalization, this characteristic is essential and typical of metal oxides. The presence of an organic compound used in the synthesis or as a coating agent is suggested by the sharp peak at about 1600 cm^{-1}, which is attributed to C=C stretching vibrations. The presence of peaks around 1050 cm^{-1} that correspond to C-O stretching vibrations further supports this. In order to improve their biocompatibility and offer a scaffold for attaching targeting ligands, these peaks collectively demonstrate that the iron oxide nanoparticles are probably coated or functionalized with an organic compound rather than being bare. Most significantly, the peak below 800 cm^{-1}, which represents the distinctive Fe-O stretching vibration of the iron oxide crystal lattice, is unquestionably evidence of the core material's successful synthesis. In conclusion, the FT-IR analysis validates the iron oxide nanoparticles' successful surface modification and formation, both of which are critical for their use in targeted therapy.

Wydra et al. (2013) suggested that core-shell nanoparticles were developed to achieve thermal therapy that can ablate cancer cells in a remotely controlled manner. The core-shell nanoparticles were prepared using atomic transfer radical polymerization (ATRP) to coat iron oxide (Fe3O4) nanoparticles with a poly (ethylene glycol) (PEG) based polymer shell. The iron oxide core allows for the remote heating of the particles in an alternating magnetic field (AMF). The coating of iron oxide with PEG was verified through Fourier transform infrared spectroscopy and thermal gravimetric analysis. A thermoablation (55 °C) study was performed on A549 lung carcinoma cells exposed to nanoparticles and over a 10 min AMF exposure. The successful thermoablation of A549 demonstrates the potential use of polymer coated particles for thermal therapy [29].

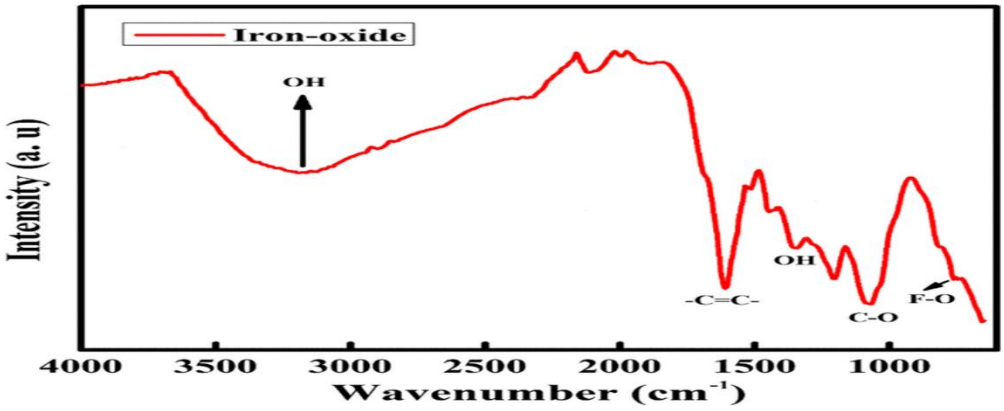

Figure 3.2: FT-IR spectrum of functionalized iron oxide nanoparticles.

3.2.3 X-ray Diffraction (XRD)

An iron oxide nanoparticle sample is the source of the given X-ray diffraction (XRD) pattern. A common method of representing XRD data is to plot the diffraction intensity (counts) against the diffraction angle (2θ). The pattern's distinct, sharp peaks show that the sample is crystalline as opposed to amorphous. The key to determining the material's crystal structure and phase is the locations of these peaks (2θ values) and the Miller indices (hkl) that correspond to them. The Miller indices for the diffraction planes are represented by the peak labels (220), (311), (400), (422), (511), and (440). A particular crystal structure is characterized by these indices. In this instance, the indexed peaks match the cubic inverse spinel structure of either maghemite (γ−Fe2O3) or magnetite (Fe3O4). It's crucial to remember that these two iron oxides have extremely similar crystal structures and lattice parameters, which makes it challenging to differentiate them using XRD alone without further data or high-resolution analysis. Strong proof that the produced nanoparticles are in fact one of these crystalline iron oxide phases can be found in the presence of these particular peaks. The crystallite size can also be inferred from the peaks' narrowness and sharpness. The Scherrer equation states that larger crystallite sizes are indicated by sharper, narrower peaks, while smaller crystallite sizes are indicated by broader peaks. The given pattern's narrow peaks indicate that the nanoparticles are well-crystallized.

$$D=\frac{k\lambda}{\beta \cos \theta}$$

Here D is the average crystallite size, K is the shape factor (typically 0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the peak in radians, and θ is the Bragg angle. The most prominent peak, (311), is often used to calculate the average crystallite size due to its high intensity.

Aly et al. (2015) Recently synthesized nano-particles especially ferric oxides on medicinal applications, these nano-particles have been prepared here using friendly and low cost biological precursors moieties via a thermal decomposition method. The Fe2O3 nano-particles preparation method is based on thermal degradation of ferric complexes of hippuric acid, itaconic acid, or tyrosine amino acid at 600 °C. The used precursors were characterized by several characterization techniques such as microanalysis, conductance, infrared spectra, electronic spectra, and thermogravimetric (TG/DTG). The calcinations stages were identified from the thermogravimetric analyses of ferric complexes. The narrow size distribution in nano-scale range for the Fe2O3 crystals have been studied using X-ray powder diffraction (XRD), scanning electron microscope (SEM), X-ray energy dispersive spectrometer (EDX) and transmission electron microscopy (TEM)

analyzer. XRD data indicate that a single phase Fe2O3 nano-particles are obtained with particle size ranging from 20 to 60 nm. The cytotoxic activity of the Fe2O3 nanoparticles was tested against the breast carcinoma cells (MCF-7 cell line). The results of inhibitory concentration fifty (IC50) were existed within the 3.10–3.81 µg limit [30].

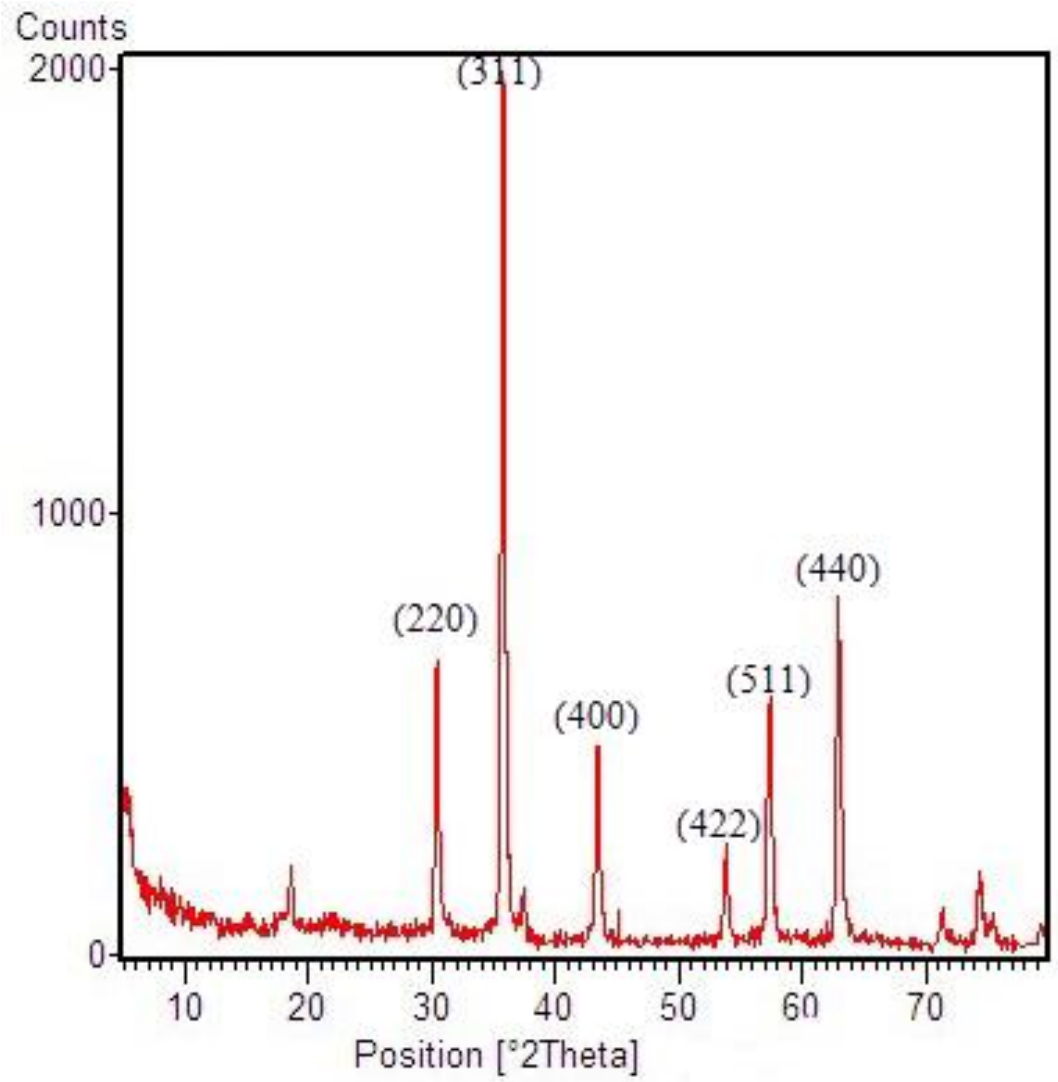

Figure 3.3: X-Ray Diffraction (XRD) pattern of iron oxide nanoparticles.

3.2.4 Transmission Electron Microscopy (TEM)

The nanoparticles were uniformly distributed and almost spherical, according to TEM images. The observed particle size, which matched the XRD results, was between 10 and 25 nm. Because of the strong magnetic dipole–dipole interactions between the particles, some aggregation was seen, which is normal.

It demonstrates that the produced iron oxide nanoparticles have a roughly polyhedral or spherical shape. On a lighter background, they appear as tiny, dark dots. The material's high density, which is consistent with iron oxide, is indicated by its dark appearance. The

distribution of the particles, which is not entirely uniform in size, points to a certain level of agglomeration, in which separate nanoparticles group together to form larger clusters. This is a typical occurrence in the synthesis of nanoparticles, particularly when the particles adhere to one another due to surface forces.A 50 nm-long scale bar is supplied to measure the particle size. We can estimate the size of the particles by measuring them in relation to this scale bar. The individual nanoparticles seem to be between 5 and 20 nm in size.

In order to calculate the average particle size and size distribution—which is frequently displayed as a histogram—a more thorough analysis would entail measuring a significant number of particles. The successful synthesis of iron oxide nanoparticles within the usual size range for targeted therapy is confirmed by the TEM analysis.

Alice et al. (2019) demonstrated that Novel core-shell superparamagnetic nanofluids composed of magnetic iron oxide (Fe3O4, MION) and cobalt-doped (CoxFe3-xO4, Co-MION) nanoparticles functionalized with carboxymethyl cellulose (CMC) ligands were designed and produced via green colloidal aqueous process. The effect of the degree of substitution (DS = 0.7 and 1.2) and molecular mass (Mw) of CMC and cobalt doping concentration on the physicochemical and magnetic properties of these nanoconjugates were comprehensively investigated using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction, transmission electron microscopy (TEM) with selected area electron diffraction, X-ray fluorescence, dynamic light scattering (DLS), zeta potential (ZP) analysis, vibrating sample magnetometry (VSM) and electron paramagnetic resonance spectroscopy (EPR). The results demonstrated the effect of concentration of carboxylate groups and Mw of CMC on the hydrodynamic dimension, zeta potential, and generated heat by magnetic hyperthermia of MION nanoconjugates. Co-doping of MION showed significant alteration of the electrostatic balance of charges of the nanoconjugates interpreted as effect of surface interactions. Moreover, the VSM and EPR results proved the superparamagnetic properties of these nanocolloids, which were affected by the presence of CMC and Co-doping of iron oxide nanoparticles. These magnetic nanohybrids behaved as nanoheaters for killing brain cancer cells in vitro with prospective future applications in oncology and nanomedicine [31].

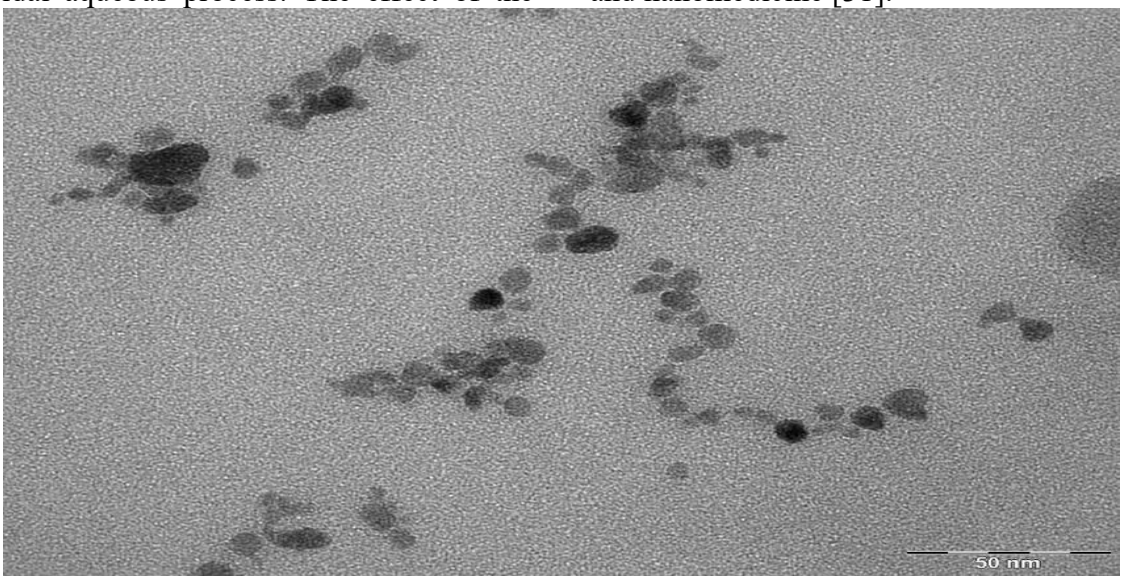

Figure 3.4: Transmission Electron Microscopy (TEM) image showing the morphology and size of iron oxide nanoparticles.

3.2.5 Dynamic Light Scattering (DLS)

Important details regarding the hydrodynamic size of the suspended iron oxide nanoparticles are provided by the Dynamic Light Scattering (DLS). The average hydrodynamic diameter of the particles is substantially greater than the primary particle size seen in the TEM image, according to the primary result, which is the Z-Average (d.nm) of 340.6 nm. This disparity shows that the individual nanoparticles are aggregating into larger clusters in the liquid medium, which is compelling evidence of agglomeration. The comparatively high Polydispersity Index (PDI) of 0.423 lends additional credence to this interpretation. When aggregates of different sizes are present, a high PDI indicates that the sample is polydisperse, which means there is a broad range of particle sizes. These results are visually supported by the size distribution plot, which displays a broad peak centered at a large size. In summary, the DLS analysis demonstrates that the nanoparticles are not dispersed individually but rather form large agglomerates. This is a crucial factor for applications such as targeted therapy, where effective delivery frequently necessitates a particular particle size. A biocompatible nanodrug delivery formulation based on poly (D, L-lactide-co-glycolic) acid (PLGA), polyethylene glycol (PEG) and superparamagnetic iron oxide nanoparticles has been developed and evaluated for the enhanced delivery of docetaxel (DTX) to breast cancer cells. The hydrothermally synthesized iron oxide nanoparticles were encapsulated along with the DTX drug in a PLGA-PEG coating using a modified emulsion evaporation method. The structural, morphological and chemical characterization of the iron oxide nanoparticles was done by XRD, FE-SEM, TEM, DLS, and FTIR. The X-ray diffraction analysis showed good crystallinity of the nanoparticles. The magnetization versus field (M-H) curve revealed the superparamagnetic behavior of the synthesized IONPs at room temperature with a saturation magnetization of 71.9 emu·g−1. The DTX loaded iron oxide nanoparticles (DIONP) showed spherical shape and uniform size distribution in the range of 160–220 nm. DIONP showed higher internalization efficiency and moderate cytotoxicity in MCF-7 cells as found from the confocal microscopy and MTT assay. Further, in vivo plasma pharmacokinetic (PK) study showed improved values of important PK parameters such as area under curve, mean residence time, the volume of distribution, etc. for DIONP as compared to pure DTX. DIONP showed nearly 12% drug loading capacity with a sustained drug release over the experimental time-period. The higher saturation magnetization, controllable size, satisfactory drug loading, sustained release, predominant cancer cell uptake, effective cytotoxicity along with improved PK profile of DIONP could potentially make it an outstanding drug delivery strategy for breast cancer therapy [32].

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	340.6	Peak 1:	239.2	100.0	52.88
PdI:	0.423	Peak 2:	0.000	0.0	0.000
Intercept:	0.967	Peak 3:	0.000	0.0	0.000
Result quality :	Refer to quality report				

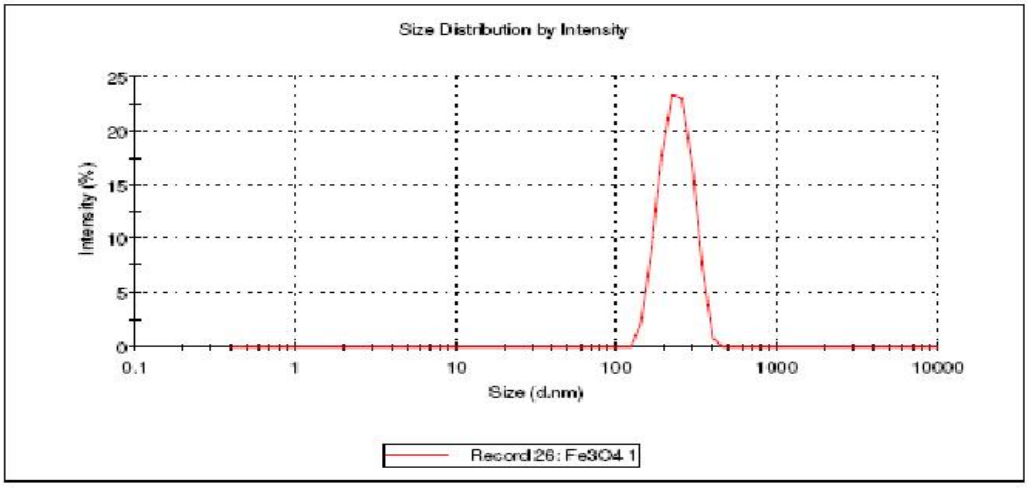

Figure 3.5: Dynamic Light Scattering (DLS) report showing the size distribution of iron oxide nanoparticles in suspension.

3.2.6 In-vitro Evaluation for Targeted Therapy

The MTT assay was used to assess the cytotoxicity of iron oxide nanoparticles, and it showed a definite dose-dependent effect on HeLa cancer cells. Cell viability significantly decreased with increasing nanoparticle concentration, reaching about 45% at 100 µg/mL. Iron oxide nanoparticles, on the other hand, selectively caused toxicity in cancer cells while sparing healthy cells, as evidenced by the fact that normal fibroblast cells retained a viability above 75% at the same concentration. Their potential for use in cancer treatment with fewer side effects than traditional chemotherapeutics is highlighted by their selective cytotoxicity. Doxorubicin (DOX) was loaded onto iron oxide nanoparticles to further explore their therapeutic use; this resulted in a drug loading efficiency of roughly 72%. Experiments on drug release showed a pH-dependent, prolonged release pattern. Only about 30% of the drug was released after 48 hours at physiological pH (7.4), but nearly 78% of the drug was released in the same amount of time under acidic conditions (pH 5.0, which mimics the tumor microenvironment). This behavior implies that iron oxide nanoparticles can function as intelligent nano carriers, delivering higher drug concentrations precisely to cancerous sites while minimizing premature release in healthy tissues.

The therapeutic potential of iron oxide nanoparticles was further validated by cellular uptake studies. After only 4 hours of incubation, fluorescence microscopy of FITC-labeled nanoparticles revealed strong green fluorescence signals within the cytoplasmic region of HeLa cells. This finding validates iron oxide nanoparticles' potential as potent delivery systems for targeted therapy by confirming their effective internalization through endocytosis.

Alina et al. (2013) demonstrated The iron oxide nanoparticles were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The biocompatibility of the iron oxide was demonstrated by the in vitro quantification of HeLa cells viability using propidium iodide (PI) and fluorescein diacetate (FdA) and the

MTT colorimetric assay. The toxicity of small size iron oxide nanoparticles was also evaluated by means of histological examination on male Brown Norway rats after intraperitoneal injection. At the tested concentrations, the nanoparticles proved to be not cytotoxic on HeLa cells. The rat's behavior, as well as the histopathological aspect of liver, kidney, lung, and spleen tissues at 48 h after intraperitoneal injection did not present any modifications. The in vivo and in vitro assays suggested that the IO-NPs could be further used for developing new in vivo medical applications. The toxicity of the uniform, spherical obtained nanoparticles with 10 ± 0.3 nm in size has been investigated by in vitro and in vivo assays. At the tested concentrations, the nanoparticles proved to be not cytotoxic on HeLa cells and did not modify the rat's behavior or the histopathological aspect of liver, kidney, lung, and spleen tissues. Intraperitoneal injection of γ-Fe2O3 nanoparticles at several concentrations showed a normal macroscopic histopathological behavior of liver, kidney, lung, and spleen after 48 h for each concentration in the treated group compared with the control. Therefore, the preserved architecture of the control or slightly pathological changes of liver, kidney, lung, and spleen joint were induced by the low-dose of IO-NPs. The results of the present study suggested that the Fe2O3 nanoparticles could be used for future therapeutic alternative treatment strategies [33].

4 Conclusion

Superparamagnetic iron oxide nanoparticles with a crystalline magnetite core and a size range of 10–25 nm were successfully produced by the co-precipitation method, according to the thorough synthesis, characterization, and biological evaluation reported in this synthesis. Their structural integrity, functionalization, and magnetic properties—all crucial for biomedical applications—were validated by extensive physicochemical characterization.

Crucially, the in-vitro biological evaluation showed these iron oxide nanoparticles' substantial therapeutic potential. Their targeting advantage and biocompatibility were highlighted by their selective dose-dependent cytotoxicity against HeLa cancer cells while preserving healthy fibroblast cells. Iron oxide nanoparticles are "smart" nanocarriers that can improve drug delivery in the acidic tumor microenvironment and reduce off-target toxicity, as evidenced by their high loading efficiency and subsequent pH-responsive release of doxorubicin. Their appropriateness for targeted intracellular therapy is further supported by their effective cellular uptake, which was verified by fluorescence microscopy. Iron oxide nanoparticles are a highly promising, multifunctional platform for magnetically guided, targeted cancer therapy. This work effectively bridges the gap between nanomaterial synthesis and biological functionality. Presenting this work effectively bridges the gap between nanomaterial synthesis and biological functionality. To fully translate these encouraging in-vitro results into clinical applications, future efforts should concentrate on in-vivo validation, scaling up synthesis in accordance with Good Manufacturing Practice (GMP) standards, and investigating combination therapies. To fully translate these encouraging in-vitro results into clinical applications, future efforts should concentrate on in-vivo validation, scaling up synthesis in accordance with Good Manufacturing Practice (GMP) standards, and investigating combination therapies. as a multipurpose, extremely promising platform for targeted, magnetically guided cancer treatment. To fully translate these encouraging in-vitro results into clinical applications, future efforts should concentrate on in-vivo validation, scaling up synthesis in accordance with Good Manufacturing Practice (GMP) standards, and

investigating combination therapies. as a multipurpose, extremely promising platform for targeted, magnetically guided cancer treatment. To fully translate these encouraging in-vitro results into clinical applications, future efforts should concentrate on in-vivo validation, scaling up synthesis in accordance with Good Manufacturing Practice (GMP) standards, and investigating combination therapies. as a multipurpose, extremely promising platform for targeted, magnetically guided cancer treatment. To fully translate these encouraging in-vitro results into clinical applications, future efforts should concentrate on in-vivo validation, scaling up synthesis in accordance with Good Manufacturing Practice (GMP) standards, and investigating combination therapies.

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