

UNVEILING THE MAGNETIC AND BIOPHYSICAL PROPERTIES OF $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NANOPARTICLE AND ITS INTERACTION WITH BOVINE SERUM ALBUMIN

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

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(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Mithrinthaa S (39230026) who carried out the project "UNVEILING THE MAGNETIC AND BIOPHYSICAL PROPERTIES OF $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NANOPARTICLE AND ITS INTERACTION WITH BOVINE SERUM ALBUMIN" under our supervision from January 2023 to May 2023.

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I, Mithrinthaa S (39230026) hereby declare that the Project report entitled **"UNVEILING THE MAGNETIC AND BIOPHYSICAL PROPERTIES OF $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NANOPARTICLE AND ITS INTERACTION WITH BOVINE SERUM ALBUMIN"** done by me under the guidance of Dr. SUDHA. S and Dr. KUNAL BISWAS at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of a Bachelor of Technology degree in Biotechnology.

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ABSTRACT

The creation of nano drugs is one of nanotechnology's most promising uses in the realm of medicine, which it has completely transformed. Typically, smaller than 100 nanometres in size, nanoparticles are used to encapsulate drug molecules to create nano-drugs. A form of nano drug that has received a lot of attention is magnetic nanoparticles because of their potential medical uses. The use of magnetic nanoparticles as a flexible and efficient medication delivery, hyperthermia, and medical imaging tool has enormous potential. The superparamagnetic nature of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Magnetic Nanoparticles has been shown, with high saturation magnetization, great biocompatibility, outstanding colloidal stability, high specific absorption rate, and a monodisperse nature. The crystal structure, particle size, and magnetic properties were investigated by XRD, FTIR, UV, Raman spectroscopy and DLS techniques. In this study, Bovine serum albumin (BSA) was coupled with $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nanoparticles (NPs). The ability to quickly quantify BSA using $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs with protein corona was analysed by SDS Page and Barford Assay a variety of methods, including dynamic light scattering, zeta potential, and FTIR, were used to validate the effective conjugation of BSA to $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs. Additionally, cytotoxicity tests were carried out, and the results demonstrate that neither $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs nor their conjugates had any harmful effects on the evaluated cells. A crucial component for effective drug delivery applications, cell uptake experiments revealed that conjugating BSA to $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs improved cellular uptake by HepG2 cells.

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LIST OF ABBREVIATION

AFM	Atomic force microscopy
BBB	Blood-Brain Barrier
BSA	Bovine Serum Albumin
CA	calcification appears
CD	Circular Dichroism
CNS	Central nervous system
CLIO	Cross-linked iron oxide
Co	Cobalt
CoFe ₂ O ₄	Cobalt ferrite Oxide
CuSO ₄	Copper(II) sulfate
Co _{0.5} Zn _{0.5} Fe ₂ O ₄	Cobalt zinc ferrite
Dia	Dynamic image analysis
DCFH-DA	Dichlorodihydrofluorescein diacetate
DNA	Deoxyribonucleic acid
DLS	Dynamic light scattering
Fe ₂ O ₃	Ferric oxide
Fe ₃ O ₄	Magnetic oxide
FWHM	Full width at half maximum
FTIR	Fourier Transform Infrared
Gd	Gadolinium
HepG2	Hepatoblastoma cell line
HDD	Hydro-dynamic diameter
HSA	Human Serum Albumin
IC ₅₀	Half-maximal inhibitory concentration
IONPS	Iron nanoparticles
KDa	Kilodaltons

MW	Molecular weight
MNMs	Magnetic nanomaterials
MnFe ₂ O ₄	Manganese ferrite Oxide
MNPs	Magnetic nanoparticle
MRI	Magnetic resonance imaging
MTC	Medullary thyroid cancer
Mn	Manganese
MEIO	Magnetism-engineered iron oxide
MTT	- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
Ni	Nickel
NiFe ₂ O ₄	Nickel ferrite Oxide
NPs	Nanoparticles
PSA	Particle size analyser
RES	Reticuloendothelial System
ROS	Reactive oxygen species
SPIONs	Superparamagnetic iron oxide nanoparticles
SDS page	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
TDD	Targeted drug delivery
TGA	Thermal Gravimetric analysis
TEG	Tri ethylene Glycol
UV	Ultraviolet
Vol	Volume
WBC	White blood cells
Zn	Zinc

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CHAPTER 1

INTRODUCTION

In fields ranging from medicine to physiology, nanotechnology and nanoengineering have the potential to significantly advance science and technology. They can be characterised by synthesis, design, characterization, and application of science and engineering (Silva, 2004). Metallic, semiconductor, and polymeric nanoparticles are the three categories into which nanoparticles can be divided based on the kind of substance. There are two metallic nanoparticles (that is gold and magnetic nanoparticles), in which magnetic nanoparticles are widely employed in medicinal applications. (Liu, 2006) The catalysts, ferrofluids, recording mediums, and biomedical materials all contain magnetic nanoparticles as their active ingredient. Magnetic nanoparticles are also employed as research tools in the fields of materials physics, biology, and medicine in addition to being the subject of significant research in the various technological applications (Soler & Paterno, 2017a).

Two key characteristics of nanoparticles (NPs) determine their magnetic properties finite-size effects (single-domain, multi-domain structures, and quantum confinement) and surface effects, which result from the crystal structure's symmetry breaking at the particle's surface oxidation, dangling bonds, and surface stain. Surface effects become more relevant when particle size reduces, and the ratio of surface atoms to core atoms increases. It is generally known that some magnetic properties, such as magnetic anisotropy, the magnetic moment per atom, Curie temperature, and the coercivity field of NPs, might differ from those of bulk material. (Obaidat *et al.*, 2014).

The desired size of the nanoparticles is typically between 10 and 50 nm in the majority of medicinal applications, a nanoparticle often exhibits superparamagnetic behaviour in this size range by unifying into a single magnetic domain to minimise its magnetic energy when the temperature is higher than a specific level known as the blocking temperature. For loading medicines or adsorbing proteins, MNPs have a high surface-to-volume ratio. Also, they have unique superparamagnetic characteristics that can react to a magnetic field outside of them. MNPs are readily

distinguishable from the mixture's components with the use of magnets. As a result, they can be used for separation processes (e.g., cell separation). Target delivery of genes and drugs is made possible by the superparamagnetic feature of MNPs since they can be precisely accumulated in a desired spot by applying an external field(X. Li et al., 2016).

Studies have been conducted on the interactions between magnetic nanoparticles and biomolecules, mostly for therapeutic purposes, medication administration, contrast imaging agents, antibacterial agents, and bio-separations. It is generally known that biomolecules cover nanoparticles in biological mediums, creating a "corona" that changes the physicochemical characteristics of the nanoparticles and endows them with biological features. Hence, the area where classical forces including electrostatic, van der Waals, covalent, hydrophobic, and steric interactions occur between a biological entity, the medium, and a nanoparticle surface is known as the "bio-nano interface." The protein corona has been the focus of extensive study because of its importance in the pharmacological activities of nanoparticles. The other three primary types of biomolecules lipids, polysaccharides, nucleic acids, and small molecules, which are similarly crucial to the biotech sector, are rarely studied(Abarca-Cabrera *et al.*, 2021).

The huge biomacromolecules known as proteins are made of peptides, which are covalently bonded chains of the amino acid building blocks. The interaction of bio-macromolecular proteins with magnetic nanoparticles has received the most research attention. MNPs and protein interactions for bio-separation and other application sectors, including their properties. These compounds are known to quickly adhere to surfaces after being incubated with them via electrostatic contacts, hydrogen bonds, hydrophobic interactions, and coordinative bonds. The properties of the nanoparticle should be examined first when analysing the interaction. The surface chemistry, including charge and hydrophobicity, influences protein binding more depending on the nanoparticle's composition (material, shape, and size). The size and surface charge of the nanoparticulate system, for example, vary after binding, as do other physicochemical characteristics. The adsorption of some proteins is influenced by the surface charge. Spectroscopy methods can be used to analyse these interactions(Hirsch *et al.*, 2013).

The field of biomedicine has some intriguing applications for magnetic nanoparticles $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$. They are smaller than or comparable to the dimensions of a cell, a virus, a protein, or a gene due to their controlled sizes, which range from a few nanometres to tens of nanometres. They can 'get close' to an interesting biological thing, in other words. they can have biological molecules added to them to cause them to engage with or bind to a biological entity, giving rise to a manageable method of "tagging" or addressing it.

The nanoparticles can be controlled by an external magnetic field gradient since they are magnetic, which implies they follow Coulomb's law. Many applications involving the transport and/or immobilisation of magnetically tagged biological or magnetic nanoparticle entities are made possible by this "activity at a distance" as well as the intrinsic penetrability of magnetic fields into human tissue. It is possible to get the magnetic nanoparticles to react resonantly to a magnetic field that changes over time, favourable outcomes in the energy transfer from the stimulating field to the nanoparticle. For example, the ability to heat the particle makes them useful as hyperthermia agents, providing lethal levels of heat energy to certain body parts like tumours, or as enhancing agents for chemotherapy and radiation. Wherein modest tissue heat causes more efficient malignant cell death. The unique physical characteristics of magnetic nanoparticles enable these and a wide range of additional potential uses in biomedicine applications (Barcena *et al.*, 2009).

Magnetic nanoparticles' (MNPs') toxicity can change based on several variables, including size, surface charge, surface coating, and concentration. MNPs are generally non-toxic and biocompatible when utilised within specific parameters. $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ MNPS toxicity is dependent on a variety of parameters, including size, shape, structure, surface modification, concentration, dosage, biodistribution, bioavailability, solubility, immunogenicity, and pharmacokinetics, it is crucial to understand this phenomenon. The huge surface-to-volume ratio may facilitate interactions with various organ components if inhaled, absorbed, or consumed. Moreover, the capacity to traverse cell barriers and tolerance to biodegradation boost the hazardous potential among these nano-entities. In toxicology research, the underlying mechanism of acute and chronic toxicity is should consider. In animal and cellular studies, the destruction of particle compounds, any ensuing stimulation of defence mechanisms, inflammation, long-

term toxicity, metabolism, and elimination (Malhotra *et al.*, 2020). Several metabolic processes can cause MNPs to become hazardous, involving the diffusion-based generation of reactive oxygen species (ROS), and protein coordination during endocytosis as a result of metal ion release and membrane depolarization.

CHAPTER 2

LITERATURE SURVEY

Magnetic nanomaterial plays a vital role in biomedical research and application. Nanomaterial less than 100 nm has unique properties like high reactivities and surface volume ratios. It has been compared to the bulk micron structure counterpart. Magnetic nanoparticles have several biomedical applications, for example, cellular therapy is involved in cell targeting, and labelling provides a tool in cell biology research to analyse the cell, target drug delivery, tissue repair, MRI (magnetic resonance imaging), and hyperthermia for cancer treatment (Tran & Webster, 2010). Efficient treatment high saturated magnetization is used such as Fe, Co, Ni or metal oxides like Fe_3O_4 , Fe_2O_3 . High saturation magnetization is possessed by pure metal; it is highly toxic and susceptible to oxidation (Tran and Webster 2010). It gives a more significant application in SPIONs and promising results in biological applications (Spoiala *et al.*, 2021).

2.1 PROPERTIES OF MAGNETIC NANOPARTICLE

MNPs are made with the property of strong magnetic and magnetized properties, such as materials enclosed with iron, nickel, cobalt, and alloy derived from the mixture of magnetic metal and oxide. Iron and its oxide play a vital role in the environment among all MNMs. For example, ferromagnetic material has a high magnetic susceptibility at room temperature and is greatly magnetized even in a weak magnetic field. Fe_2O_3 and Fe_3O_4 nanoscale is usually $<20\text{nm}$. They are superparamagnetic materials that can be magnetized; it is exposed and applied in a low-intensity magnetic field externally, with no hysteresis. The magnetization curve immediately decreases to zero when the magnetic field is removed (Zhou *et al.*, 2016) (Ngomsik *et al.*, 2005) (Jang & Lim, 2010) (Thurm & Odenbach, 2002). Inverse spinel structure of Fe_3O_4 nanoparticle, the divalent transition metal ions known as Mn^{2+} , Co^{2+} , and Ni^{2+} are doped and furtherly turned into Fe_3O_4 magnetism. As a result, MnFe_2O_4 , CoFe_2O_4 , and NiFe_2O_4 nanoparticles have been used in biomedical applications. Bimetallic and metallic exhibit superparamagnetic or ferromagnetism which gives promising results in magnetic bioanalysis, excellent in

the separation of magnetic and T2 MRI contrast enhancement(Huang *et al.*, 2019)(Lee *et al.*, 2007) (Sun, 2006).

The enchantment character of magnetic is applied in separation and labelling, in magnetic vectors, magnetic heating, hyperthermia, and the enhancement of contrast agents. An MNP is used in both therapeutic and diagnostic applications. MNPs are a prototypical tool for theranostic application, and the magnetic field used as an aid for drug delivery is termed magnetic drug targeting(Dagata *et al.*, 2018).

The MNP character can be evaluated by magnetization measurement as a function of the field ($M \times H$) curve and temperature. This magnetic property depends on nanoparticle arrangement, an intrinsic characteristic of individual particles like crystallinity, chemical composition, stoichiometry, shape, interaction, and magnetic anisotropy. The interparticle exchange has a strong impact, which controls the distance between particles, the design of magnetic nanocomposites with specific properties should be considered. In nanocomposite (via self-assembly), the nanoparticle's volumetric fraction or mass fraction is used to control the interparticle distance. The production of the sample with a variable fraction of nanoparticles is permitted by particle-particle interaction. This method is explored in the study(Papaefthymiou, 2009)(Rebolledo *et al.*, 2008)(Paterno *et al.*, 2012)(Paterno *et al.*, 2009)(Pichon *et al.*, 2011). Susceptibility measurement is used to evaluate the level of interparticle interaction. Dormann and Gitle Mann proposed a model that describes a magnetic susceptibility system composed of particle which does not interact. Considering the inversion of the magnetization orientation of each nanoparticle taken by time, each NP follows Arrhenius's behaviour, using the Arrhenius curve is used to evaluate the frequency of the imaginary component of ac susceptibility(Soler & Paterno, 2017).

Diamagnetic, paramagnetic, and ferromagnetic materials are the three types of magnetic materials. When a diamagnetic substance is placed in magnetic field lines, the substance is compelled to avoid the magnetic field lines and does not attach to the external magnetic field. This is due to the non-uniform magnetic field of diamagnetic material, which transitions from a stronger to a weaker field, creating repulsion. Examples of diamagnetic materials include copper, gold, silver, mercury, argon, and so on. Paramagnetic materials are attracted to the external magnetic

field. The substance is compelled to pass the magnetic field line. This is because the non-uniform magnetic field of paramagnetic material changes from a weaker to a stronger field with weak attraction. Aluminium, chromium, CuSO_4 , MnSO_4 , and alkaline earth metals are a few examples of paramagnetic materials. Ferromagnetic materials have a strong magnetic field attachment. In the specimen, the magnetic lines are compelled to condense. This is due to the non-uniform field of ferromagnetic material, which moves with tremendous attraction from a weaker to a stronger field. Examples of ferromagnetic materials include steel, iron, cobalt, nickel, and gadolinium.

2.2 INTERACTIONS OF MNP WITH BIOLOGICAL SYSTEM

MNPs play a vital role in clinical and biological applications; nano-magnetism is the most challenging research area. Cobalt ferrite is giving promising results in biomedical applications, for example, drug delivery, magnetic hyperthermia, biosensors, and MRI(Rajput *et al.*, 2013)(Baldi *et al.*, 2007)(Sajjia *et al.*, 2010) in CNS diseases MNP, so promising results in target administration. For example, chemotherapy used in brain tumours is helpful for gene therapy, or malignant cells are destroyed by mechanical force by nano surgeons(Zhao *et al.*, 2010)(DAgata *et al.*, 2018). IONPs are the ability to reach the brain through the olfactory neuronal pathway with the help of sensory nerve ending; translocation takes place in the axon bypassing the BBB or direct injection for hyperthermia treatment.

IONPs are deposited in the olfactory bulb, striatum, and hippocampus(Wu *et al.*, 2013) after intranasal instillation, which induces some oxidative stress in the striatum. Fluorescent IONPs is be used in intraperitoneal injection or inhalation and pass-through BBB into the brain. It was documented in fluorescence microscopy and MRI(Petters *et al.*, 2014) (Kim *et al.*, 2006). A sensitive monoisotopic detection system gives promising results in the research area, clinical diagnostics, DNA sequencing, and fundamental molecular biology; it provides new opportunities in automated biological assays and ultrasensitive. Biological staining and diagnostic in fluorescent biosensing probes other their nanoparticle usage. It has two groups of specific interaction methods to conjugate the MNP with nucleic acids. The first one is on binding DNA/ RNA nanoparticles recognized by molecular. The second

one is on chemical reduction and electrostatic interaction, where the interaction is less specific in this type. Design MNPs are formed in the desired field of application. MNP-DNA interaction follows: nanoparticle surface in the DNA molecule is oriented, or aligned molecules have a mutual position of nanoparticles. Nature bonding between DNA and MNP results in a change in Ph value, the addition of ionic strength, or the addition of compound according to the desired field of application. DNA molecules bind nanoparticles through electrostatic interaction with the surface. Synthesis of the magnetic(Tiwari *et al.*, 2015) nanoparticle surface properties is based on electrostatic interaction.

Strong-oriented and particular binding biomolecules to nanoparticles can be achieved using surface-functionalized MNPs. Thiolate and aminated molecules are attained by covalent immobilization. These groups, such as sulfhydryl, carboxyl, amino, and azido, are formed in the surface nanoparticle. Traditional methods form covalent bonds, bioconjugation, and click chemistry applications involved in the thiol-disulfide exchange, carbodiimide activation, aldehyde-amine condensation, azide-alkyne cycloaddition, etc. Different manners blocked the unreacted functional groups in the immobilization of nucleic acids to avoid nonspecific interaction on the surface of MNPs. High-affinity noncovalent interaction biotin-streptavidin or protein aptamers are used to bind the specific MNPs to DNA. The surface of MNPs and nucleic acid needs preliminary modification with appropriate molecules. The complementary binding of oligonucleotides immobilizes MNPs and targets nucleic acid molecules; an operation principle underly certain diagnostic assays. Functionalization of the surface of nanoparticles with double-stranded DNA is intercalated by molecules, paving the way to achieve complex MNPs with DNA. (Ben Djemaa *et al.*, 2019) (Gandhi & Roy, 2019). Magnetism is used in emerging technology in magnetic separation to efficiently separate micrometre-sized Ferro and paramagnetic particles from biological or chemical suspension. Magnetic separation tech is used to purify natural compounds such as nucleic acids, proteins, and cell organelles, cells, and regrowing.

MNP can interact with DNA in a variety of ways, including electrostatic contact, covalent bonding, and assembly on DNA molecules. Numerous processes can result in the creation of MNP-DNA, including the electrostatic coating of the MNP surface by the nucleic acid, high-affinity noncovalent interactions between the

nucleic acid and pre-functionalized surfaces, and complementary hydrogen bond formation between the oligonucleotide molecules (down) or long single-stranded nucleic acid molecules immobilized on MNPs (up) (Tiwari *et al.*, 2015).

Theragnostic holds enormous potential for magnetic nanoparticles. Therefore, it is vital to assess how it interacts with the blood protein BSA to enhance its application in biomedicine. Both spectroscopy (FTIR, UV/visible, CD) and computational methods were used to investigate these relationships. The fluorescence tests showed that the quenching of the fluorescence of BSA by Manganese ferrite NPs was static in character, indicating the binding. The addition of nanoparticles increased the absorbance of BSA due to the creation of a ground-state combination between them, according to UV/Visible spectroscopy. Binding constants from Benesi-Hildebrand plots were equivalent to those from fluorescence investigations. When NPs were added, both FTIR and CD of BSA displayed a change in amide bond bands, indicating that BSA may have partially unfolded as a result of the binding. The molecular docking investigations confirmed once more that NPs and BSA had a stable interaction.

As a result, we now have a better grasp of the potential interactions between NPs and the blood protein albumin, which will help formulate these nanoparticles with medicine for biomedical applications.

2.3 TOXICITY ANALYSIS OF MNP

In the heart, the most common damage with cardiac arrhythmia. In the digestive system, the primary organ affected is the liver. Genotoxicity and hormone imbalance occur in WBCs. Dermal profile damage occurs due to long-time exposure to the nanoparticles. The effect is also observed in the reproductive system, eye, ear, pulmonary injury, and urinary profile. DNA damage in living systems is due to the indirect inducement of cell inflammation and activates oxidative stress known as MNPs. The physicochemical properties of MNPs induce toxicity when it interacts with biological systems.

The principal reason for toxicity is the size of the MNPs. The study suggests liver and spleen are trapped via macrophage phagocytosis when MNPs are more

extensive than 100nm. Compared to rod shape nanoparticles, spherical-shaped nanoparticle shows less toxicity. Increasing intracellular reactive oxygen levels in the host cell is initiated by the toxicity of MPs-toxic effects MNPs such as DNA damage, oxidative stress, and apoptosis. Trapping of the renal spleen port during transportation is reduced by reducing the size of MNPs. Surface coating of nanoparticles plays a significant role in the toxicity MNPs coatings such as polymers, dextran, Pluronic acids, and Polyethylene glycol, resulting in reduced toxicity and improved stability and distribution of nanoparticles. Toxicity of MNPs based on dosage duration of stay in the organ(Jiang *et al.*, 2019)(Schwalbe *et al.*, 2006)(Khanna *et al.*, 2015).

2.4 VARIOUS APPROACHES AND CHALLENGES OF MNPs

Drug delivery is the modern technology for drug targeting; in recent years, drug targeting drastically increased by MNP application. External magnetic field or magnetizable combination with MNPs such as Co-ferrite implant allowed for delivery of the drug to the desired targeted area and fixed in the local site; when the medicine is realized from the local site, it activates the magnetic drug delivery. Eliminate the side effect and reduce the required dosage. It is possible by sending the drug to a specific area. Organic polymer and inorganic metals or oxides required in modifying the surface help in a biocompatible and suitable further attachment, such as attaching various bioactive molecules(Lu *et al.*, 2007)(Jeong *et al.*, 2007) (Faraji *et al.*, 2010).

Complex interaction depends, it is the success of targeting and delivery. The property of complex interaction is applied to MNP and magnetic field, including hydrodynamic condition, MNP concentration, drug-particle binding, target site location, and dept and injection method. Biomedical applications aim to study mechanical and rheological cell properties and cellular function, and recent emerging relies on magnetic actuation of membrane and cellular components on molecular signal pathways(Lubbe *et al.*, 2001)(Issa *et al.*, 2013).

Physical targeting describes localized agents and target areas because the composition, size, or characteristics are not explicitly designed for biological receptors. Targeting, in many cases, is based on the nonspecific accumulation in

some areas related to another site. Localized agents and areas externally can be targeted by physical targeting; specific external applications are ultrasound, heat magnetic field, etc., which help in drug-delivering systems or diseased tissue. It gives promising results in controlled action/ accumulation in the target area or delivering and releasing agents against the target cell or tissue. Site-specific prodrugs are involved in chemical targeting, the localized agent to the target area. The agents use enzymic or chemical reactions; they can direct the site to a chemical reaction. It leads to the agent's control, targeting release, and action. Biological targeting pays the way for an agent to target the area with the help of peptides, antibodies, other compounds, or proteins, as an affinity to organs, another biological target, or receptor sites in a specific manner. Tissue, cell, or another particular promoter in the vector are targeted to localized by gene expression(Singh & Lillard, 2009). Active and passive targets are the two types of targeted drug delivery. Aptamers, antibodies, and ligands are former contained it is functionalized or conjugated to the drug-loaded MNPs.

The drug had nanoparticles guided to the target, which is the object of the site-specific. The retention effect and enhanced permeability or localized body system are possible through this passive target delivery. Retention of the affected part and drug permeability is increased. It is the main advantage of passive drug delivery. Drug-loaded nanoparticle injection is directed to the disease target. This is possible in localized delivery. The motive of targeted drug delivery is a therapeutic technique and treatment. Chemotherapeutic agents in the human body behave like foreign agents, but they deliver the drug to the disease target in the circulatory system. Foreign objects enter living systems such as drug molecule systems, and the thing is removed through the mechanism and sent through the excretory system. The specific dose of the drug become difficult to reach the target disease site and cure the particular disease. A low-dose side effect is drug resistance. A nonspecific chemotherapeutic agent can cause severe damage to human tissue.

Chemotherapeutics has several advantages in targeted drug delivery. MNPs are used, which helps in rectifying the issue because their external magnetic field gives the best results in specific drug delivery. Some advantages of MNP used in targeted drug delivery are target identification, drug delivery only to the affected area, and customizing the drug amount. The concentration of nanoparticles, blood

flow rate, circulation time, drug delivery, and effective treatment are the various factor of contribution. MNPs can influence the external magnetic field. The drug is loaded with MNP and introduced into the circulatory system; the external magnetic field guides this to deliver the drug to a specific point. MNP is loaded with drugs or therapeutic agents according to their biocompatibility; it is used as a carrier or in MTC. MTC is introduced in veins due to its colloidal suspension. The drug moves toward the target site due to the magnetic field gradient detached. Drug delivery is complex. It has to undergo certain factors such as biocompatibility, the physiochemical impact of drug loaded in MNP, size, target distance and dept, magnetic field strength, flow, and vascular blood supply.

All factors influence targeted drug delivery. The great impact of targeted drug delivery can be achieved by optimization of the size and nature of MNPs. MNPs are linked or encapsulated with a conventional therapeutic agent like doxorubicin and methotrexate for effective treatment and delivery of prostate cancer, arthritis, and malignant breast. The typical description of Targeted Drug Delivery (TDD) has been shown in Figure 2.6. MRI is used to monitor drug delivery and targeted drug delivery and disease can be monitored by bio-imaging.(Widder *et al.*, 2019)(McNamara & Tofail, 2015)(Barcena *et al.*, 2009) (Mirza *et al.*, 2020) (Dobson, 2006).

2.5 BIOMEDICAL APPLICATIONS AND CHALLENGES OF MNPs

Therapeutic and diagnostic area biomedical applications play a paramount role. MNMs show a positive impact in biomedical applications due to their applied magnetic field. There is no limitation for penetration in real-time monitoring or gene or drug delivery. Biomolecules or hydrophilic macromolecules linked with several modified routes are developed to improve the biocompatibility and stability of MNMs. MNMs detected target biological entry by MRI, and in research, laboratory imaging-guided photothermal therapy and drug delivery were developed.

In biomedical application, MNMs gives promising results in the research field. MRI is used in clinical applications in addition to chemotherapy and photothermal therapy providing agents. It serves nano-platforms to kill tumour cells(Zhu *et al.*, 2018). This biomedical application can be used in both *in vitro* and *in vivo* conditions, such as injection enclosed with colloidal nanoparticles is introduced into the

bloodstream it's delivered to the target site through magnetic field gradients. Once it reaches the targeted area, it will change into a theragnostic agent. This can be used in diagnosis and therapy(Yoo *et al.*, 2011).

The distinct characteristic of MRI is the imaging modality. It has excellent intrinsic in soft tissue contrast and radiation at a safer level. Hardware technology was recently developed, and a 10ms scan time can be achieved by sub-millimetre resolution new implementation in MRI. For tissue enhancement, the contrast was made using extraneous calcification appears (CA) that shortened the longitudinal (T1) and transverse (T2) relaxation time. Unpaired electrons possessed by the element provide dipole-dipole interaction and magnetic moment; it is used in protons relaxation enhancement. Gadolinium superseded manganese which was used first. The significant relative magnetic moment of gadolinium is due to the several unpaired electrons. Larger molecules are shielding chelate the Gd from direct contact with water protons. Mobility was affected by increased molecule activity, and Gd magnetic moment in the vicinity the protons spend their time. Inner-sphere relaxation theory governs these factors, explaining the T1 and T2 data in low concentrations of paramagnetic calcification.

Paramagnetic was surrounded by electronic spin relaxation and relative motion of proton water, the characteristic of time constant was given to each of its own, for dipole-dipole interaction the necessary fluctuation where provided. The more considerable magnetic moments material, such as ferromagnetic particles and iron oxide or ferrite substituted like Gd, Zn, Mn, etc., can enhance the efficiency of calcification appears (CA). Giant magnetic spin material is exposed when the volume is reduced to the nanoscale. Super para MNPs present the relaxation theory of protons; modifying the idea of paramagnetic calcification appears (CA) can develop the superparamagnetic NP; paramagnetic calcification appearance takes two different media into account. It includes the size and dependence of magnetic moment applied on the magnetic field, magnetic anisotropy presence, the extent of distribution and particle size, and the shell and surface nature of the particle. It is strongly modified by the magnetic properties of the Np and hydrodynamic. An MNP is high in T2 relativity (concentration of MNPs per rate of relaxation) MNP. Its colloidal suspension is considered and used in MRI calcification appears(Wang *et al.*, 2001).

MRI imaging as a magnetic contrast agent consists of metal alloys and oxides. The specific targeting of MNPs ensured the desired tissue for the effective development of a contrast agent. RES eliminates the unwanted MNP structure, but significant challenges are posed by the non-specific accumulation of the spleen, lymph node, and liver. Surface function and target agent were involved in specific targeting. Iron oxide nanoparticle employs contrast agent with or without particular targeting. Tumour cells can be selectively imaged by precise targeting; for example, they are several targeted nanostructures, such as chlorotoxin conjugated Poly ethylene glycol-coated iron oxide particles, anti-carcino embryonic antigen conjugated iron oxide nanoparticle, which is used to enhance the contrast in magnetic imaging. MRI shows more sensitivity *in vivo* by using the MNP. It exhibits high magnetization.

MFe_2O_4 is an iron oxide nanoparticle engineered with a series of metal-doped magnetism, where MFe_2O_4 (M^{+2}) cation of Fe, Co, Ni, or Mn. In mice, the susceptible target was exhibited by Herceptin-conjugated manganese doped magnetism-engineered iron oxide (Mn-MEIO); it shows even the tiny tumour and sufficient sensitive image. Herceptin-conjugated Mn-MEIO and CLIO (cross-linked iron oxide) were injected various times to get an MR image. As a result, Mn MEIO showed colour-changing contrast from red to blue, and time increased for advanced targeting. In CLIO, there is no change observed. High MNP and the targeting agent provide a powerful platform for ultrasensitive cancer detection. Multifunctional nanoparticles exhibit the impact of multifunctional merged with various metals like plasmonic and magnetic (quantum dots). For example, to employ magnetic or optical images, the dumbbell structure is connected with the plasmonic or MNP; the same approach was exhibited in nanostructure for the dual purpose of therapy and imaging (Singamaneni *et al.*, 2011).

Nanotechnology offers promising results in medicine and environmental fields, but this technology has unintended effects on the environment in humans and the natural ecosystem. The advantage of new technology was brought up in health, sustainability benefits, and environments but must be examined before implementation. The technology should undergo specific medical, ethical, legal, and applicable criteria to the climate; MNM monitors and analyses the quality. MNPs

play a vital role in biomedical applications, magnetic energy storage, and spintronics application(Jayavarthanana *et al.*, 2017).

2.6 CHALLENGES ASSOCIATED WITH MNMS

The challenge to be overcome is a large-scale development and issues related to toxicity because be a threat to living systems because they can act as a contaminated carrier and interact with living organisms that can cause a hazardous reaction. Reducing the toxicity is possible by modifying it into biodegradable and biocompatible material. The current challenge of handling MNPs should be considered. The economic issues of MNP are that the adsorption stage is low, and the pollutant removal of contact time is short compared to conventional absorbents; in this case, it is profitable, but MNPs will perform with other materials, such as adsorbents, at low cost. Here, the financial barrier appears. Higher cost of absorbent is used in agriculture and industrial waste. MNPs give promising results in removing hazardous contaminants, and MNPs are environmentally and economically beneficial(Gomez-Pastora *et al.*, 2014).

The recent study is on the safety implication of nanoparticles, but there needs to be more information or result obtained. Radial trouble, which notes is mainly caused by particulate matter. The matter increases by decreasing the diameter of nanoparticles. For example, a dust explosion is caused by a fine particle that causes high surface reactivity. An excellent particle is known as a nanoparticle. The metal's low boiling point can easily be exposed to a dust explosion. In this case, the particle is noted, but in every case, the particle won't tell independently in the form of a single particle. In a dust explosion, particle size increase won't become the solution. Extremely high agglomeration can be caused by fine particles such as 1 μ m or smaller nanoparticles. In some cases, a large particle can cause behaviour conversely. This point is to be taken into consideration when it comes to the implementation of the nanoparticle. The performance of nanoparticles gives promising results in applications like biomedical, information technology, environmental science, energy production, and material. It offers opportunities for new materials to be designed, fabricated, and discarded. But we must consider safety measurements of production, process, and discard. To know the toxicity of

the nanoparticle, we have conducted experiments to analyse the toxicity of the particle in a minimal concentration, with threshold concentration to be standardized in each material. If it is not fixed, it should not be used at any concentration, meaning the engineering process should not be carried out (Makino, 2018) (Li *et al.*, 2003).

CHAPTER 3

AIM AND SCOPE

- To analyze the biophysical character of cobalt zinc ferrite
- To understand the conjugation of cobalt zinc ferrite and BSA
- To evaluate the cytotoxicity effect of BSA and cobalt zinc ferrite
- To determine the influence of cobalt zinc ferrite on BSA in an *in vitro* study
- The possible interactions of NPs with blood protein albumin, which will be helpful to develop these nanoparticles into a drug-loaded formulation for biomedical applications.
- The purpose of the study is to examine the therapeutic use in the treatment of various diseases
- To investigate cobalt zinc ferrite in BSA for their further biomedical use

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS REQUIRED

$\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ were purchased from SRL (Sisco Research Laboratories), a biological reagent (99.99% purity). BSA (Bovine Serum Albumin) were purchased from SRL. All chemicals were used in the present study without further purification. Hepg2 cell line purchased from NCCS, Pune. Double distilled water was used throughout

Table 4.1 Specifications of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ provided by Sisco Research Laboratory

Specification	Results
Appearance (colour)	Brown
Appearance (Form)	Powder
Assay	Min 99.9%
OD	50nm

4.2 METHODS

4.2.1 Physical Characterisation

Physical characterisation of nanoparticles is essential to understanding their properties and behaviour, which can be utilised to optimise their production, processing, and applications.

4.2.1.1 UV-Visible spectroscopy

An analytical examination of various types of solvents and compounds is done using a technique known as UV-VIS spectroscopy. The analytical approach is based on calculating how much monochromatic light is absorbed by colourless substances throughout spectrums near the ultraviolet path. The process required to ascertain the "identification, strength, quality, and purity" of such chemicals is included in the pharmaceutical analysis. (Verma & Mishra, 2018). $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$

NPs (powder sample) were taken for evaluation and the characteristic absorbance range from 200-1200 nm was measured in this measurement. Absorption spectra were recorded using a double-beam spectrophotometer (JASCO, V670 from Japan).

4.2.1.2 FTIR

FTIR spectroscopic technology offers precise, significant structural and functional data gleaned from the examination. The vibrational characteristics of amino acids and cofactors, which are susceptible to minute structural changes, are investigated using Fourier transform infrared (FTIR) spectroscopy (Berthomieu & Hienerwadel, 2009). As prepared the solution of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs at concentration was subjected to KBr pelletizing using a motor pistil and the sample was subjected to FTIR instrumentation (Agilent Cary630 FTIR) at a range of wavelength 400 to 4000 per cm. FTIR plots were obtained for the presence of different available function groups present in the nanomaterial.

4.2.1.3 Raman spectroscopy

The investigation of inelastic scattering of light is called Raman spectroscopy. The substance under inquiry and the incident radiation field exchange energy, which leads to inelasticity. The method offers crucial knowledge regarding the vibrational state of matter, among other things (Rani *et al.*, 2018). These films' Raman spectra often exhibit two unique peaks known as the D- and G- peaks. The position, width, and intensity ratio of the two peaks are utilised to characterise the thin film material. For instance, an increase in the quantity and/or size of sp^2 clusters are linked to an increase in the I_D/I_G ratio (Petrova *et al.*, 2011). Raman spectroscopy is usually performed to understand the available decade in the sample and detect analysis. Prepared powder samples of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs were subjected to Raman spectroscopy and defect intensity was evaluated using a 785 nm (Renishaw, UK, Invia0719-17) in spectral frequency 100 –3,500 cm^{-1} with a spectral resolution of 0.75 cm^{-1} (FWHM).

4.2.1.4 DLS

The hydrodynamic diameter of nanoparticles in solution is measured using dynamic light scattering (DLS), which also offers information on the aggregation

condition of the particles in solution (Paul *et al.*, 2012). Dynamic Light Scattering (DLS) measurements were performed using (Microtrac) equipment using a 4 mW He-Ne LASER ($\lambda = 632.8$ nm) fitted with a thermostatted sample chamber. The sample was added to a 1.5 mL DTS0112 low-volume disposable size cuvette (path length 1 cm). A particular hydrodynamic diameter and size distribution were derived using the software programme provided by the manufacturer. The measured data are the average of 25 runs, each of which was averaged for 15 s.

4.2.1.5 Zeta Potential and PSA

Colloid science refers to the potential difference between a particle's surface and the surrounding liquid or solid as having a "zeta potential." It is a measure of the electrostatic attraction or repelling force between particles and is affected by variables including pH, ionic strength, and surface charge (Bohara *et al.*, 2015a). As prepared nanomaterial dissolved in a concentration of the aqueous solution, sonicated for 30 min were subjected to zeta potential and PSA analysis (SZ-100V2 HORIBA, nanoparticle Japan).

4.2.1.6 XRD

X-ray diffraction is a method for examining crystalline materials' structure. When X-rays are focused on a sample, the resulting diffraction pattern reveals details about the atom arrangement within the substance. The unique arrangement of atoms in the crystal lattice causes X-rays to diffract in various directions when they interact with a crystalline sample. The atom locations and the distance between them are ascertained by detecting and analysing the diffraction pattern. This data can be used to determine the material's crystal structure, phase composition, and other physical characteristics (Ch. Vinuthna, D. Ravinder, 2013). $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ X-ray diffraction (XRD) patterns were acquired using a (ThermoFisher Model - Equinox 3000) run at 40 kV and 20 mA with nickel-filtered Cu K radiation ($\lambda = 1.54178$). The samples were recorded at a scan speed of $20^\circ \text{ min}^{-1}$ in the 2θ range of 5°C to 80°C . (The step size is $0.025^\circ/\text{step}$) with an exposure of 0.1 s/step . Different lattice planes against specific diffraction angles were calculated and used to determine the intrinsic details of the structure based on the diffractogram.

4.2.1.7 AFM

AFM scans the surface of a material using a pointed probe that has a radius of a few nano-meters. A three-dimensional image of the surface topography is created as a result of the probe's interaction with the surface through a mix of attraction and repulsive forces, which are measured (Lopez *et al.*, 2012). The sample $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ were dissolved in double distilled water and sonicated, acquired aqueous solution was board cast in a glass coverslip and air dried before subjecting to AFM. An NTMDT Ireland Atomic Force Microscope in AC non-contact mode in the air operating at room temperature was used to observe the morphology of nanoparticles. Using an NTMDT Ireland, cantilever with a 50 nm Co-Cr coating, a spring constant of 0.01-0.5 N/m, a resonance frequency of 140-390 KHz, and a tip radius of less than 6 nm, able to perform magnetic imaging.

4.2.1.8 TGA

A technique called thermal gravity analysis (TGA) is used to examine a sample's thermal and gravimetric properties. As a sample is exposed to a controlled temperature increase or reduction, its weight is measured. Changes in the sample's weight during this procedure can reveal chemical reactions, phase transitions, or other thermal phenomena (Ch. Vinuthna, D. Ravinder, 2013). Using a Perkin Elmer STA6000 THERMAL SYSTEM with a heating rate of 10 °C min⁻¹ in the temperature range of 25-800°C under an N₂ atmosphere, thermogravimetric tests were carried out (flow rate 30 mL min⁻¹). The powder samples, weighing between 8 and 10 mg, were heated. Weight loss was measured during this procedure as a function of temperature differential.

4.2.2 Comparative Analysis of BSA And HSA

It is crucial to investigate how albumin and nanoparticles interact biologically. Human serum albumin (HSA) and bovine serum albumin (BSA) are structurally and functionally identical. BSA and HSA are more similar than 83% and share more than 76% of their sequence. A total of three homologous sub-domains makes up the 583 amino acids that make up BSA. 1–183 residues, 184–376 residues, and 377–583 residues makeup domain I, domain II, and domain III, respectively.

Different molecules can bind in two hydrophobic pockets, one in domain IIA and one in domain IIIA. The discipline of theragnostic has a lot of potential for magnetic nanoparticles. Therefore, it is vital to assess how it interacts with the blood protein BSA to enhance its application in biomedicine. Magnetic nanoparticle aims to develop core-shell nanostructure, corroborating characteristic with synthesizing route. This way, we could discover new functionalization and provide new applications and performance in the biomedical field (Gandhi & Roy, 2019).

Table 4.2 Comparative analysis of BSA and HSA

BSA	HSA
The molecular weight of BSA is 66.5 KDa	The molecular weight of HSA is 66 KDa
The amino acid sequence of BSA is 583	The amino acid sequence of HSA is 585
BSA is a Compact structure with a small radius of gyration	HSA as a Compact structure
The isoelectric point (PI) of BSA is 5.1	The isoelectric point (PI) of HSA is 4.9
BSA consist of 2 Tryptophan residues	HSA consist of Tryptophan residues

4.2.3 Conjugation Of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA

The $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs interact with the BSA, leading to changes in the protein's conformation and function. BSA can cause $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ to agglomerate, changing their size, shape, and surface characteristics. For conjugation of BSA in $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPS, $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs of 1mM were prepared in double distilled water and the solution is sonicated for 30 min to get a stable solution, after the temperature drop BSA is added to the solution and incubate overnight in the refrigerator. After conjugation BSA and $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs were purified by performing centrifugation (13,000rpm for 30 min) for the removal of weakly absorbed BSA, the supernatant containing free BSA which is discarded, the pellet is resuspended in distilled water. All biophysical

characterization assays and in vitro studies are performed after the purification of BSA with $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ N NPs. The schematic diagram example of the conjugation of cobalt zinc ferrite with BSA in **Fig 4.1**

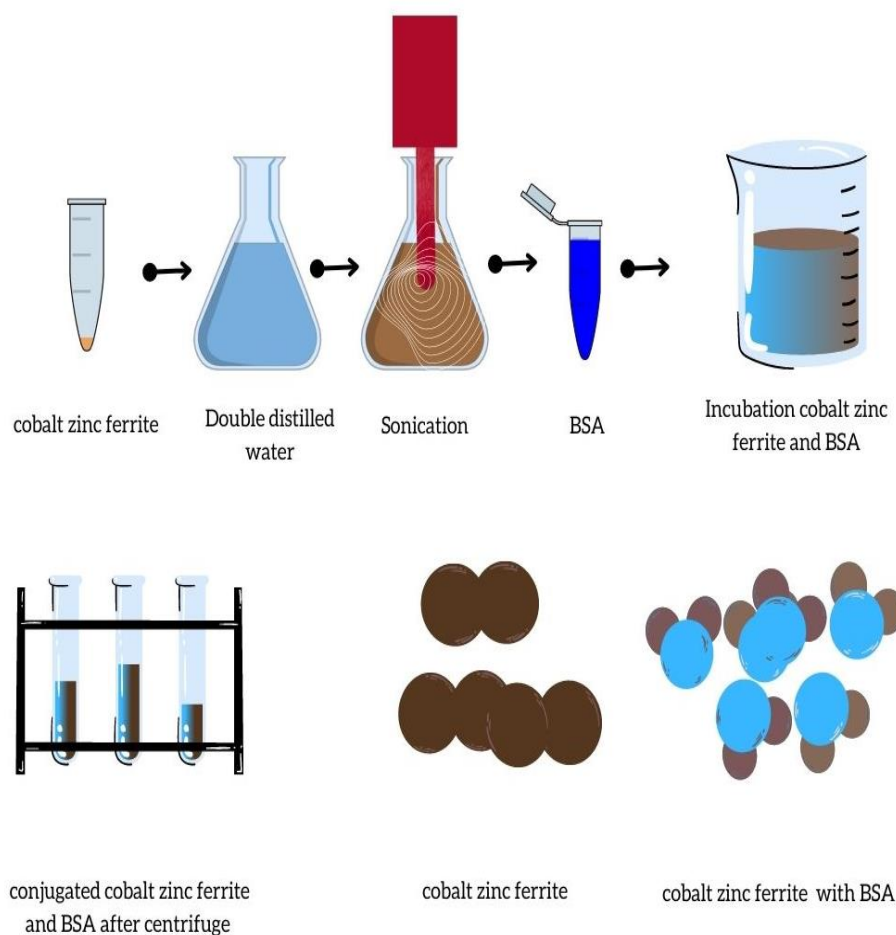


Fig 4.1 Conjugation of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA. (Created by Canva)

4.2.4 Biophysical Characterisation

Biophysical characterisation of nanoparticles is the process of determining a nanoparticle's physical and chemical characteristics utilising a variety of methods.

4.2.4.1 UV-Visible spectroscopy

The absorbance spectrum of a sample is measured using UV-visible spectroscopy. The absorbance spectrum is a depiction of the sample's absorbance at various light wavelengths. Information on a molecule's stability in various environmental circumstances can be found using UV-visible

spectroscopy(Paul *et al.*, 2012). A protein's shape or stability may change, which may be indicated by changes in the protein's absorbance spectrum. The prepared solution of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs at a particular concentration interacted with BSA at laboratory conditions and was examined for strength, quality, and purity. Absorption spectra were recorded using a double-beam spectrophotometer (JASCO, V670 from Japan).

4.2.4.2 FTIR

The composition and structure of ferrites can be better understood using the Fourier transform infrared (FT-IR) spectroscopy technique. The interaction of infrared radiation with matter is the subject of infrared spectroscopy(Ch. Vinuthna, D. Ravinder, 2013). The prepared solution of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs at a particular concentration interacted with BSA at laboratory conditions and was examined for the extension functional group in the interactive system. The sample was subjected to FTIR instrumentation (Agilent Cary630 FTIR) at a range of wavelength 400 to 4000 per cm.

4.2.4.3 DLS

In biophysical analysis, DLS is frequently used to examine the size distribution of macromolecules like proteins. Additionally, it can be used to examine the state of particle aggregation and track changes in particle size or shape over time(Guindani *et al.*, 2020). Particle average diameters and polydispersity index of the uncoated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs and BSA conjugated were measured by DLS (Microtrac).

4.2.4.4 Zeta Potential

The electrical potential difference between a particle's surface and the surrounding fluid is measured by zeta potential analysis of colloidal systems of proteins. Many details regarding the stability, aggregation, and interaction of particles in a solution can be obtained from the size and sign of the zeta potential(Guindani *et al.*, 2020). The surface charge of the uncoated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs and conjugated was analysed by zeta potential measurements (Microtrac).

4.2.5 Conjugation Analysis

Conjugation analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs and BSA will demonstrate the interaction and determine by molecular mass, the distribution of conjugated molecules, and the stability of the conjugate.

4.2.5.1 SDS Page

SDS, a detergent that binds to proteins and gives them a consistent negative charge density, is applied after a protein sample has been denatured. Applying an electric field induces the negatively charged proteins to move through a matrix of polyacrylamide gel, where they are subsequently sorted according to their size (Kushwah *et al.*, 2017). The effectiveness of removing the free protein from the conjugates was evaluated using the SDS-PAGE of the conjugates. Protein presence was determined using the Coomassie Blue stain.

4.2.5.2 Bradford assay

To ascertain the amount of protein in a sample, the Bradford assay, a calorimetric protein assay, is frequently used. The Coomassie Brilliant Blue dye is used in the assay, and it is bound to the amino acid residues in proteins. This binding results in a shift in the dye's absorption spectrum and a colour change (Ho *et al.*, 2015). Standard BSA concentrations were employed as the calibration standards for protein tests, and analysis kits were used. In triplicate, the tests were carried out in 96-well microplates. For Bradford protein tests, the absorbance was measured at 562 nm and 595 nm after the assays had been incubated for the allotted amount of time. Plots of the concentration calibration curves and comparisons with the NP-based protein assay were made.

4.2.6 Toxicity Analysis

A cell line's toxicity analysis often entails assessing how different substances, such as chemicals or environmental stressors, affect the cell line's viability, proliferation, and functionality.

4.2.6.1 MTT assay

A calorimetric technique called the MTT assay is employed in cell culture to

gauge cell viability and proliferation. MTT stands for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. A common technique for assessing cell viability, cytotoxicity, and proliferation in vitro is the MTT assay. It is a convenient assay that can be used to evaluate the effectiveness of medicines, test substances, and environmental contaminants(Alshahrani *et al.*, 2021). 1×10^6 HepG2 cells were seeded onto 96-well microplates, which were then cultured at 37°C for 48 hours in an environment with 5% CO₂ to allow the cells to reach 70-80% confluence. After that, the medium was changed, and the cells were exposed to various sample concentrations while being incubated for 24 hours. After 24 hours, the morphological alterations of untreated (control) and treated cells were seen and captured using a digital inverted microscope (20X magnification).

4.2.6.2 ROS assay

A ROS test can be carried out on cell lines to determine the concentrations of reactive oxygen species (ROS) within the cells. DCFDA, a nonfluorescent substance, was used to identify intracellular ROS generation. As this substance enters cells, an esterase deacetylates it, creating a non-fluorescent substance(Iqbal *et al.*, 2019). Cells were seeded in a 96-well, black plate, and incubated for 14 hours at 37 degrees Celsius in humidified air containing 5% CO₂. Under appropriate lighting with a wavelength of 630 nm, the same procedures were followed with labelled NPs in HepG2 cells. In Dulbecco's modified Eagle media, the cells underwent a single gentle washing. Cells were loaded with 100 L of 5 M DCFDA and left in the dark for 30 min at 37 C. The ROS fluorescence was then measured using a Wallac 1420 Victor Plate Reader after the cells had been subjected to 10 J/cm² of light fluence for 2 min. Given the previously mentioned treatments, the cells were raised on Petri dishes and activated with blue light (488nm).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 PHYSICAL CHARACTERISATION

5.1.1 UV-Visible spectroscopy

UV-Visible spectroscopy was used to evaluate the optical properties of the samples $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles (Andhare *et al.*, 2020). Impurity centres, particle size, bandgap, lattice parameters, and surface roughness are all factors that influence absorbance. **Fig 5.1** UV-Visible spectra $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ (Absorbance ~ 486 stemming from the particles in liquid solution). With increased zinc concentration, the absorption spectra show a greater absorption peak that has shifted towards a higher wavelength; this shift denotes a change in the optical bandgap brought on by the influence of quantum confinement. The electrical structure of molecules and other materials and their concentration in solution is frequently determined using UV-Visible spectroscopy. A UV-Vis spectrum, which shows the absorption of light as a function of wavelength, is commonly used to present the resulting data (Begum *et al.*, 2018).

Cobalt zinc ferrite nanoparticles have a band gap energy of 2.273 eV. Physically, in the direct band gap of hole momentum and electron, there are the same conduction and valence bands; the electron the mean the energy of 2.273 eV to emit a photon from the valence band to the conduction band. The value of band the gap energy of cobalt ferrite was smaller than that of magnetite. The presence of Co ion that changed Fe in the octahedral position enhanced the electron state in the outside orbital area and caused the decrease in band gap energy (Kurniasari *et al.*, 2017).

The UV–Visible absorption and photocatalytic results produced by several ferrite samples are in good agreement, indicating that the band gap of ferrite materials significantly affects their photocatalytic activity. However, in $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ the sample exhibits a significant reduction in photocatalytic activity. Absorbance peaks were observed at ~ 486 nm respectively.

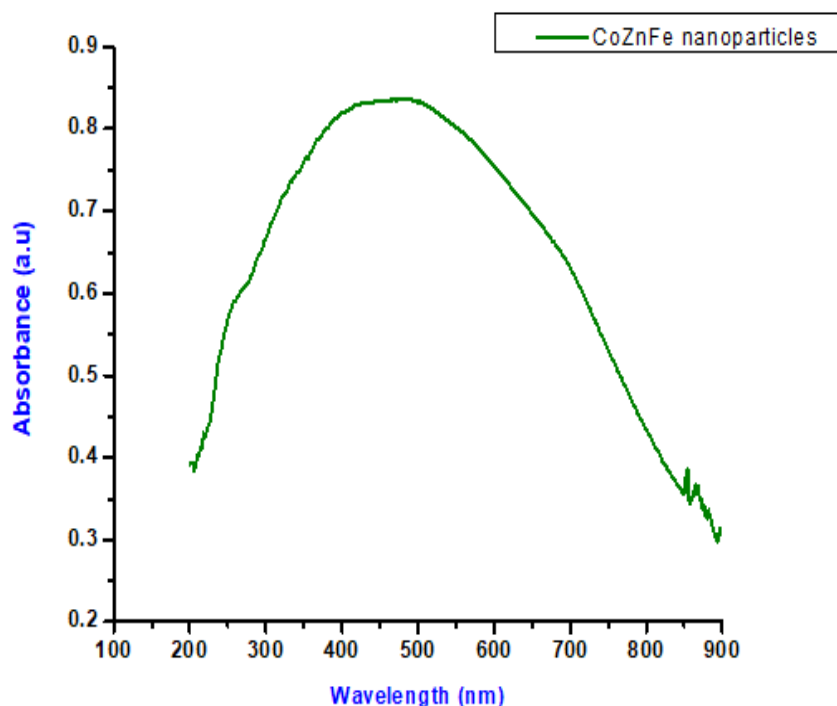


Fig 5.1 UV-Visible spectra $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ (Absorbance ~ 486 stemming from the particles in liquid solution).

5.1.2 FTIR

Tetrahedral and octahedral clusters are explained by FT-IR spectra, which also support the existence of the M-O stretching band in ferrites (Ch. Vinuthna, D. Ravinder, 2013). **Fig 5.2** FT-IR of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles (wavenumbers of $\sim 3373 \text{ cm}^{-1}$ corresponds to stretching vibrations of the surface hydroxyls groups absorbed onto the surface of the material and O-H groups in the absorbed water, indicating the adsorbed moisture. Peaks at $\sim 1566 \text{ cm}^{-1}$ denote H-O-H bending vibrations or -OH vibrations from the aqueous water molecules. Smaller and weaker peaks ~ 500 to 800 cm^{-1} wave number indicate the vibrations of ferrite groups (tetrahedral and octahedral) sites of positive ions in the ferrite. The downshifting in the wavenumbers of smaller peaks $\sim 700\text{-}800 \text{ cm}^{-1}$ indicates the incorporations of Zn content and further results in a distancing between the Fe^{3+} and O^{2-} in the tetrahedral sites, resulting in the overall formation of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nano-structures.

Cobalt ions have a significant predilection for octahedral sites, whereas zinc ions typically prefer to occupy tetrahedral sites. An increase in Fe^{3+} ions at

octahedral sites is indicated by the observed shift in the wavenumber of the octahedral band with zinc concentration. In other words, zinc ions reached their respective site, where they subsequently replace cobalt ions in a less direct manner. The wave number of the tetrahedral band drops as a result of the process used to move Fe^{3+} ions (lower atomic mass) from one site to another site. The decrease in decreased mass and the displacement of Co^{2+} ions (0.745) at octahedral sites with Fe^{3+} ions (0.645) with smaller ionic radius are the result of this mechanism position of the octahedral band was seen to vary. Zn-substituted cobalt ferrites had a similar outcome(Appa Rao *et al.*, 2019). The resulting spectrum demonstrates how the radiation is absorbed or transmitted in relation to frequency or wavelength. The functional groups and chemical bonds that are present in the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ is determined using this spectrum(Preetha *et al.*, 2013).

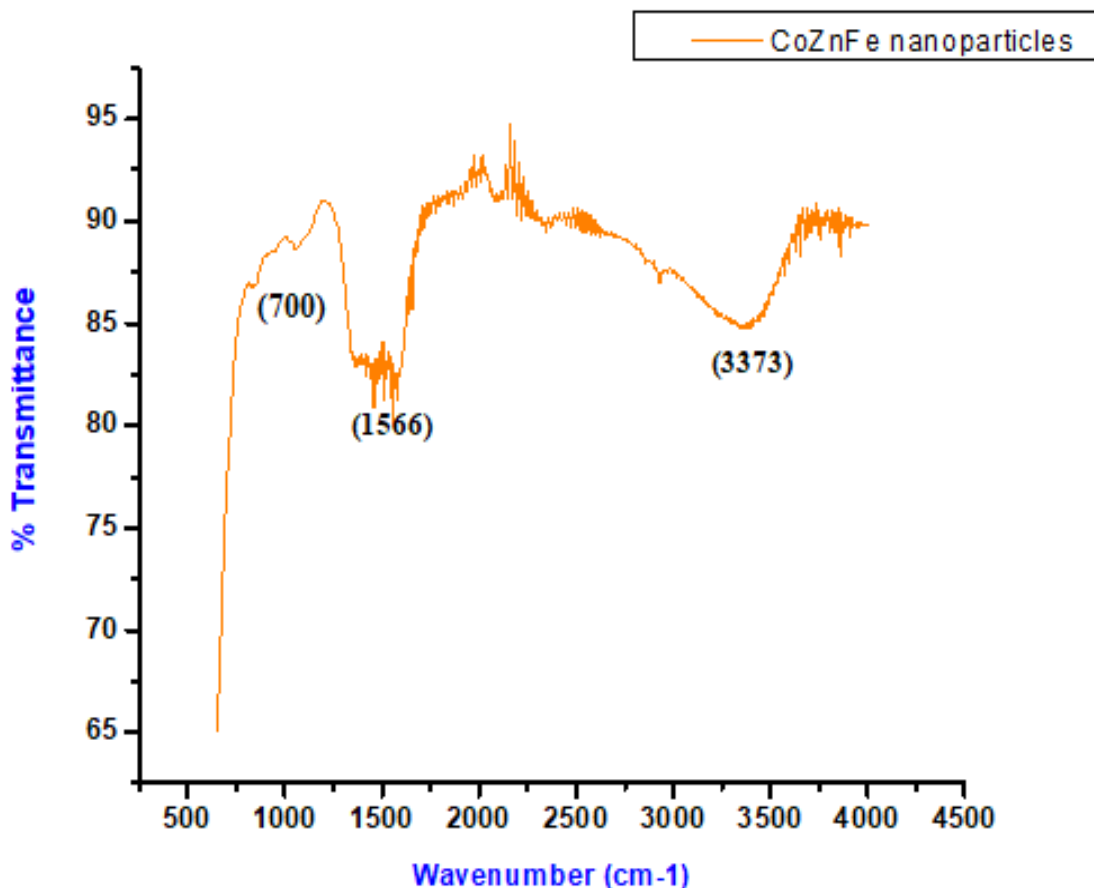


Fig 5.2 FTIR of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles.

5.1.3 Raman spectroscopy

The structure of pure and cobalt-substituted zinc ferrites made using a straightforward co-precipitation process was further elucidated using Raman spectroscopy. Raman's investigation of pure and cobalt-substituted $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs under 800 °C calcination. In the region of 200-800 cm^{-1} , two distinct bands are seen in pure zinc-ferrite products. The band found at 645 cm^{-1} is related to the zinc-ferrite product's A_{1g} phonon vibration mode, whereas the band in the region of 155 to 700 cm^{-1} is assigned to phonon vibration mode. The value of D and G peaks were respectively 189 cm^{-1} and 319 cm^{-1} , 470 cm^{-1} and 845 cm^{-1} ; and 1326 cm^{-1} and 1560 cm^{-1} . **Fig 5.3** Raman spectroscopy of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles (I_D/I_G gives the intensity of defects in the material). The Raman spectrum's D-band (defect-induced) and G-band (graphitic) peaks are referred to as the I_D/I_G ratio and their intensities, respectively. The disorder or faults in a material are frequently assessed using the I_D/I_G ratio.

A potent method for revealing the structural characteristics of ferrites is Raman spectroscopy. Only five of the 39 Raman modes are active in spinel ferrites, which is mostly related to the motion of the oxygen ion at the respective site. The mixed ferrite structure has the Oh 7 (Fd³m) space group, which produces 39 Raman modes (Pawar *et al.*, 2016). Raman spectra. In $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ there are three major broad bands and two additional minor modes with weaker peaks. As a characteristic of the cubic spinel structure, the five bands detected can be attributed to Raman spectra in active optical modes (1A_{1g}, 1E_g, and 3T_{2g}).

The symmetric stretching of oxygen atoms along Fe-O (and Zn-O) bonds at the tetrahedral sites is what causes the A_{1g} mode. The oxygen atom's symmetrical bend about to the metal ions produces the E_g mode. The octahedral group vibrations are represented by the T_{2g}(2) and T_{2g}(3) modes (Siu *et al.*, 2011). In ferrites, tetrahedral A-site metal ions are represented by Raman modes that emerge in the 630–700 cm^{-1} wavelength range, whereas octahedral metal ions are represented by Raman modes in the 310–610 cm^{-1} range. The distribution of divalent and trivalent cations is described by a shift in the band wavelength and cation migration.

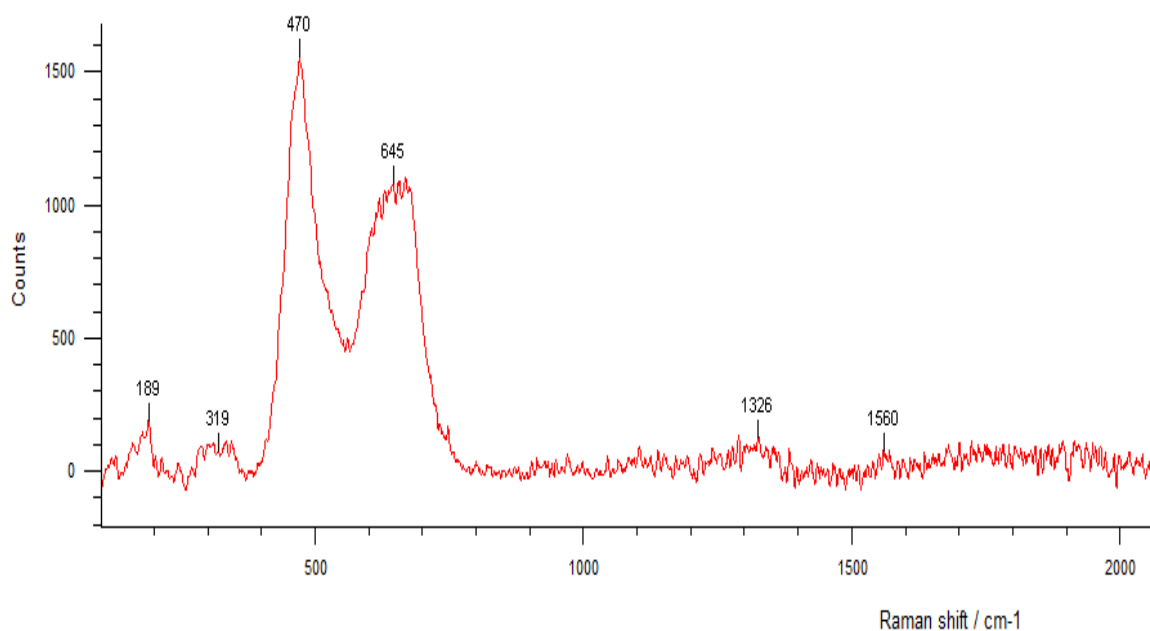


Fig 5.3 Raman spectroscopy $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ of nanoparticles (I_D/I_G gives the intensity of defects in the material).

5.1.4 DLS

The measurement of the intensity of dispersed light as a function of time, dynamic light scattering operates. The Brownian motion of the particles in the sample is what causes the dispersed light (Bohara *et al.*, 2015). The size distribution of the particles in the sample can be ascertained by examining the time-dependent fluctuations in the scattered light. The average hydrodynamic diameter for $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs is determined by the average maximum of the DLS size (HDD). A limited size distribution between 13 and 30 nm and a polydispersity of 0.2 has been seen in the HDD, which suggests that the particles are monodispersed in nature. **Fig 5.4** DLS analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs. The size distribution of the particles in the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs is ascertained by analysing the intensity variations in the scattered light. The method is sensitive to particles as small as a few nanometres and as large as several micrometres (Jitkang *et al.*, 2013).

The observed Co/Fe molar ratio is stable and similar to stoichiometric $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ in terms of its composition. The hydrodynamic diameter significantly increased as a result of the DLS observations (Baldi *et al.*, 2007). Instead of a true increase in the biological shell around the particles, this rise can be attributed to the dipolar magnetic forces between the particles. The Brownian motion on which the

DLS approach depends is significantly impacted by the dipolar interaction, which rises with the particle magnetic moment and hence with the size.

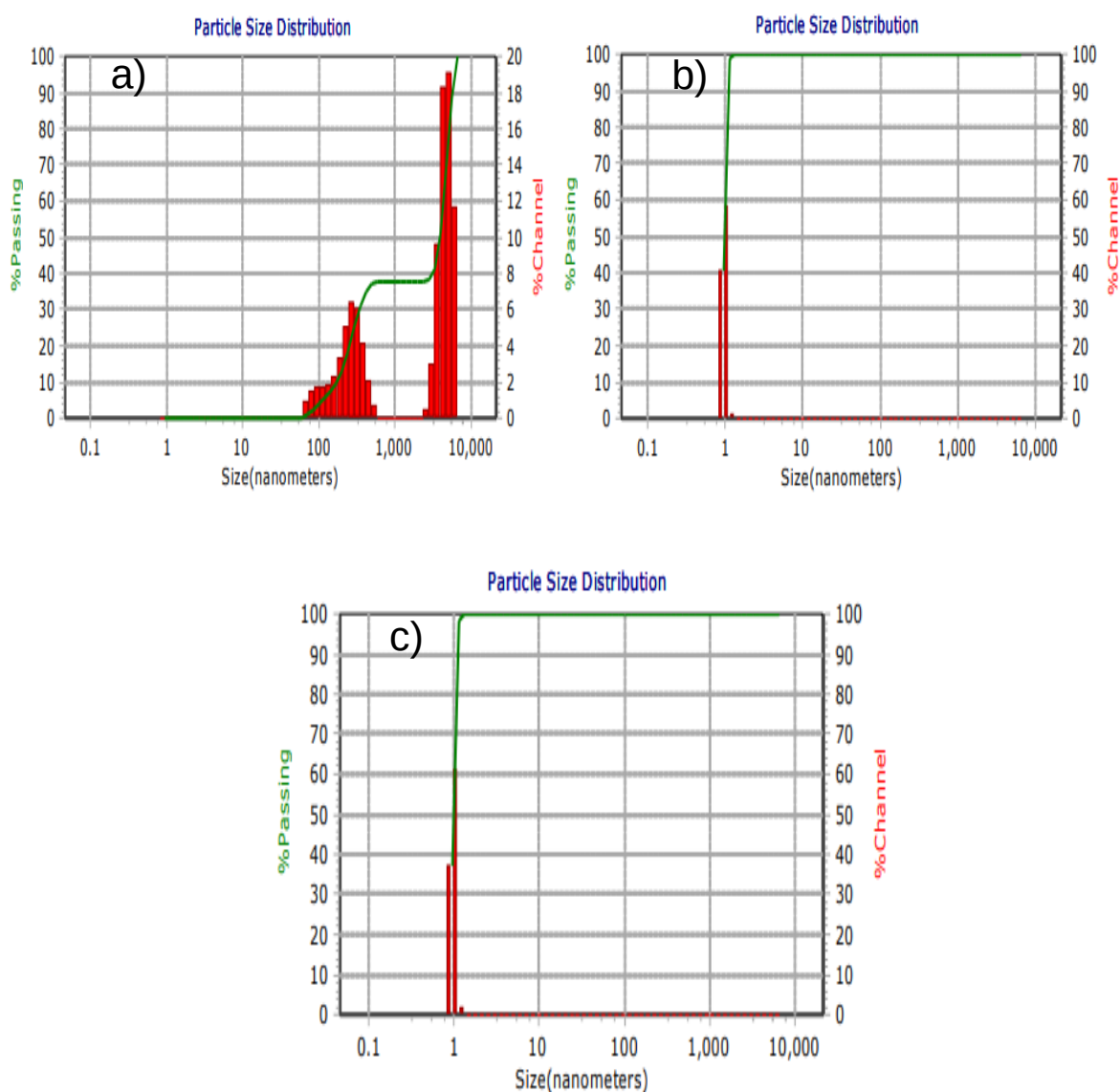


Fig 5.4 DLS analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs (a - Dia(nm)-4610& 247.1, Vol% - 62.2& 37.8, Width- 1882&231; b – Dia(nm)-0.97, Vol%- 100, Width -0.2; c - Dia(nm) 0.98, Vol% - 100, Width – 0.19).

5.1.5 Zeta Potential and PSA

The maximum of the zeta potential distribution plot, which is at around -48.5 mV, at high temperatures, the molecules on the surface may polarise into R-O⁻ and H⁺ ions, and this H⁺ is responsible for the positive surface and improved water

dispersibility. Due to electrostatic and steric interactions between the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs, the particle can remain stable in a colloidal solution, which is important for water stability and is highly desired in biomedical applications. **Fig 5.5** Zeta Potential analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs The surface charge of the particles can be determined by measuring the zeta potential, and the stability of the system can also be evaluated (Bohara *et al.*, 2015). Strong particle repulsion, which can resist aggregation and maintain stability, is indicated by a high zeta potential (either positive or negative).

It was noted that the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs was colloiddally stable. The material's average colloidal stability was found to be -48.5 mV. In this study, colloidal stability has been found, which defines how much surface charge is present over the material during experimentation. It is well known that $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs is a possible candidate for the net electrostatic interaction between the material and the cellular architecture due to its differential particle dispersion and colloidal stability. The colloidal stability also makes it possible to determine the material's thermodynamic free energy linked with its inherent atomic configuration. **Fig 5.6** PSA analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs.

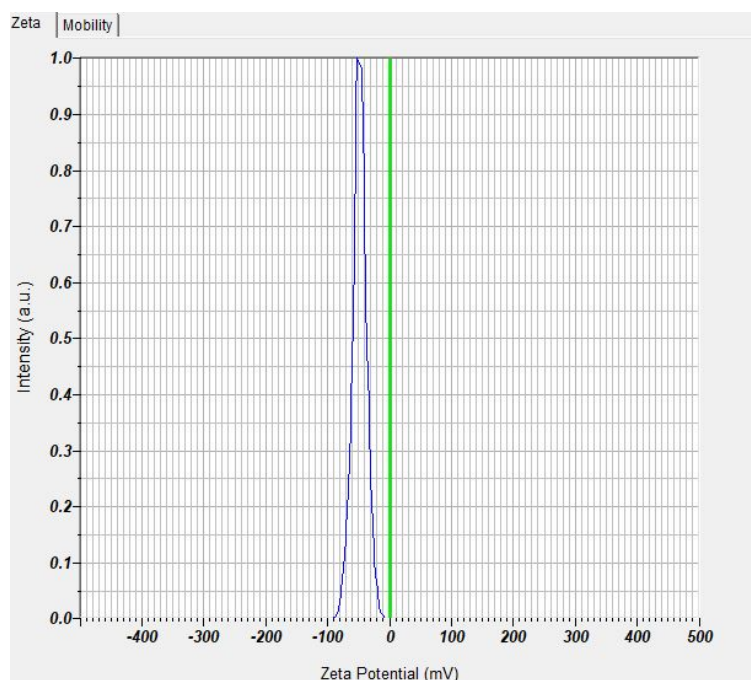


Fig 5.5 Zeta Potential analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs.

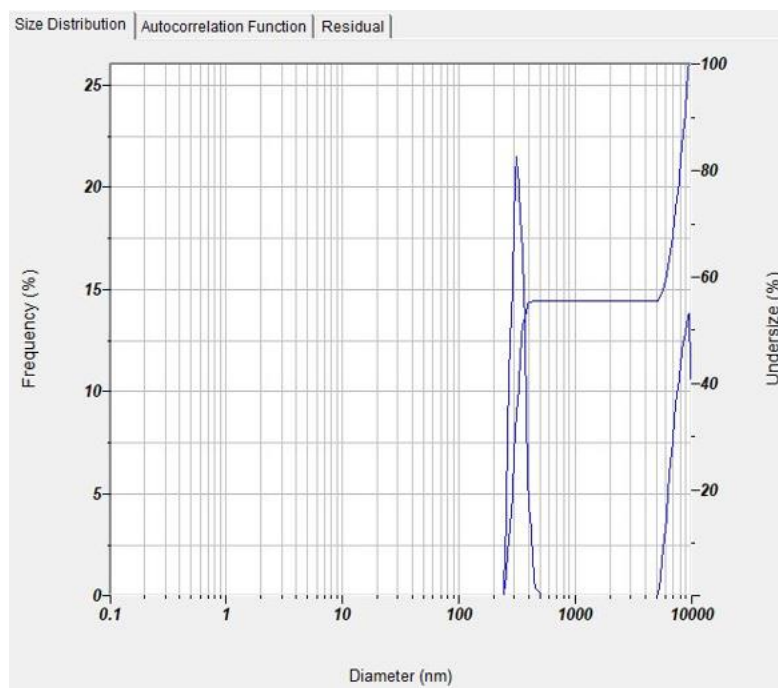


Fig 5.6 PSA analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs.

5.1.6 XRD

$\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs nanoparticle XRD patterns. The existence of reflection planes with (h k l) values of (220), (311), (222), (400), (422), (511), (440), (620), and (533) in all samples can be attributed to cubic spinel, according to XRD patterns. Structure for every sample with Fd-3m space group on display, which is verified. The formation of the cubic spinel structure in a single phase without an impurity peak is confirmed by the X-ray diffraction pattern (El Foulani *et al.*, 2019). XRD pattern illustrating the cubic spinel structure. Due to the crystallite's nanoscale size, the diffraction peaks are broad. **Fig 5.7** XRD of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs nanoparticles, lattice planes are exhibited at different diffraction angles, indicating inherent crystallinity in the samples (Akbari *et al.*, 2011).

$\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ has a lattice constant of 8.38. Up to $x = 0.2$, the lattice constant drops as the Zn concentration rises. Cation stoichiometry provides a theoretical justification for this (Sathishkumar *et al.*, 2010). Because the ionic radius of Ni ions (0.69 Å) is less than that of Co^{2+} cations (0.72 Å), the lattice constant in $\text{Ni}_x\text{Co}_{0.5-x}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs decreases when Cobalt is replaced by Ni, as predicted by Vegard's equation. Fe^{2+} production or probable presence in the octahedral sites may

be the cause of the increase in lattice constant for the value of $x = 0.3$. The lattice constant rises because the ionic radius of the Fe^{2+} ion ($0.74 \text{ \AA}$) is greater than that of the Fe^{3+} ion ($0.64 \text{ \AA}$). Tetrahedral and octahedral site cations, respectively, have intensities that are more sensitive to them. Fe^{3+} ions favour both tetrahedral and octahedral sites, and Co^{2+} ions prefer octahedral sites. The cation distribution changes when particle size decreases to the nanoscale. Both tetrahedral and octahedral positions are occupied by the Co^{2+} ion. figuring out the composition of the phases and the quantity of each is present in a $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$.

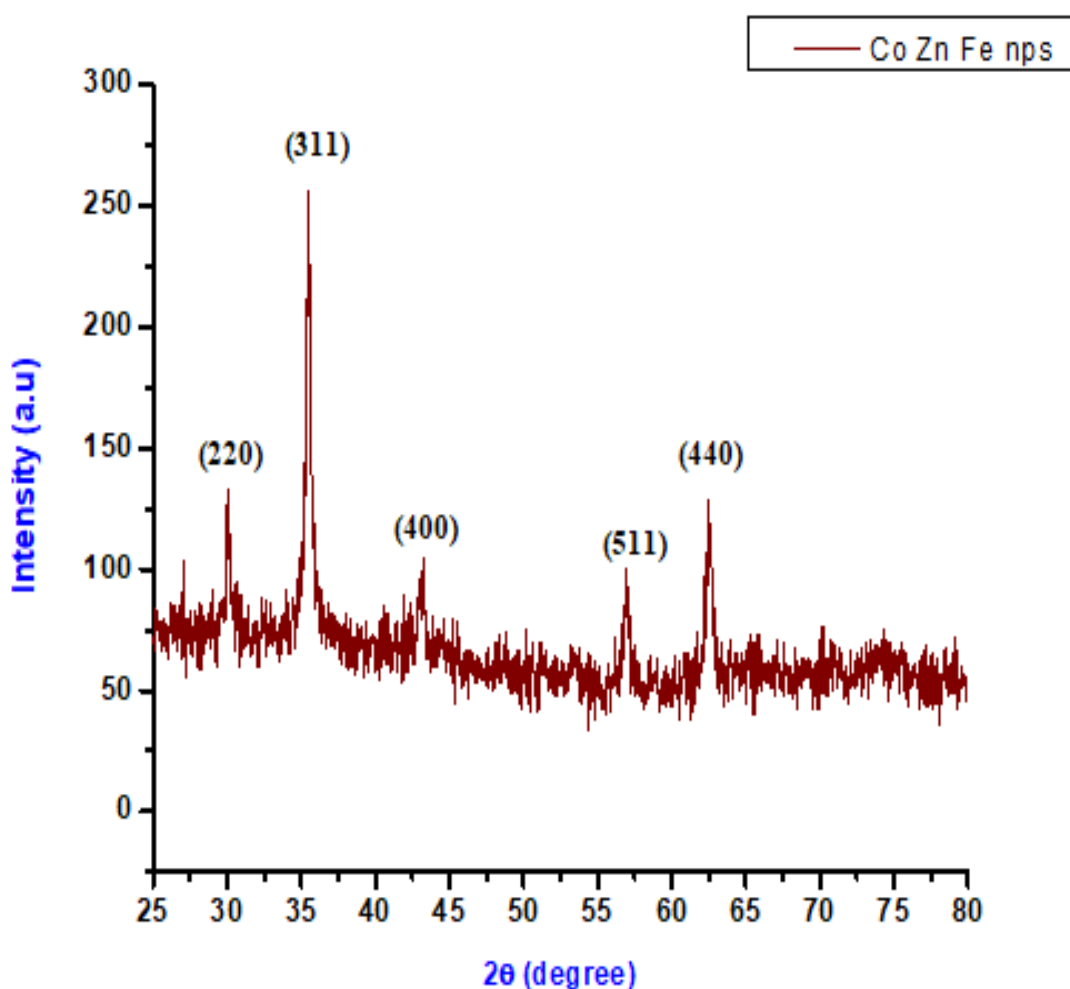


Fig 5.7 XRD of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs nanoparticles, lattice planes are exhibited at different diffraction angles, indicating inherent crystallinity in the samples.

5.1.7 AFM

The surface of aggregates of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles was observed during the analysis of magnetic particles. The crystal size of the nanoparticles as determined by XRD is less than the mean diameter calculated from the height and measured by AFM (Lopez *et al.*, 2012). This can be accounted for by the quantitative nature of the analysis and the result reveals that some of the 'particles' are conglomerates, which could produce particle conglomerates with a disproportionately greater height. the conglomerates of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticles' magnetic domains. The measurement of the samples and the tip were magnetised by an external field that was vertical to the substrate plane (Kaminskiene *et al.*, 2013). According to the interpretation of magnetic domains, a bright zone is an attracted force, while a dark zone reflects a domain's repellent force. **Fig 5.8** Magnetic domains of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticle conglomerates. **Fig 5.9** 2D AFM topography image of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$.

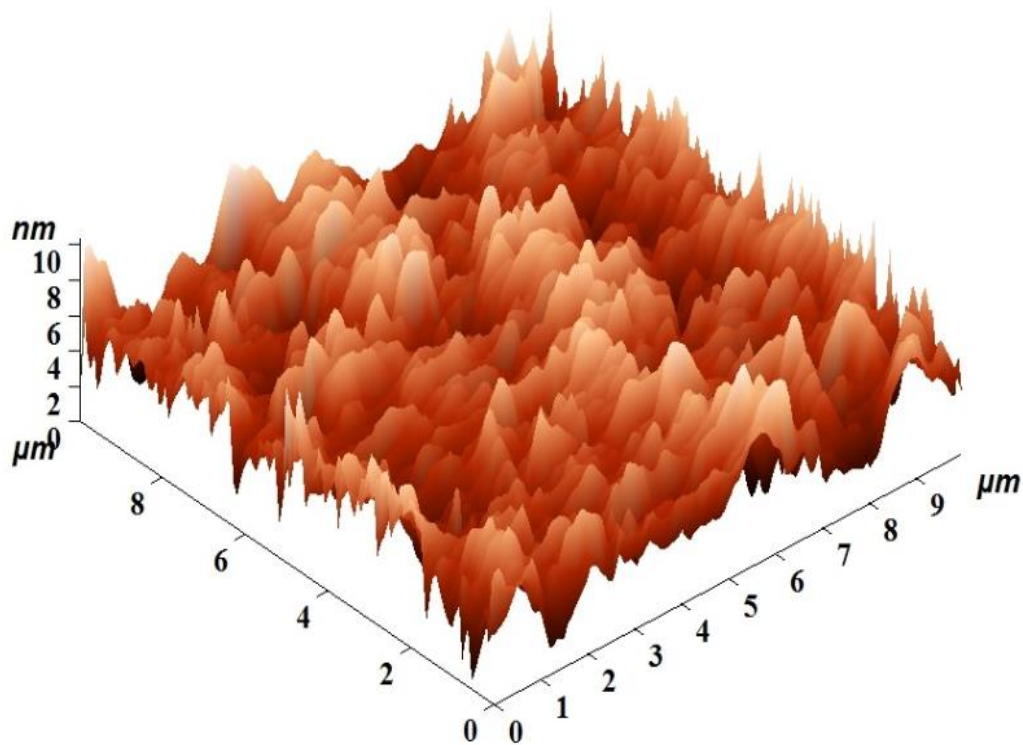


Fig 5.8 Magnetic domains of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticle conglomerates.

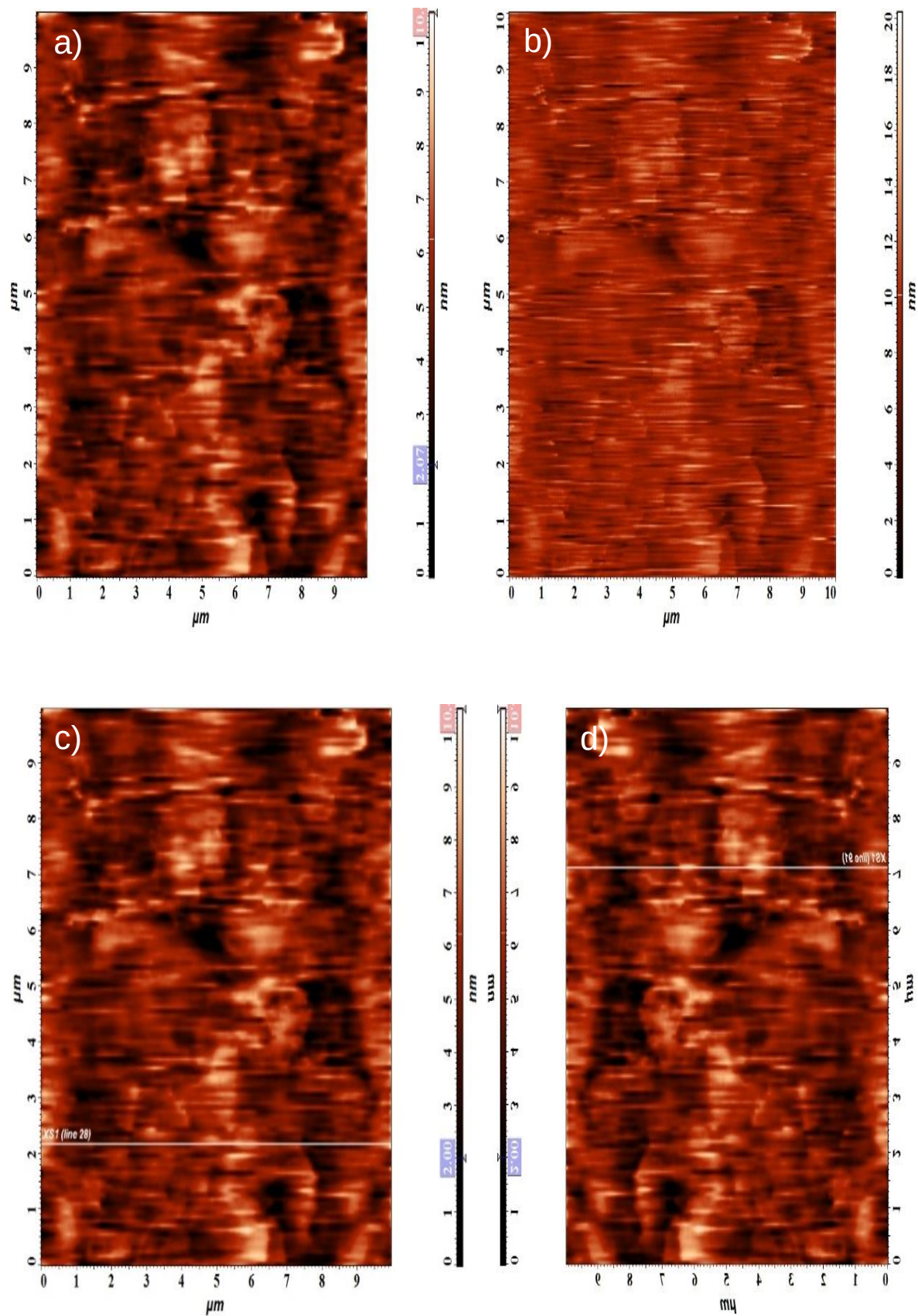


Fig 5.9 2D AFM topography image of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$. (a and b -few layer $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ sheet; c and d – monolayer of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$)

5.1.8 TGA

Thermogravimetric analysis is a method for determining how much a sample weighs changes as it is heated, cooled, or maintained at a constant temperature under controlled conditions. TGA can be used to assess a material's thermal stability, impurity levels, and other physical characteristics.(El Foulani *et al.*, 2019) TGA analysis, after the samples were calcined, the moisture content decreased Its weight is continuously measured while it is continuously heated. The material's thermal stability and the temperature at which breakdown or other thermal events take place are shown by the weight loss curve that results.

The graph of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ from the thermo-gravimetric (TG) study ranges from 30 to 80°C. Weight loss began at 100°C. and increased progressively with a minor difference. When the temperature reached 80°C, no further weight loss was seen, indicating that nano ferrite does not contain hydroxyl groups. With the initial weight loss of only 4.14% over the temperature range between 40 and 800°C, the TGA curves demonstrated breakdown as a function of temperature. Due to the subsequent drying of the material, the initial weight loss of 0.292% between 40 and 100 °C was attributed to the physical loss of water absorbed. **Fig 5.10** Thermal analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. Thermal stability of a substance, information on its chemical make-up, and the temperature and rate of its decomposition. It can be used to figure out how much ash, moisture, and volatile substances are in a $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ and provide details about the material's structure and composition(Ledo-Suarez *et al.*, 2006).

Cobalt zinc ferrite gel precursor TG-DTA curves. The TG curve shows that there are two distinct phases of weight loss. Chemical reactions that release heat are known as exothermic reactions and chemical reactions that absorb heat are known as endothermic reactions. With a weight loss of 7% and an endothermic peak on the DTA curve, the first thermal event in the temperature range of 30-120 C can be attributed to sample dehydration(Poorbafrani *et al.*, 2015). Between 120 and 280 C, the exothermic peak in the DTA curve was linked to the beginning of cobalt ferrite crystallisation and the decomposition of nitrates (which resulted in a 41% weight loss).

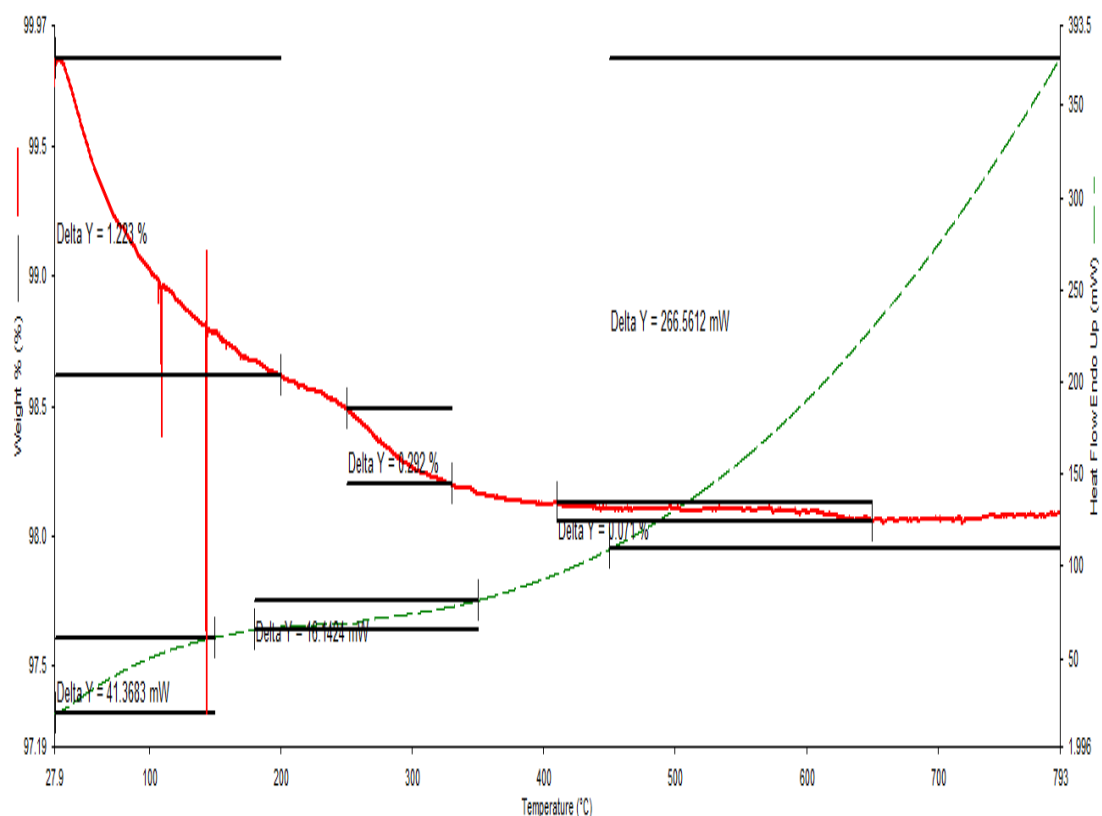


Fig 5.10 Thermal analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps.

5.2 COMPARATIVE ANALYSIS OF BSA AND HSA

The most prevalent protein in the bloodstream, serum albumin, binds and transports a variety of exogenous and endogenous compounds, including fatty acids, nutrients, hormones, and medicines with limited solubility. Seventeen disulfide bonds are used to cross-link the 585 amino acids that make up human serum albumin (HSA) in which BSA have 583 amino acids. It is thought to have three distinct domains, designated I, II, and III, each of which is composed of two subdomains (a and b) that share structural motifs. Brown's comparison of the amino acid sequences of bovine serum albumin (BSA) and human serum albumin (HSA) reveals that their general structural properties are comparable, with the sequence variation being primarily conservative structural variations (Gelamo & Tabak, 2000). The secondary and tertiary structures of the two proteins differ somewhat, even though their general structures are similar. Compared to HSA, BSA has a structure that is more compact and has a lower gyration radius. The three homologous domains that make up the BSA molecule are split into nine loops by seventeen disulfide links. The first domain of BSA contains Trp 134, and the second domain has

Trp 213(Roy *et al.*, 2012). A BLAST search in the PDB using the Swissport sequence ALBU_BOVIN (P02769) from bovine serum albumin indicated 76% similarity with HSA. This shows that there are many sequence similarities and structural similarities between the two proteins. The sequences of BSA and HSA contain numerous conserved sections and motifs, which adds evidence to their closeness. **Fig 5.11** Comparative analysis of BSA and HSA using Blastp.

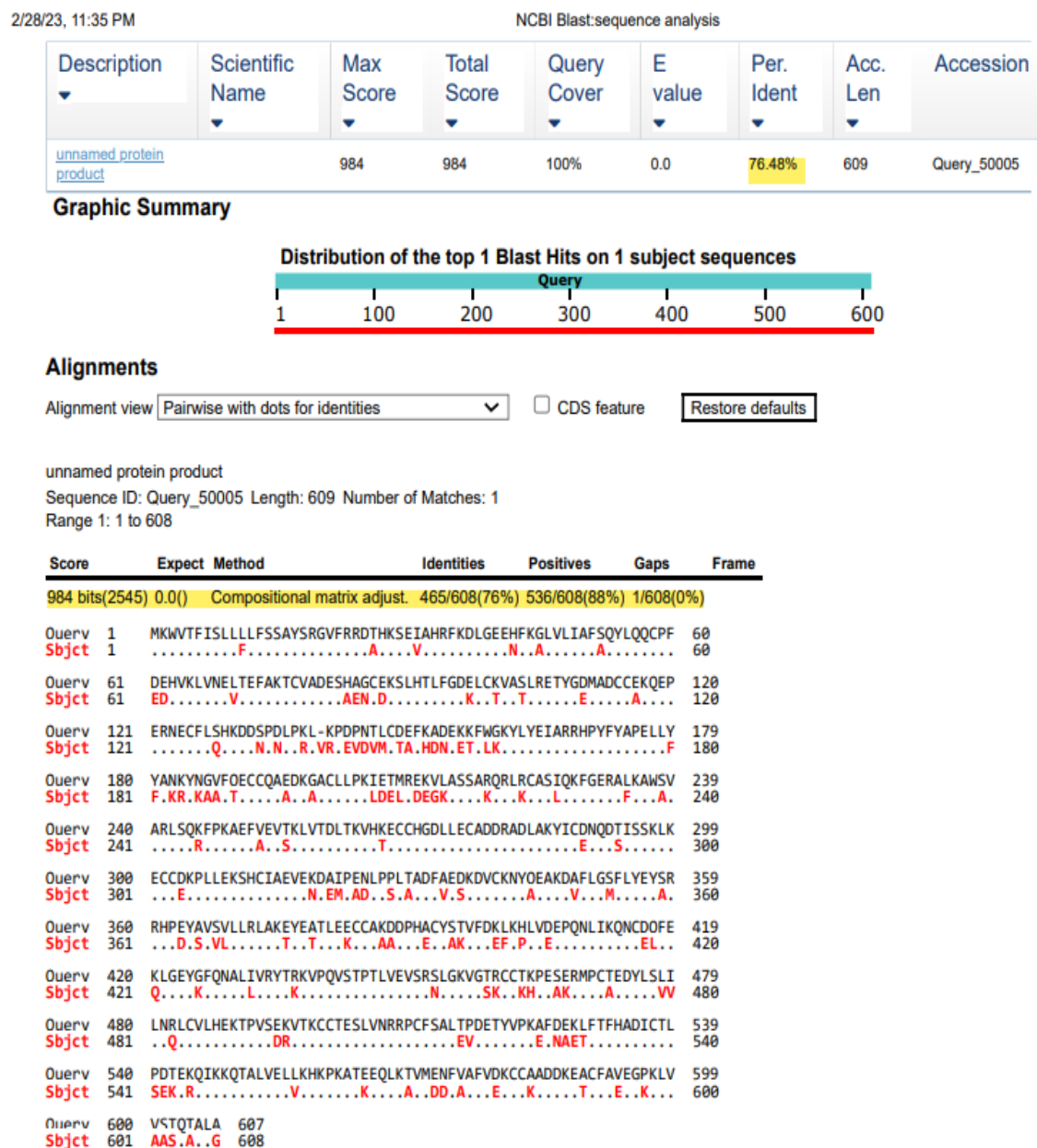


Fig 5.11 Comparative analysis of BSA and HSA using BlastP.

5.3 BIOPHYSICAL CHARACTERISATION

5.3.1 UV-Visible spectroscopy

When the protein corona forms, changes in the absorbance spectra of the nanoparticle-protein combination can be seen using UV-Visible spectroscopy. The protein-nanoparticle interaction's strength and stability, as well as the kinds of proteins that are adhering to the surface, can all be inferred from the spectra. Using basic UV/visible spectroscopic techniques, it is simple to investigate protein conformational change and complex formation.

Two absorption peaks were visible for BSA a robust peak at 210 nm that revealed the framework conformation and a faint signal at 278 nm that revealed the aromatic amino acids (Try, Trp, and Phe) of the protein. Changes in the microenvironment have an impact on chromophore absorption, indicating the distinctive p / p^* transition brought on by variations in the solvent's polarity. Increased unfolding is a result of the polypeptide strand's extension, which reduces its hydrophobicity, as seen by the rise in absorbance at 210 nm (Appa Rao *et al.*, 2019). The fact that the peaks shift towards shorter wavelengths may indicate that the contact is mediated by van der Waals or hydrogen bonds. Changes in the absorbance spectrum can be used to identify proteins when they cluster into insoluble or partially soluble aggregates. Changes in the absorbance spectrum may follow the development and be used to examine the size, stability, and structure of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ (Rashtbari & Dehghan, 2021).

Tryptophan, tyrosine, and phenylalanine residues give BSA a prominent 210 nm absorption peak. The $\pi\text{-}\pi^*$ transition causes the peak to appear. Investigation of the binding is done using the variation in BSA absorption. the BSA absorbance spectra with and without various $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NP concentrations. With each subsequent addition of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs, the absorbance of BSA gradually rises. This implies the formation of a ground-state complex between the two. This ground-state complex involves a large number of non-covalent interactions. When nanoparticles are added, the amino acid residues' hydrophobicity

(microenvironmental) increases. **Fig 5.12** UV-Visible spectra $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA (Absorbance ~ 210 stemming from the particles in liquid solution).

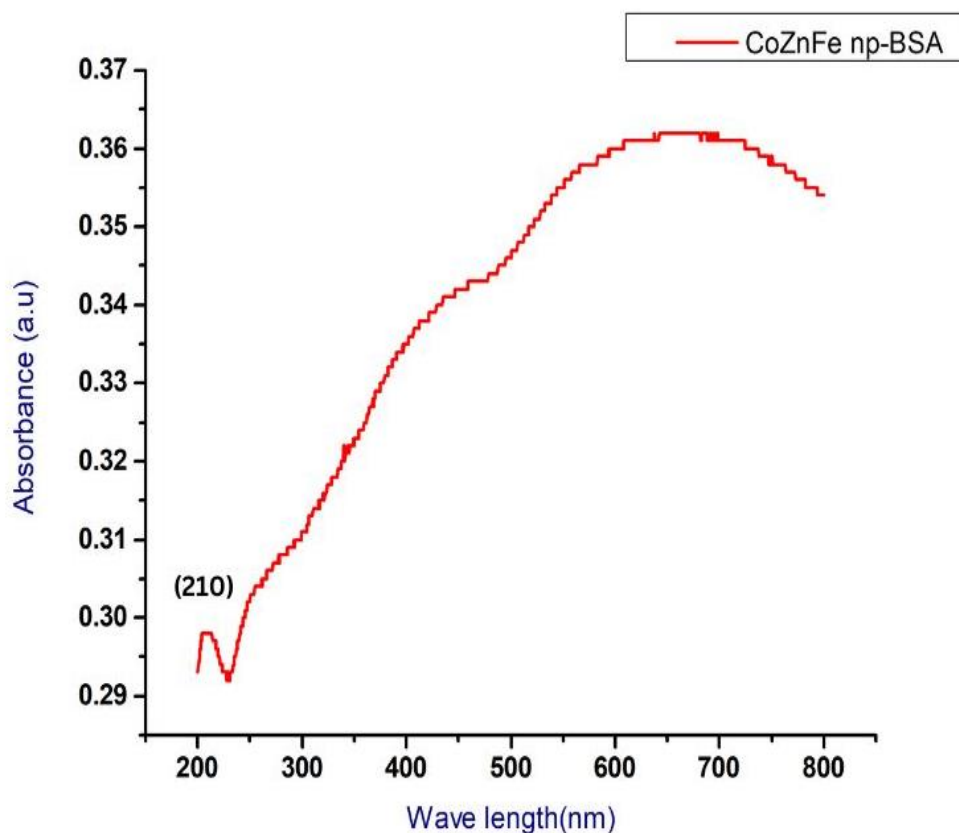


Fig 5.12 UV-Visible spectra $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA (Absorbance ~ 210 stemming from the particles in liquid solution).

5.3.2 FTIR

The samples' FTIR spectra show two strong bands in the range of 590^{-1} and 621^{-1}cm that is related to the stretching vibration modes of the metal-oxygen absorption bands Fe-O bonds in the crystalline lattice of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. For ferrites, they are distinctively evident in all spinel forms. This happens because the octahedral and tetrahedral sites of the metal have stretching vibration bands in this area. Moreover, FTIR spectra display an absorption band between 1247 cm^{-1} and 1650 cm^{-1} that relates to the stretching vibration of the carboxyl group (C=O) that is present in the molecule of BSA. That reveals information about secondary structural alterations in the BSA, which can reveal the degree of interaction with the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticle. For instance, a change in the BSA structure as a

result of interacting with the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoparticle may be indicated by a drop in the intensity of the amide I band at about 1650 cm^{-1} and an increase in the intensity of the amide II band at about 1540 cm^{-1} (Ahmad *et al.*, 2015).

FTIR absorption spectroscopy enables the identification of the spinel structure and verifies it. The peak at 1508 cm^{-1} that indicated the existence of amide II was more complicated than amide I. The symmetric bending vibration peak was discovered at 1427 cm^{-1} , while the peak associated with the amide III stretching vibration was discovered at 1263 cm^{-1} . (J. Li *et al.*, 2010).

The hydrogen bonding between the amine groups of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps and the hydroxyl group of the exudates was seen in the FTIR spectrum. The emergence of distinct spectral lines for amides ($1638.2; 1645.52\text{ cm}^{-1}$), carbohydrates' carbonyl groups ($3336.0; 41.868\text{ cm}^{-1}$), and a carboxylic group ($1543.3; 1384\text{ cm}^{-1}$) supports the interactions of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps with exudates. By adsorbing $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps to BSA, the release of Fe^{3+} and Co^{2+} ions is decreased. **Fig 5.13** FTIR $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA.

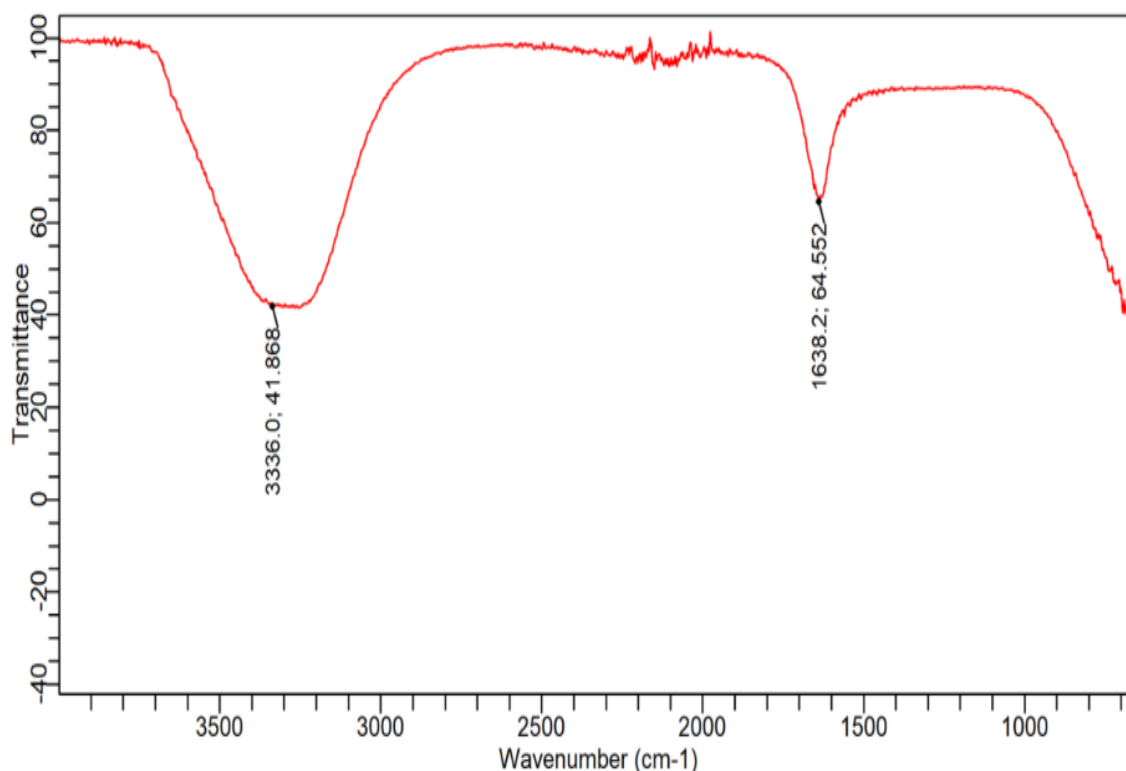


Fig 5.13 FTIR $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA.

5.3.3 DLS

A method for determining the size of particles, usually in the submicron range, is dynamic light scattering. Brownian motion is measured using DLS, which correlates it to particle size. The typical size of synthesised $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs and BSA- $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs has been determined by analysing DLS spectra. This is so that it is clear how the concentration and viscosity of the solution affect the mean hydrodynamic diameter of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ core, which is determined by the DLS approach and encircled by the organic and solvation layers. By using dynamic light scattering, the average diameter of BSA is determined. Similar values have already been reported (Kannan *et al.*, 2021). BSA molecules are affixed to the surfaces of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$, according to the DLS findings

It was measured how much BSA altered the size of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. BSA adsorption on the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps surfaces causes a size growth of up to 14%. This exemplifies the creation of a tightly packed protein corona on the surface of NPs. Moreover, the protein conformations undergo an induced structural change as a result of the BSA with $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps adsorption. Also, understand that the quantity of proteins that are adsorbed is influenced by the protein concentration that is present in the system (Bohara *et al.*, 2015). Because serum albumins have a negative charge at a pH of 7.4, an increase in the negative charge, therefore, signals the adsorption of BSA. This suggests that $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps and BSA molecules have developed electrostatic forces. Moreover, the interaction and charge reduction between BSA and $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps are also influenced by the van der Waals forces and steric contact.

The BSA- $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs complex continues to display a fairly monomodal size distribution profile with an appropriate level of polydispersity, which is noteworthy to observe in the current situation. The presence of the protein (BSA) does not cause the nanoparticles $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs to aggregate, which suggests that the nanoparticles are stable in the solution while the protein is present. Additionally, the significant rise in hydrodynamic diameter after the protein addition explains the contact between the BSA in $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NP in **Fig 5.14** DLS analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs conjugated BSA.

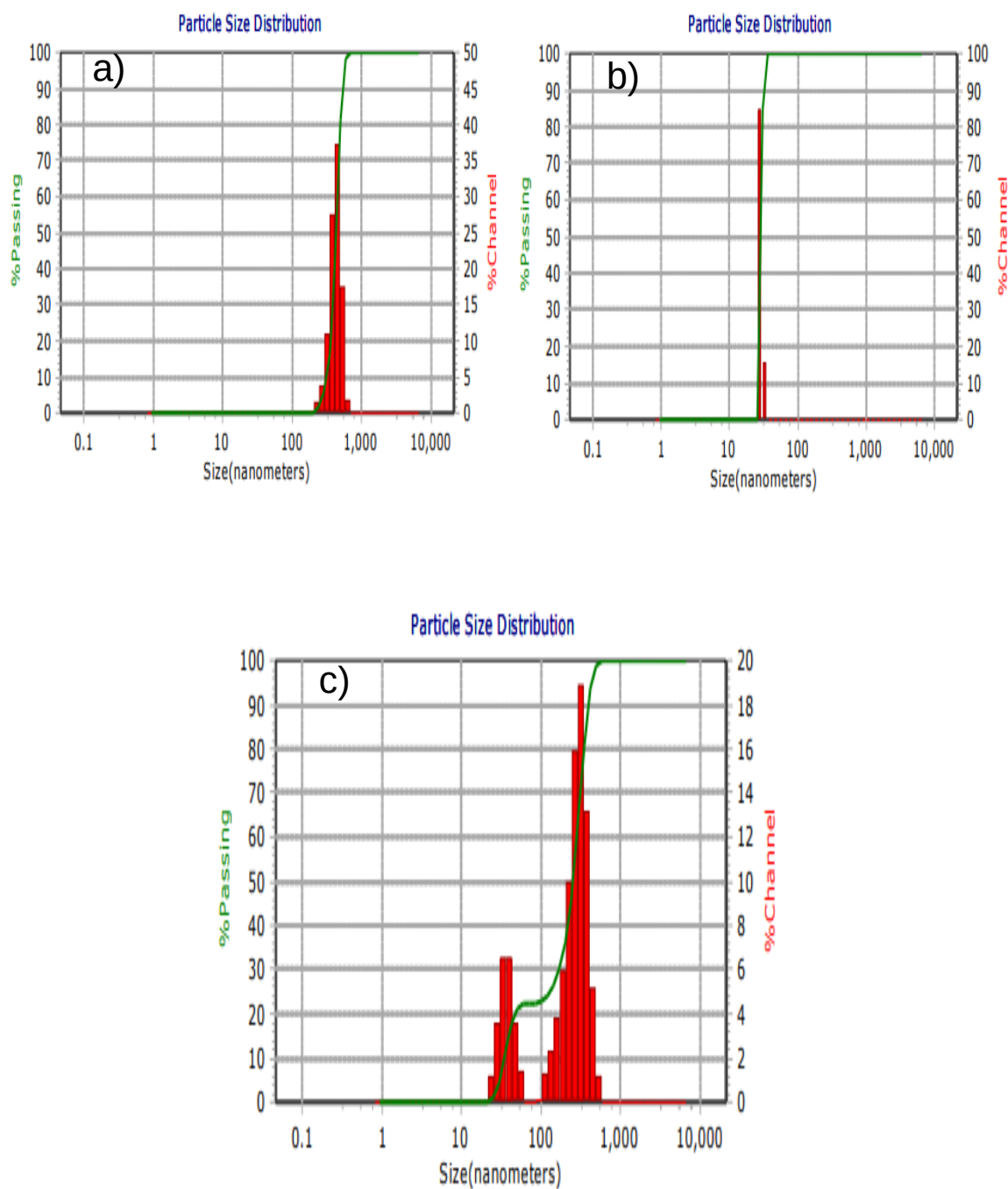


Fig 5.14 DLS analysis of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs conjugated BSA (a- Dia(nm)- 421, Vol%-100, Width -153.8; b- Dia(nm)- 28.33, Vol% -100, Width- 3.74 ; c- Dia(nm) -287.4 & 36.2, Vol% - 77.4 & 22.6 Width 173 & 16.01).

5.3.4 Zeta Potential

Zeta potential analysis results demonstrate the interaction between BSA and $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps surfaces. Moreover, the surface chemistry and behaviour

within the physiological environment are altered by the adsorption of BSA with $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. Value of the zeta potential, showing strong stability. The conjugates maintained a consistent size distribution after BSA conjugation. This rise is probably due to the development of a BSA layer around the NPs. After conjugation with BSA, the surface charge's zeta potential value is reduced. Given that BSA has a net negative charge, the drop in the surface charge value is attributed to this charge (Guindani *et al.*, 2020). This trend suggests that BSA was successfully conjugated to the surface of the NPs, along with the rise in mean particle diameter. A zeta potential analyser was used to examine the impact of changes in the surface charge of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. when BSA was present. The surface charge of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps was measured to be ± 48.5 mV and the presence of citrate ions on their surface accounts for their negative charge (Clogston & Patri, 2011). When a protein connects to a nanoparticle's surface, it can change the zeta potential and so reveal details about the type and strength of the BSA- $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ interaction (Nikam *et al.*, 2014).

The strong repulsive forces between the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps made it abundantly evident that the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps are extremely stable. BSA was found to have a surface charge of ± 10.88 mV (Karthiga *et al.*, 2017). When $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps interacted with the highest concentration of BSA, it was found that their surface charge changed to ± 56.29 , which is more stable. Electrostatic interaction was what caused the net charge of the BSA conjugated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps complex to alter. the interplay between $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps and BSA. As a result, the zeta potential tests verified that BSA was adsorbed onto the surface of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps. **Table 5.1** Particle analysis of zeta potential. **Fig 5.15** Conjugative analysis of zeta potential

S.NO	PARTICLE	mV
1	$\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$	± 48.5
2	BSA	± 10.88
3	$\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4 + \text{BSA}$	± 56.29

Table 5.1 Particle analysis of zeta potential.

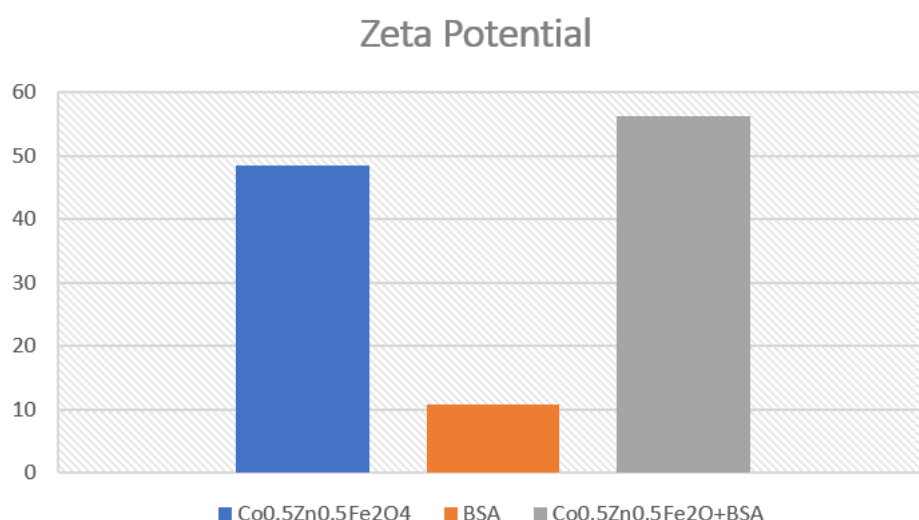


Fig 5.15 Conjugative analysis of zeta potential.

5.4 CONJUGATION ANALYSIS

5.4.1 SDS Page

Protein molecules exposed to radiation undergo chemical modifications as a result of the by-products of water radiolysis, which result in hydrated electrons and hydroxyl radicals(Akhavan *et al.*, 2010). The polypeptide chain of the irradiated BSA has slightly broken down, and there are very few aggregated molecules. There is a considerable increase in the aggregated proteins that could not pass through the gel over at the doses of (arrows) (Palma-Albino *et al.*, 2021). The cross-linked by-products of the damaged protein are responsible for these larger molecular weight aggregates(Chaudhary *et al.*, 2016). The BSA are linearized and denaturized with SDS, a detergent that also breaks up non-covalent connections, and then they are separated according to their molecular weight using a polyacrylamide gel matrix.

The protein profile of the protein concentrates and fractions was examined using SDS-PAGE. This study showed several bands ranging in size from 16 to 90 kDa, many of which were visible bands for proteins with molecular weights of 18, 20, 25, 50, and 58 kDa. In the 100 and 80µg, respectively, the intensity of the 25 kDa and 18 kDa bands was higher. **Fig 5.16** SDS Page analysis of Protein concentration.

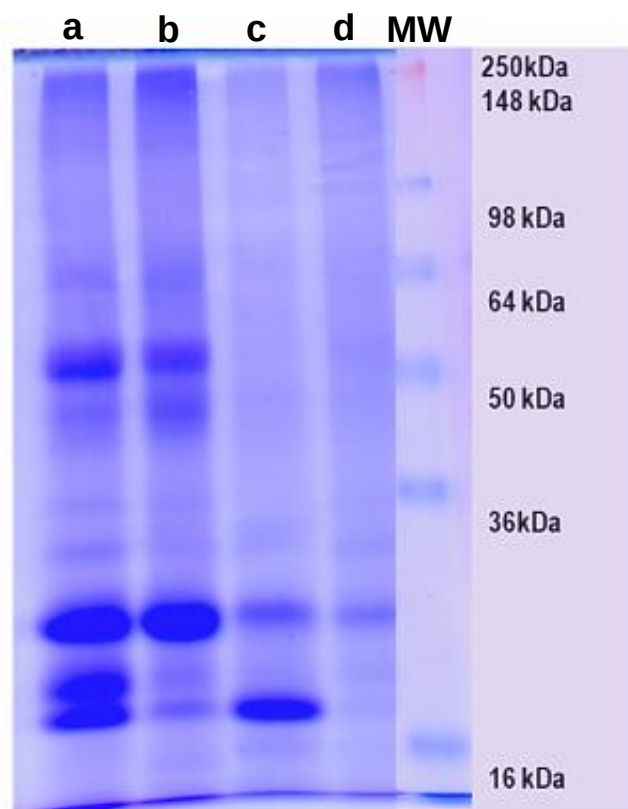


Fig 5.16 SDS Page analysis of Protein concentration (a- 100ug/ml; b- 80ug/ml; c -40ug/ml; d-20ug/ml; MW-molecular weight).

5.4.2 Bradford assay

The sequential Bradford assay's linear operating range was more constrained and demonstrated saturation at BSA concentration (Ho *et al.*, 2015). Vary protein Concentrations are shown in Fig, each row representing an of the others, changing colour at varying doses following the appropriate incubation times. For each microplate, BSA concentrations rise from right to left. A steady variation in the absorbance of a single dye colour in the microplate served as a representation of the BSA concentration calibration curves for the Bradford assay (Lopez–Luna *et al.*, 2018). Here, a rise in the brown and blue intensity of the Bradford assay respectively indicated an increase in BSA concentrations. As a result, only one wavelength, which corresponds to the dye's Analyst peak absorption (Gupta *et al.*, 2020), was used to quantify their absorption. **Fig 5.17** Calorimetric analysis of different concentrated conjugated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA. **Fig 5.18** Protein concentrated

curve of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA. **Fig 5.19** Bradford assay absorbance value of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ + BSA.

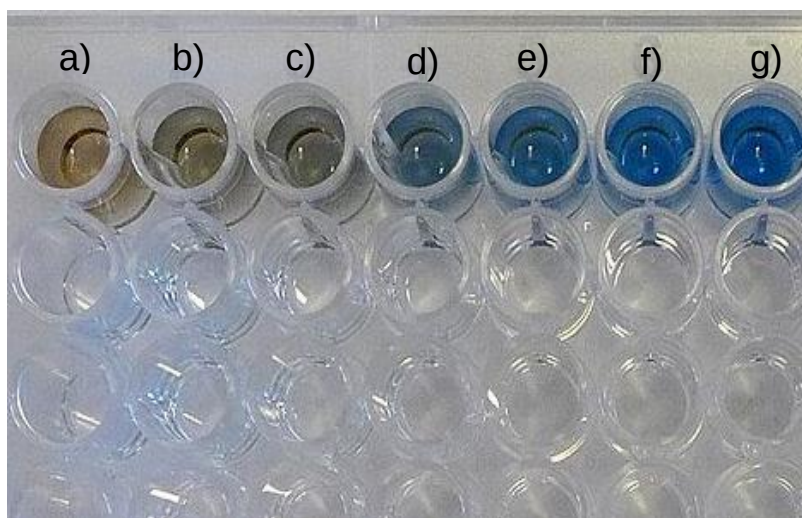


Fig 5.17 Calorimetric analysis of different concentrated conjugated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA (a – blank; b- 5ug/ml; c - 10ug/ml; d – 20ug/ml, e – 40ug/ml; f – 80ug/ml; g – 100ug/ml).

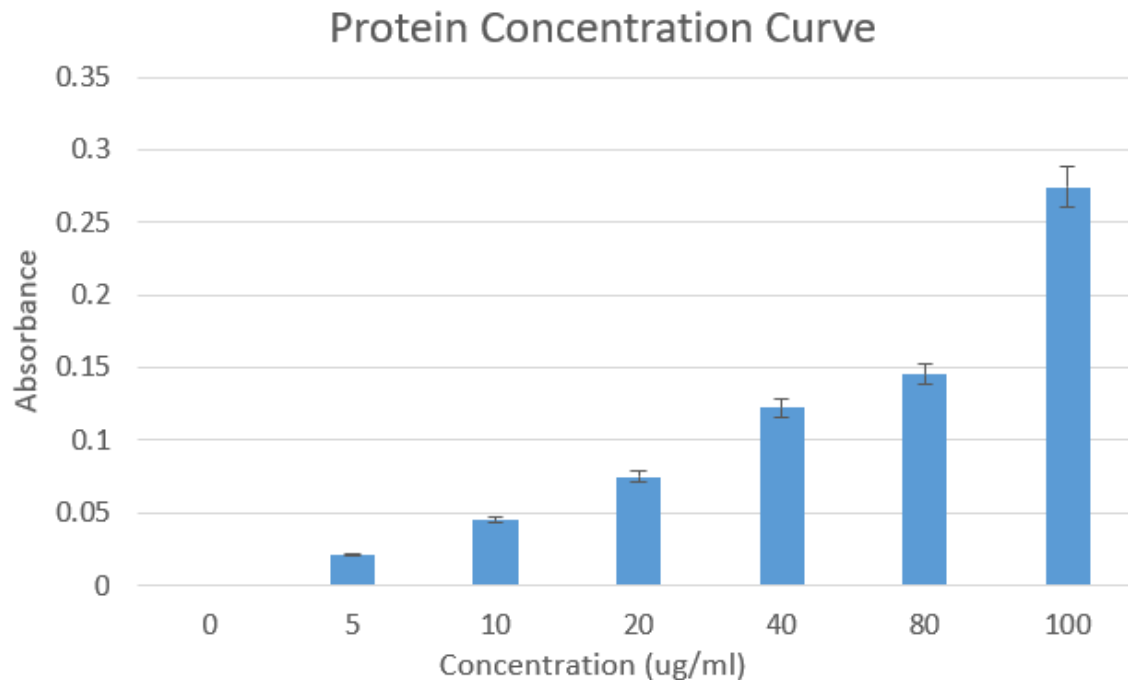


Fig 5.18 Protein concentrated curve of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA.

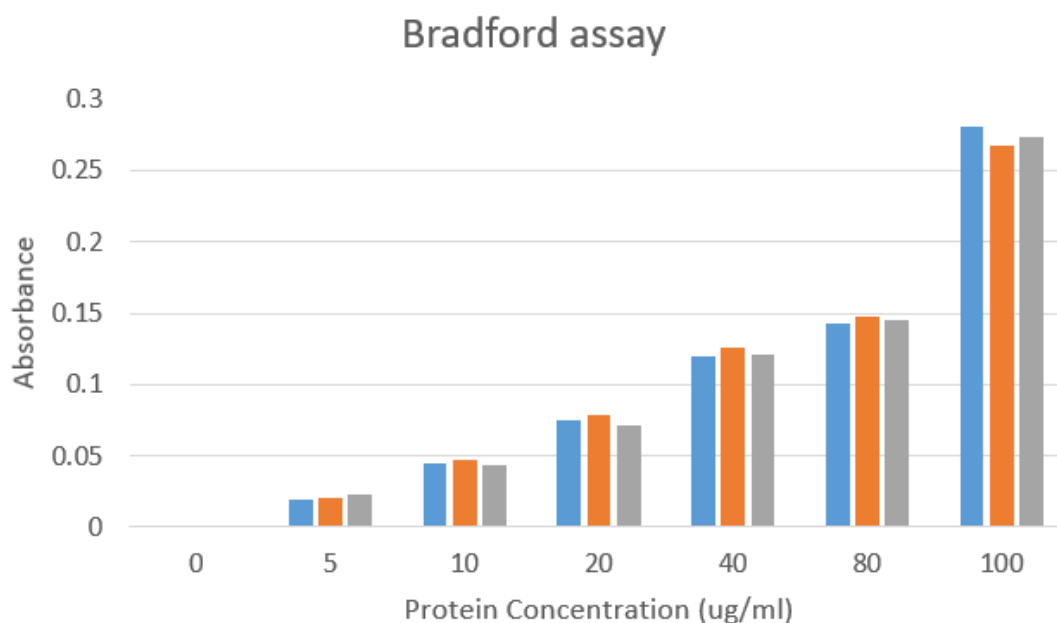


Fig 5.19 Bradford assay absorbance value of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ + BSA.

5.5 TOXICITY ANALYSIS

5.5.1 MTT assay

To determine the effects of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps on cell viability, cells were incubated following 5, 10, 20, 40, 80, and 100 $\mu\text{g/ml}$ treatment. After incubation, cell viability was evaluated by applying the 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium. The data obtained showed that there was a statistically significant difference in cell viability at 5, 10, 20, 40, 80, and 100 $\mu\text{g/ml}$ doses compared to the control group. On the other hand, there was a statistically significant for the use of nano drugs by analysing the cell viability of the group exposed to the dose of 40 $\mu\text{g/ml}$ $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps seen to be suitable for the use of nanomedicine. **Fig 5.20** calculated the value of IC_{50} ,

The graph represents in form of a straight line (R^2 almost equals 1)

$Y=mx-c$ where $m= 0.7912x$ and $c= 5.2944$

To calculate the IC_{50} value substitute $y=50$ from the equation

Where IC_{50} is found to be **69.88 $\mu\text{g/ml}$**

Therefore, in the HepG2 cell lines, several cell-based experiments were carried out to assess the impact of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ MNPs on cellular activity. Since only metabolically active cells can reduce MTT, the MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide) assay can be used to verify cell growth and death (Ch. Vinuthna, D. Ravinder, 2013). According to the results of this study, cell viability is dose and time-dependent, and it steadily declines as $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ MNP concentration and incubation time increase. The cell viability for HepG2 is up to 82% by MTT assay after 24 hours of incubation with the observed concentration of 40 $\mu\text{g/ml}$ of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ MNPs. **Fig 5.21** Cell Viability Dose-Response curve of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ treated HepG2 cells. **Fig 5.22** MTT absorbance value of HepG2 cell line cultures. **Fig 5.23** Microscopic images of HepG2 cells when treated with different concentrations of Pristine $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$.

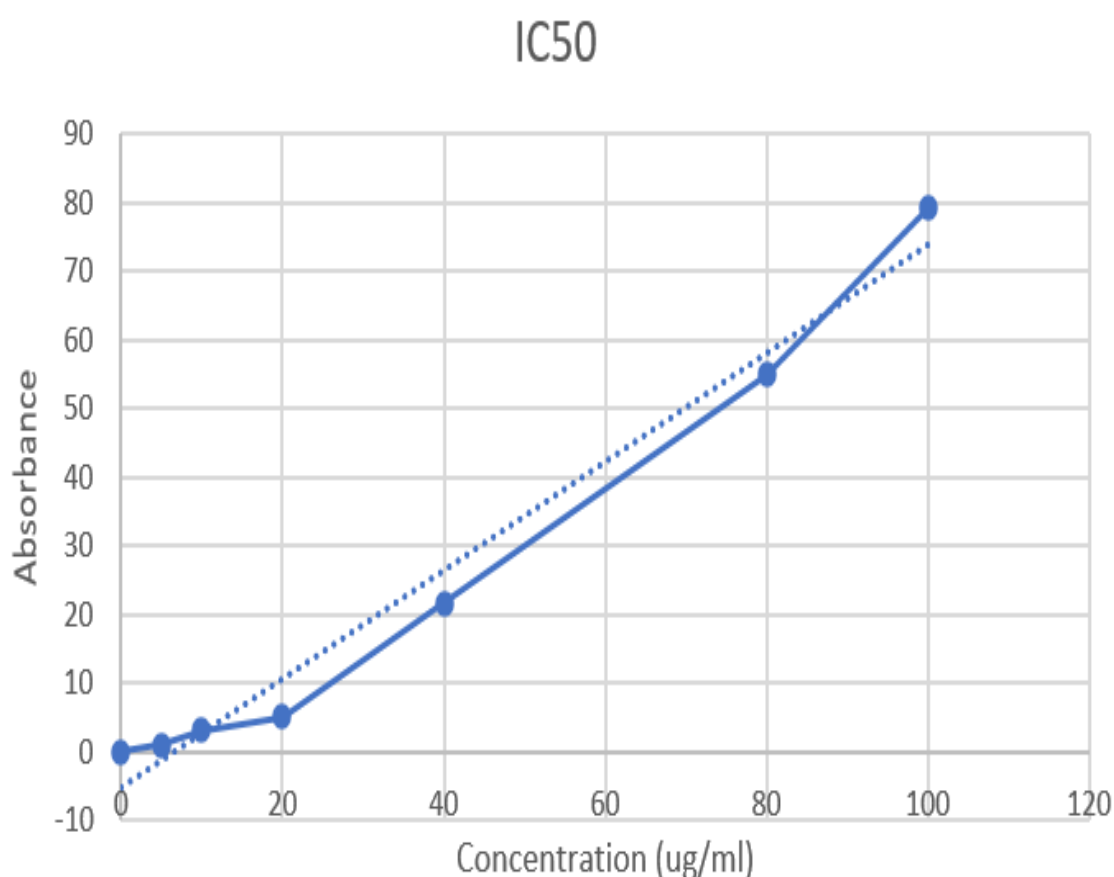


Fig 5.20 Calculated the value of IC_{50} .

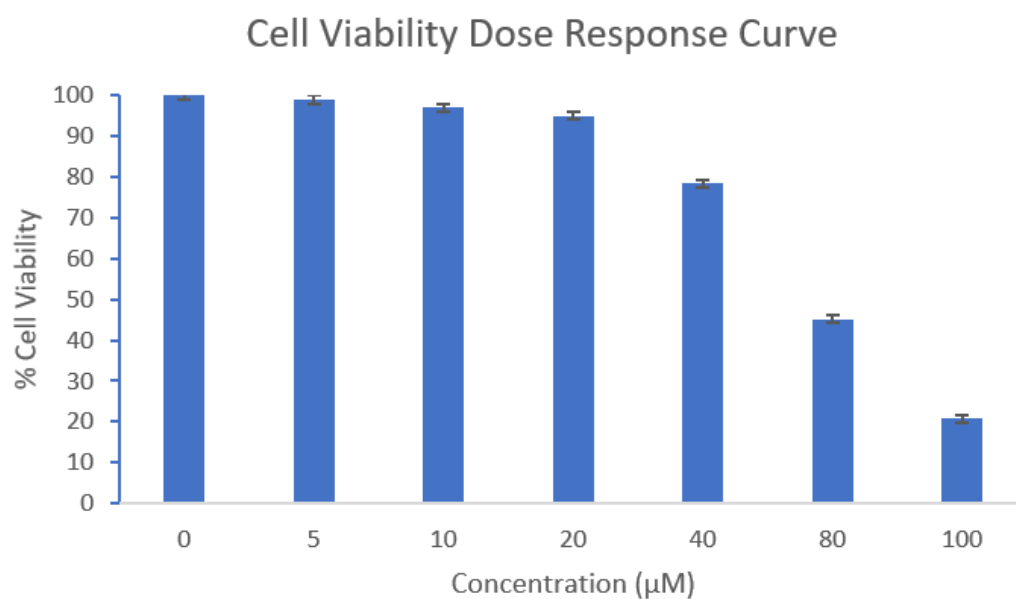


Fig 5.21 Cell Viability Dose-Response curve of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ treated HepG2 cells.

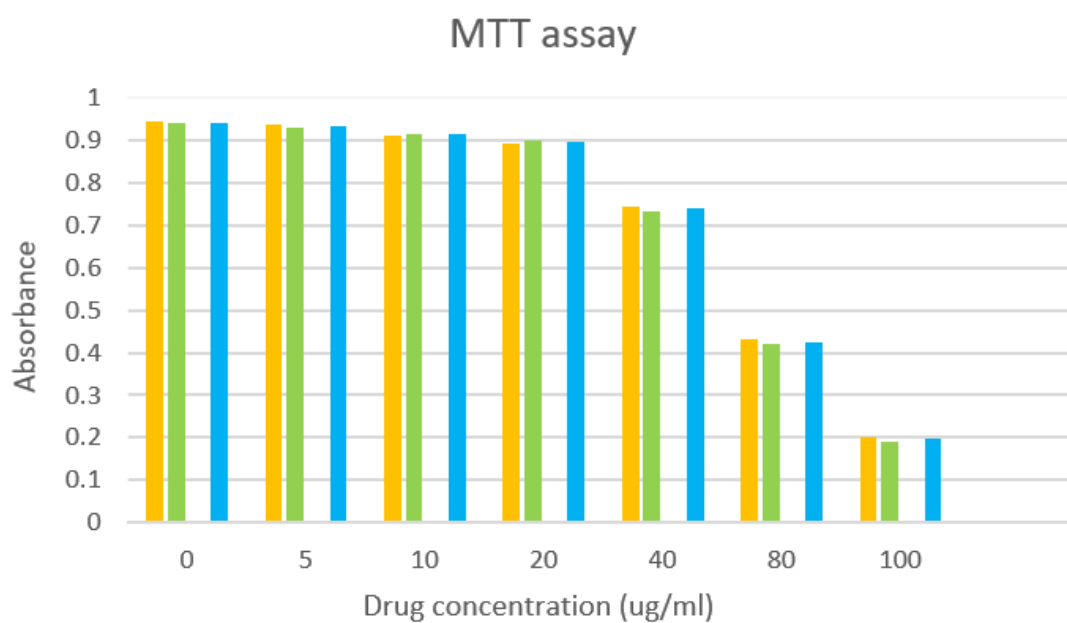


Fig 5.22 MTT absorbance value of HepG2 cell line cultures.

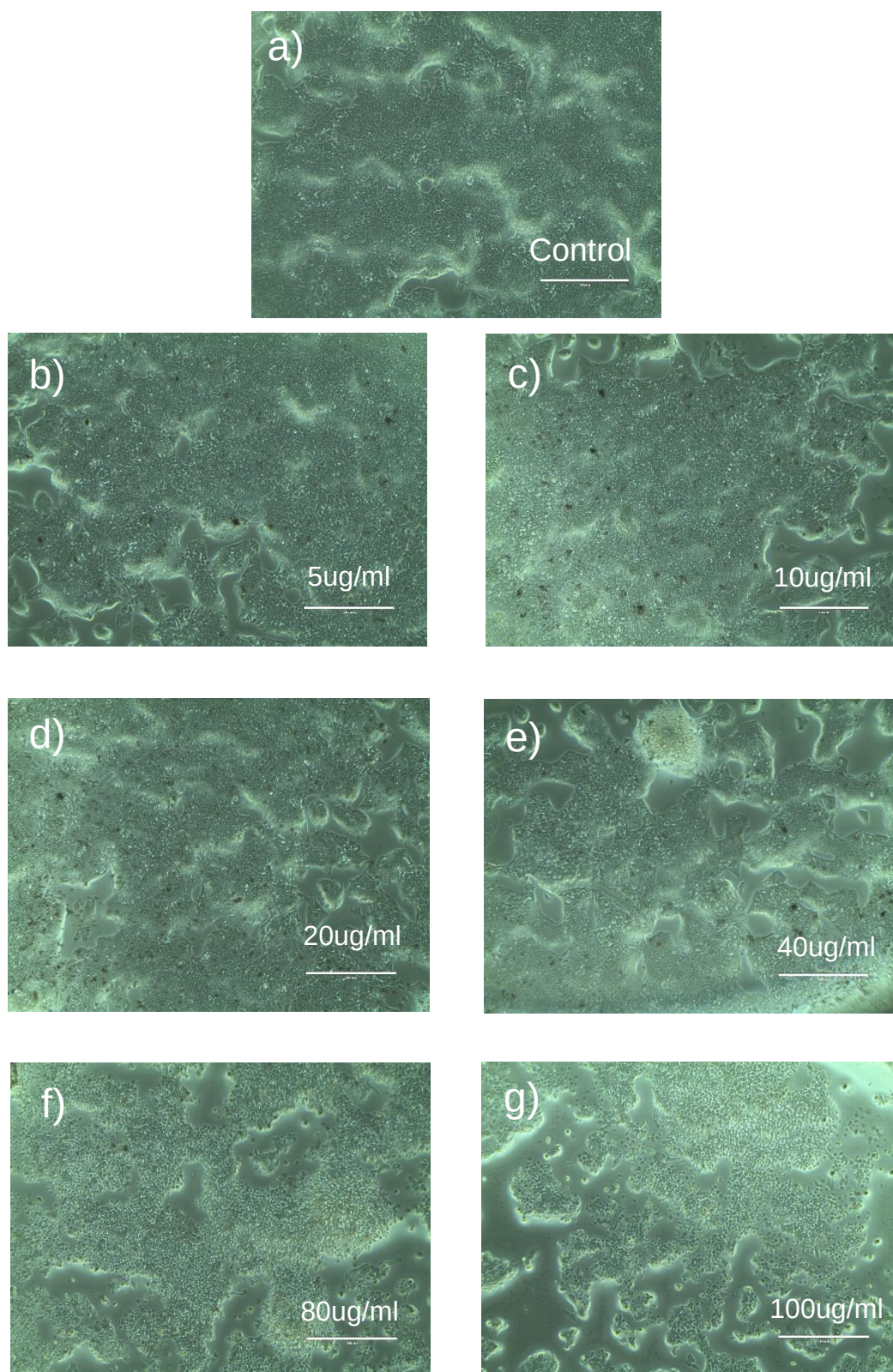


Fig 5.23 Microscopic images of HepG2 cells when treated with different concentrations of Pristine $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ (a- control; b- 5ug/ml; c- 10ug/ml, d- 20ug/ml; e- 40ug/ml; f- 80ug/ml; g-100ug/ml).

5.5.2 ROS assay

The relationship between the absorbance and incubation time for the ferrite NPs. As the incubation time increased, the drug-absorbing ability of the cells increased. In a 96-well flat-bottom microtiter plate, 1×10^5 HepG2 cells were incubated for 0 to 49 h. The absorbance of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs with a concentration of 5-100 $\mu\text{g}/\text{ml}$ is shown. After incubation, the absorbance reached and after 24 and 49 h respectively.

The maximum cellular absorbance was observed at 100 $\mu\text{g}/\text{ml}$. After 24–49 hr the Observed $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ Nps in the HepG2 cell model exhibited a sudden homogeneous/saturation trend in the absorbance, indicating that the maximum absorbance of the cells occurred at a concentration. Several previous reports showed that ferrites have nominal effects on the toxicity of malignant cell models in the absence of light/laser(Alaraby *et al.*, 2020). High fat or high glucose media can cause oxidative stress, as can hydrogen peroxide or tert-butyl hydroperoxide. To allow for probe absorption and intracellular conversion to the fluorescent chemical, the cells are treated and then incubated with a fluorescent ROS probe, such as DCFH-DA, for a predetermined amount of time.

Based on the physical and chemical properties of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$, it could be expected that an increased number of delocalized electrons on their surface and, hence, an increased hydrophobicity could lead to an enhanced formation of ROS. This in turn could be attributed to an inductive effect as a result of a dense electron cloud. As a result, data using the DCFH-DA assay showed that 100 $\mu\text{g}/\text{cm}^2$ $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ led to a significantly increased intracellular production of ROS following a 24 and 48 h exposure, The increased production of ROS and the increase in oxidative stress following $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ exposure was also reported. (Kowsalya *et al.*, 2021). **Fig 5.24** ROS assay Fluorescence intensity is an accurate measure of ROS production in the hepG2 cells, oxidative stress. The effectiveness of antioxidants in lowering ROS levels and shielding hepG2 cells from oxidative damage can also be assessed using the ROS test.

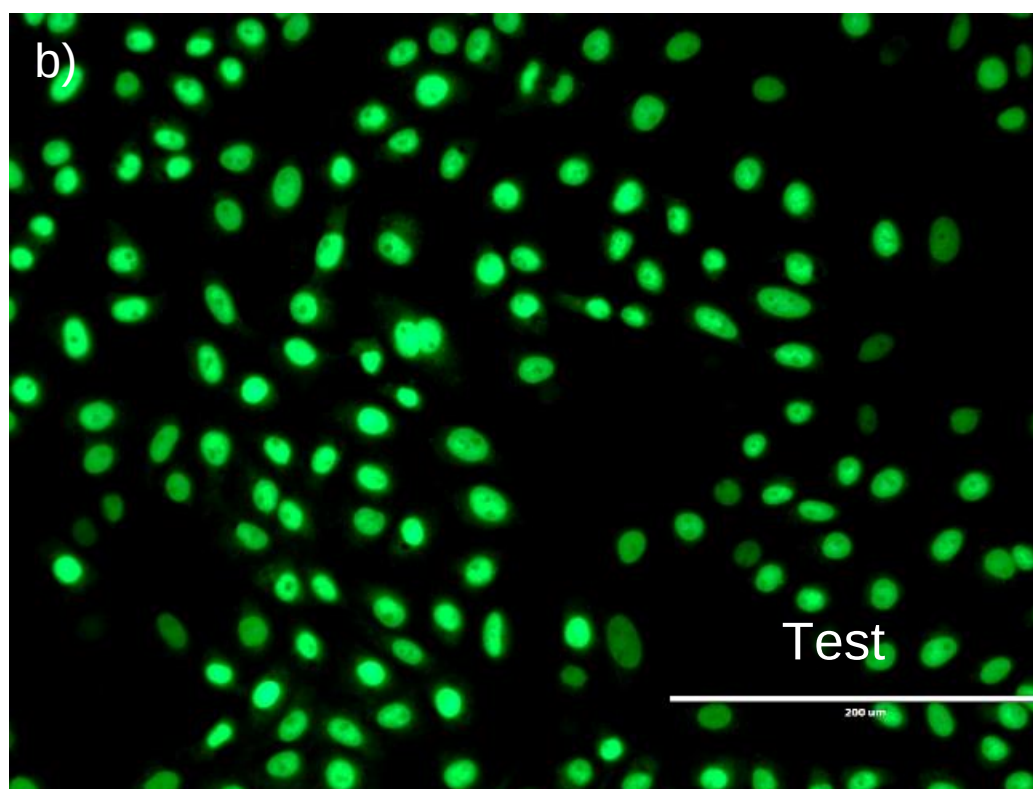
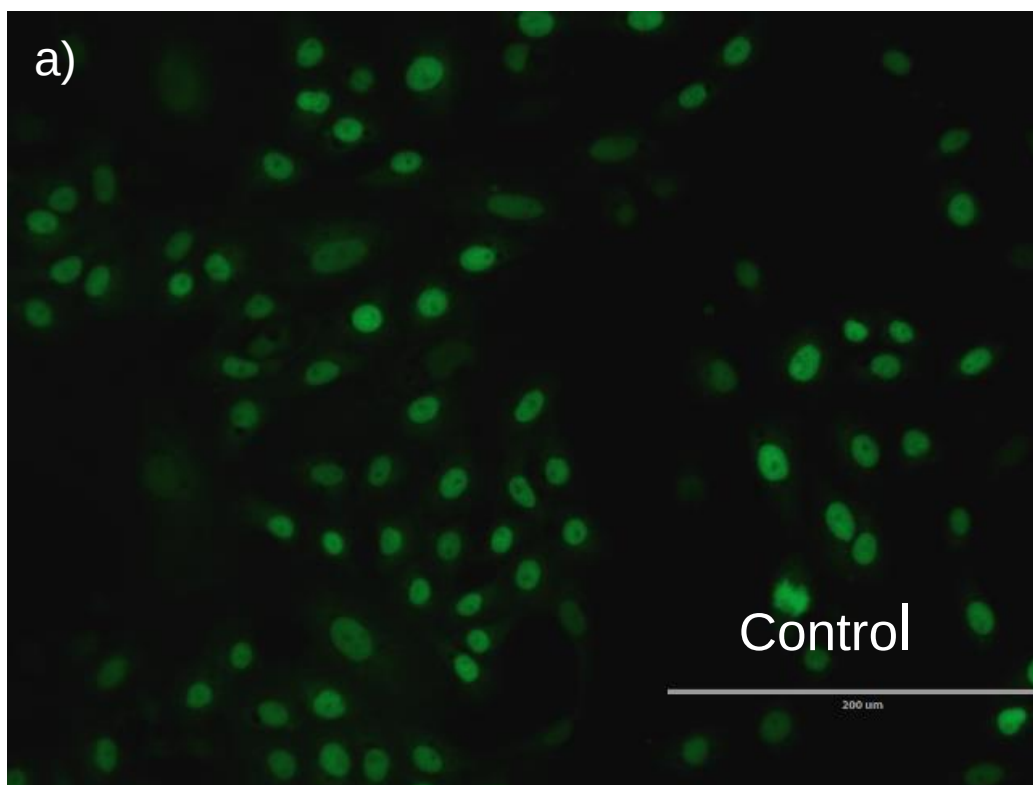


Fig 5.24 ROS assay (a-control; b- Test).

CHAPTER – 6

SUMMARY AND CONCLUSION

The $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$, X-ray powder diffractometry (XRD) was used to determine and characterise the Ferrite structural and morphological characteristics. The existence of a single phase with a cubic spinel crystal structure (21-28 nm) and the average particle size of the nano-powders are both confirmed by the X-ray diffraction pattern. TGA analysis, The Zn^{2+} substitution in ferrites is reported to be responsible for the observed fluctuation in crystallinity and rise in lattice constant. The unit cell parameter is found to increase linearly with increasing concentration of zinc due to larger ionic radii of Zn^{2+} ion. When the powder was calcined at 500 °C, its crystalline form was revealed. impact of Zn^{2+} substitution on the cobalt Zinc ferrite nanoparticles' structural characteristics. The evaluation of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ dispersion status using UV-Visible spectroscopy is effective. FT-IR spectra explain tetrahedral and octahedral clusters and confirm the presence of the M-O stretching band in ferrites. The ratio of the intensity of the D- Raman peak and G- Raman peak (I_D/I_G) is used in the characterization of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$. Atomic force microscopy was used to determine the nanoparticle size and detailed map of the surface topography.

Diverse spectroscopic methods were used to examine the interaction between BSA and $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$. The data led us to the conclusion that the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs attach to the BSA through ground state complex formation, which results in static FL quenching, and that the binding and adsorption process is largely exothermic and spontaneous in nature. The investigation of the secondary and tertiary structures of BSA after its interaction with $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs was done using FTIR and UV-visible. The development of a substantial BSA corona layer on $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs surfaces was confirmed by, DLS, and zeta potential studies. The creation of the protein corona was confirmed by a comparison of theoretical data and experimental quenching. Additionally, the physical characteristics of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs are significantly impacted by this protein corona (adsorption) production.

The SDS-PAGE investigation showed that the creation of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ can help prevent the aggregate of radioactive protein to some extent. The effectiveness of protein from the conjugates was evaluated using SDS-PAGE of the conjugates. Calorimetric analysis of different concentrated conjugated $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ with BSA was carried out by Bradford assay the colour change is absorbed.

The MTT and ROS assays validated the conjugates' biocompatibility and toxicity. BSA conjugated to $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs imparted an increase in cellular absorption by HepG2 cells, according to cell uptake assays, which is a desired property for drug delivery application in the treatment and biomedical applications. The surface engineering of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs conjugation method has proven to be quite successful, and it may be used to modify the surface of $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ NPs and BSA. Further research can be carried out based on *In vivo* analysis using Rats, zebrafish, and *Caenorhabditis elegans*.

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ANNEXURE

The book chapter “**Emerging graphene-based nanomaterials as DNA biosensor(s) using lab-on-chip, computational approaches, and robotics**” was submitted on 12th August to Elsevier, CRC Press (Taylor & Francis) and it was **ACCEPTED** on 1st February, 2023 for publication.



Acceptance for the Contributed book chapter

1 message

Dr. Yugal Kishore Mohanta <ykmohanta@gmail.com>

Wed, 1 Feb, 2023 at 8:02 pm

To: Dr. Kunal Biswas <kunalbiswas.irc@sathyabama.ac.in>, Dr. Kunal Biswas <kunal.sapiens@gmail.com>

Dear Dr K Biswas
Greetings!

I am glad to inform you that the submitted chapter from your team EMERGING GRAPHENE-BASED NANOMATERIALS AS DNA BIOSENSOR(S) USING LAB-ON-CHIP, COMPUTATIONAL APPROACHES, AND ROBOTICS has been accepted for publication in the book Graphene Nanomaterials in Food, Health & Agriculture sectors published by CRC Press.

The final proof will be sent to you by the CRC publisher for your final corrections.

Best Wishes
Editorial Team

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Presented the article “*in vitro* Evaluation of cobalt zinc ferrite cytotoxicity and cytocompatibility utilizing a hepatocyte cell line: A Review” on 12th GCORT’ 23 conducted by Anna university and the abstract has been published GCORT proceeding by “Association of pharmacy professionals”

POSTER PRESENTATION (virtual mode)

IN VITRO EVALUATION OF COBALT ZINC FERRITE CYTOTOXICITY AND CYTOCOMPATIBILITY UTILISING A HEPATOCYTE CELL LINE: A REVIEW

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Magnetic nanoparticles (NPs) show significant potential in biomedical applications such as hyperthermia, biosensors, magnetic resonance imaging (MRI), and drug delivery. Magnetite (Fe₃O₄) has been the most studied magnetic nanoparticle over the years. It has gotten simpler in recent years to create new, more potent varieties of magnetic NPs. Due to their intriguing magnetic and electrical characteristics with outstanding chemical and thermal stability, spinel ferrite nanoparticles (NPs) are significant. We have explored the crystal structure, dielectric, transport, and magnetic characteristics of cobalt zinc ferrite as a follow-up to our searches for suitable magnetic candidates for multiferroic composite structures. However, the inadequate understanding of these NPs' biological responses may ultimately limit their potential for use in biomedicine. To ensure that magnetic NPs have positive effects in the field of biomedicine, it is therefore vital to investigate their biological fate and potential harmful response. Toxicological studies with a hepatocyte cell line. Finding a viable alternative *in vitro* model to accurately predict the *in vivo* toxicity is therefore essential. Human cell lines are good *in vitro* models and sensitive tools for high-throughput toxicity screening, with the potential to reduce animal use in toxicity studies. The creation of mechanistic data that may be used for toxicity evaluations using cell lines is a possibility. To investigate the cytotoxicity and cytocompatibility of NPs, we have used extensively utilized *in vitro* cell culture models, liver (HepG2) cells. As a result, this review was carried out to assess the toxicity of the complex of Cobalt Zinc Ferrite nanoparticles on liver cells.



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GCORT'23

GLOBAL COLLOQUIUM ON ONCOLOGY RESEARCH AND THERAPY

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CERTIFICATE

THIS CERTIFICATE IS AWARDED TO

MITHRINTHAA S

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

Has participated / Presented a poster presentation in the
Global Colloquium on Oncology Research and Therapy -
Virtually / Physically organised by the Department of
Pharmaceutical Technology, BIT Campus, Anna University,
Tiruchirappalli, held on 11 & 12 February 2023.

Dr. S. Latha
Convener

Dr. P. Selvamani
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Dr. A. Puratchikody
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Dr. T. Senthilkumar
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Submitted an abstract on “**Evaluation of cobalt zinc ferrite nanomaterials towards biological macromolecules on mesenchymal stem cell: A review**” on 27 -29th March was submitted for ICONN’23 conducted by SRM University and the abstract has been **published** in the ICONN’23 proceedings

7th International Conference on Nonoscience and Nanotechnology (ICONN 2023), SRM IST, Chennai.

Evaluation of cobalt zinc ferrite nanomaterials towards biological macromolecules on mesenchymal stem cell: A review

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Abstract

Nanomaterials are currently the topic of significant research due to their vast range of potential uses in biological, optical, and electronic sectors. In biological applications, the magnetite phase is preferred over the maghemite phase because of increased magnetization saturation and magnetic susceptibilities. Cobalt-zinc ferrite, a magnetic compound with the same crystalline structure as magnetite, is more resilient to oxidation and hence more appealing for long-term applications, such as in electronics and biological research. Due to their small size, which allows them to reach nearly every part of the body, including the circulatory system and cells, these nanoparticles are constantly being researched in the biological and medical fields. Mesenchymal stem cells, which have therapeutic potential, were used to study the biological effects of different SPIONs. The findings showed that SPIONs have a potent potential to cause oxidative damage to lipids, proteins, and DNA in tagged cells. In this, we exclusively focus on biological macromolecule damage in mesenchymal stem cells and the cytotoxicity effect of cobalt zinc ferrite.

Reference

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Certificate of Participation

This is to certify that Mr. / Ms. / Dr. **Mithrinthaa** has participated and presented a paper entitled **Evaluation of cobalt zinc ferrite nanomaterials towards biological macromolecules on mesenchymal stem cell: A review** in the 7th International Conference on Nanoscience and Nanotechnology (ICONN-2023) organized by Department of Physics and Nanotechnology, SRM IST, India during March 27- 29, 2023, in association with Shizuoka University, Japan; National Yang Ming Chiao Tung University, Taiwan; GNS Science, New Zealand; University of Rome Tor Vergata, Italy; Asian Consortium on Computational Materials Science (ACCMS), Japan; Indian Ceramic Society; Indian Physics Association (IPA); Solar Energy Society of India (SESI); Innovation, Science & Technology Foundation - Tirupati (ISTF-T) and co-sponsored by Defence Research and Development Organization (DRDO), India; Council of Scientific & Industrial Research (CSIR), India; The Indian Science Congress Association (ISCA) and Springer Nature.

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Submitted the review article “**Exploring the potentials of magnetic nanoscale material for different biomedical applications: a review**” on 26th October in **BioNanoScience** Journal.

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Mithrintha Shankar <mithrinthaashankar@gmail.com>

BioNanoScience: Decision on "EXPLORING THE POTENTIALS OF MAGNETIC NANOSCALE MATERIAL FOR DIFFERENT BIOMEDICAL APPLICATIONS: A review"

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To: mithrinthaashankar@gmail.com

20 April 2023 at 07:45

Dear Dr Shankar,

Re: "EXPLORING THE POTENTIALS OF MAGNETIC NANOSCALE MATERIAL FOR DIFFERENT BIOMEDICAL APPLICATIONS: A review"

The corresponding author has been asked to revise the above submission on which you are listed as a contributing author. Meanwhile, we would be grateful if you would carefully check the author details, including spelling and sequence of given and family names; email and affiliation:

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If you spot any errors, please contact the corresponding author directly to ensure the revision moves smoothly through the submission process.

Kind regards,

Editorial Assistant
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EXPLORING THE POTENTIALS OF MAGNETIC NANOSCALE MATERIAL FOR DIFFERENT BIOMEDICAL APPLICATIONS: A review

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ABSTRACT

Magnetic nanotechnology manipulates magnetic fields and has distinct physical and chemical properties characteristics. Iron, nickel, and cobalt are known to be standard MNPs. The application of MNPs is biomedical, biotechnology, environmental remediation, and MRI medical. MNP plays a vital role in drug targeting and drug delivery system. This review paper includes the biomedical application of MNPs, explaining the interaction of MNPs in living organisms and analyzing the toxicity. And enhance the detail about the drug delivery system, such as targeted drug delivery, and explain the biocompatibility. The compatibility of MRI and the MNP is described in detail. MRI, CT, and bioimaging demonstrated MNP applications in particular. By this, we could analyze drug delivery, the interaction of tumors, and the toxicity effect. We could explain the synthesis and fabrication methods of MNP, such as bottom-up, top-down, and green synthesis. And challenges associated with nanoparticles have been described, such as environmental toxicity, safety measurements, and stability.

Keywords: Drug delivery, TDD, Toxicity, MRI, CT, bioimaging

ABBREVIATIONS

BBB-Blood brain barrier

CNS- Central Nervous System

CT-Computerized Tomography

DNA- DeoxyRNA

DMS- Dilute magnetic semiconductor

GMR- Giant magnetoresistance

GION-Gold iron nanoparticle

HDD- Hard disk drive

IONP- Iron nanoparticles

MNM- magnetic nanomaterial

MRI- Magnetic resonance imaging

MNP- magnetic nanoparticle

OI- Optical imaging

PEG- Polyethylene glycol

PDA- Plasmid DNA

QCMNPs-Quercetin-coated MNP

RNA- Ribonucleic acid

SPECT- Single photon emission CT

TMR-Tunnelling magnetoresistance

1. INTRODUCTION

MNM plays a vital role in biomedical research and applications. Nanomaterials have unique properties like high reactivities and surface volume ratios. It has been compared to the bulk micron structure counterpart. The size of nanomaterial is less than 100nm, MNPs have several biomedical applications, for example, cellular therapy is involved in cell targeting, and labelling provides a tool in cell biology research to analyse the cell, target drug delivery, tissue repair, MRI (MRI), and hyperthermia for cancer treatment [1]. Efficient treatment of high saturated magnetization is used such as Fe, Co, Ni, or metal oxides like Fe_3O_4 Fe_2O_3 . High saturation magnetization is possessed by pure metal; it is highly toxic and susceptible to oxidation [1]. It gives a more significant application in SPIONs and promising results in biological applications[2].

The character of MNPs possesses a superficial effect and anisotropy; it gives a better primordial understanding and helps develop biomedical applications like MRI [3,4]. MNP achieves unique and significant properties, especially in their stability due to their surface functionalization [4]. Research gives excellent results in MNPs due to their protective behaviour and adaptation to several chemicals and molecules [6,7]]. MNPs show promising results in a magnetic field, antiferromagnetic materials such as MnO, CoO, NiO, and CuCl_2 , and ferrimagnetic materials such as Fe_3O_4 and Fe_2O_3 .

Cobalt ferrite gives promising results in medical science. The main reason is their high magneto crystalline anisotropy origin at spin-orbit (L-S) combined at crystal lattices. Cobalt ferrite is used to isolate DNA[7]. CoFe_2O_4 gives promising results in nanomedicine; it is a hard magnetic material with high curie temperature and high coercivity at room temperature. It shows excellent chemical stability, wear resistance, mechanical hardness, ease of synthesizing, and electrical insulation[7]. It is used in DNA biosensors and drug delivery and has a more significant application in hyperthermia.

Ferrimagnetic material proposes various applications in targeting the brain and cell labelling. IONPs consist of superparamagnetic properties; it has the potential to carry a drug; IONPs are used to trace medicines within the body using MRI (MRI), as well as it is used to target a selective tissue. [8].IONP is used in CNS (CNS) applications in drug delivery and imaging platform, and the toxicity of nanoparticles is a concern in CNS. IONP is used to track and monitor traumatic brain injury and image various inflammation in CNS. IONP is mainly

used in brain imaging applications and involves CNS drug delivery. The most common compound of IONP is Maghemite (Fe_2O_3) and magnetite (Fe_3O_4)[8]; both are iron oxide that has a surface defect and atom vacancies as polar amphoteric OH decoration, they are traceable in surface manipulation and functionalization [9] due to their polymorphism involved in temperature induces the transition, which is unique biochemical, catalytic, and magnetic properties that provide a biomedical application[10]. Amino silane-coated IONPs (AmS-IONPs) are used in drug delivery applications in the brain. [8] MNP aims to develop core-shell nanostructure, corroborating characteristics with synthesizing route. This way, we could discover new functionalization and provide new applications and performance in the biomedical field.

2. SYNTHESIS AND FABRICATION OF MNPS

The MNP can be synthesized with numerous phases and compositions, including pure metal such as Fe and Co, spinel-type ferromagnets (MgFeO_4 , MnFe_2O_4 , and CoFe_2O_4), alloys (CoPt_3 and FePt) and iron oxides like Fe_3O_4 and Fe_2O_3 . Many publications show influential high stable, shape-controlled, and monodisperse MNPs. Several popular methods are used to synthesize high-quality MNPs, such as co-precipitation, thermal decomposition, reduction, micelle, hydrothermal, and laser pyrolysis[11].

2.1 BOTTOM UP

Bottom-up methods are also constructive; the material is built from atoms to clusters to nanoparticles.[12]. Direct self-assembly of nanoparticles was the most promising method for bottom-up fabrication of nanostructure material; the highly flexible approach is used to charge magnetically aerosolized (such as suspended in a gas) the tunable size and composition of NPs are self-assembled into nanostructure by using in the combination of the magnetic and electric field. Spark ablation and self-assembly can generate Co, Ni, and Fe in different structures, ranging from dimension nano chains to macroscopic three-dimensional networks. The design and simulation are being compared. Interparticle magnetic dipole-dipole interaction concludes that magnetization of the nanoparticle is self-assembly—direct self-assembled by the external magnetic field into the different macroscopic structures and aligned nanoparticles. As a result, the electric and magnetic field is combined in the deposition of aerosol. It can be used in direct bottom-up self-assembly in nanostructure with distinct morphology and composition[13].

2.2 TOP-DOWN

The destructive method is another name for the top-down approach. With this technique, the bulk material is reduced to tiny nanoparticles. This manufacturing process relies on the removal, division, or miniaturization of bulk material to create a suitable structure with the right attributes. Making uneven forms and microscopic particles with it is unsuccessful. The biggest flaw with this approach is how challenging it is to get the right particle size and form.[14].

Lithography, for example, is used to make nanowires that are not smooth and have many impurities and surface imperfections. Examples of such methods include gas phase condensation, atomic force manipulation, high-energy wet ball milling, and aerosol spraying. The schematic diagram example of the top-down and bottom-up synthesis of nanomaterial is in **Fig 1**.[15].

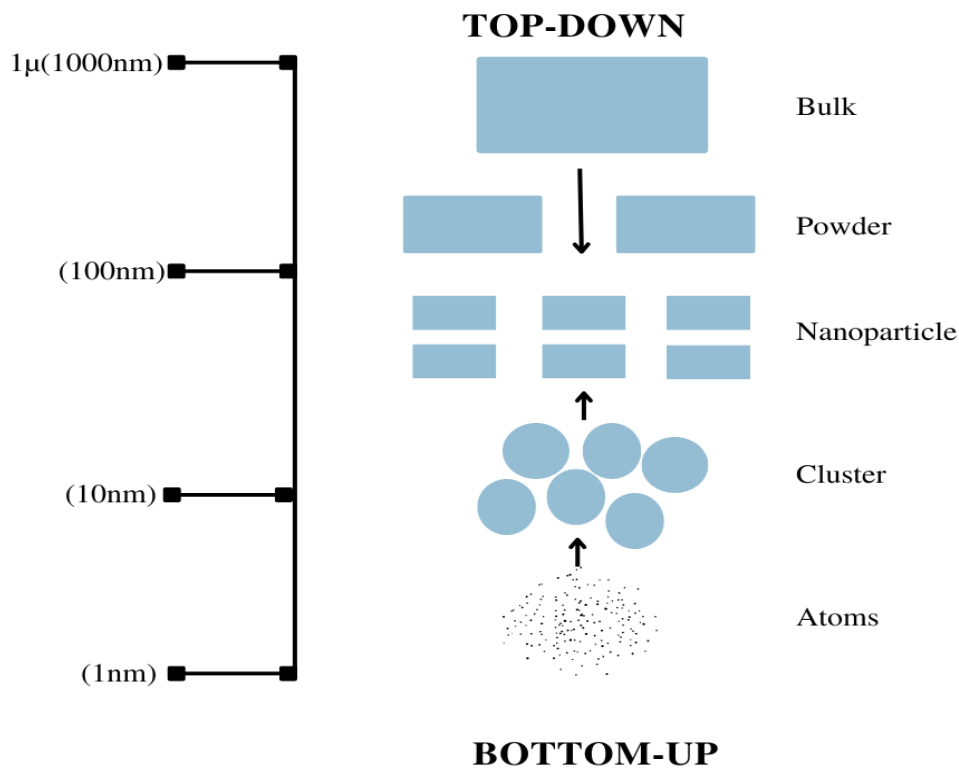


Fig 1 Approach of top-down and bottom-up synthesis

2.3 GREEN SYNTHESIS

Green synthesis is also known as biosynthesis, which is environmentally friendly and an approach to synthesizing nanoparticles; they are biodegradable and non-toxic in synthesis by using bacteria, fungi, plant extracts, etc., along with precursors; the nanoparticle is produced in a conventional chemical method for bio-reduction. [12], such as the single-step method used in the MNP of extracellular plant-mediated synthesis. The plant extract is prepared and mixed with the aqueous iron solution in controlled conditions. The harvested leaves are rinsed with deionized water, which removes mud and dust, and are dried at room temperature. Stable MNPs are grown and prepared by aqueous leaf extract; this reaction attends at vigorous stirring at room temperature[16].

Using *k.alvarezii*, Fe_3O_4 NPS is synthesized. In a 2:1 ratio, a solution of Fe^{3+} and Fe^{2+} is added to seaweed extract to obtain a yellowish colloidal solution. After that, a dropwise addition of NaOH at 0.1M is made while the mixture is continuously stirred. The solution's pH is held constant at 11. The solution is agitated for one hour to ensure a thorough reaction and to homogenize the process. Synthesized Fe_3O_4 NPs are separated using a permanent magnet after being rinsed in deionized water numerous times. The nanoparticle was then dried in an oven for 24 hours at 70°C after that. To facilitate further characterization, the dried nanoparticles were kept in airtight containers.[17]. The schematic diagram of the green synthesis of the nanoparticle is shown in **Fig 2**

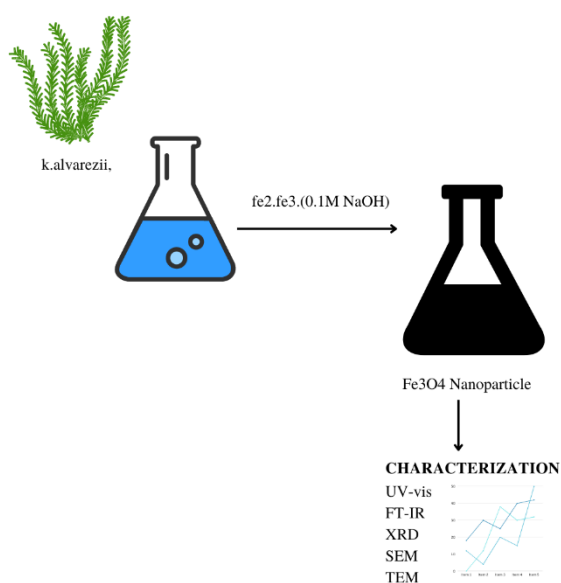


Fig 2 Green synthesis of MNPs

3. PROPERTIES OF MNMS

MNPs are made with the property of strong magnetic and magnetized properties, such as materials enclosed with iron, nickel, cobalt, and alloy derived from the mixture of magnetic metal and oxide. Iron and its oxide play a vital role in the environment among all MNMs. For example, ferromagnetic material has a high magnetic susceptibility at room temperature and is greatly magnetized even in a weak magnetic field. Fe_2O_3 and Fe_3O_4 nanoscale is usually $<20\text{nm}$. They are superparamagnetic materials that can be magnetized; it is exposed and applied in a low-intensity magnetic field externally, with no hysteresis. The magnetization curve immediately decreases to zero when the magnetic field is removed. [17-20]. Inverse spinel structure of Fe_3O_4 nanoparticle, the divalent transition metal ions known as Mn^{2+} , Co^{2+} , and Ni^{2+} are doped and furtherly turned into Fe_3O_4 magnetism. As a result, MnFe_2O_4 , CoFe_2O_4 , and NiFe_2O_4 nanoparticles have been used in biomedical applications. Bimetallic and metallic exhibit superparamagnetic or ferromagnetism which gives promising results in magnetic bioanalysis, excellent in the separation of magnetic and T2 MRI contrast enhancement [21-23].

The enchantment character of magnetic is applied in separation and labelling, in magnetic vectors, magnetic heating, hyperthermia, and the enhancement of contrast agents. An MNP is used in both therapeutic and diagnostic applications. MNPs are a prototypical tool for theragnostic application, and the magnetic field used as an aid for drug delivery is termed magnetic drug targeting[20].

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The MNP character can be evaluated by magnetization measurement as a function of the field ($M \times H$) curve and temperature. This magnetic property depends on nanoparticle arrangement, an intrinsic characteristic of individual particles like crystallinity, chemical composition, stoichiometry, shape, interaction, and magnetic anisotropy. The interparticle exchange has a strong impact, which controls the distance between particles, the design of magnetic nanocomposites with specific properties should be considered. In nanocomposite (via self-assembly), the nanoparticle's volumetric fraction or mass fraction is used to control the interparticle distance. The production of the sample with a variable fraction of nanoparticles is permitted by particle-particle interaction. This method is explored in the study[25-29]. Susceptibility measurement is used to evaluate the level of interparticle interaction. Dormann and Gitle Mann proposed a model that describes a magnetic

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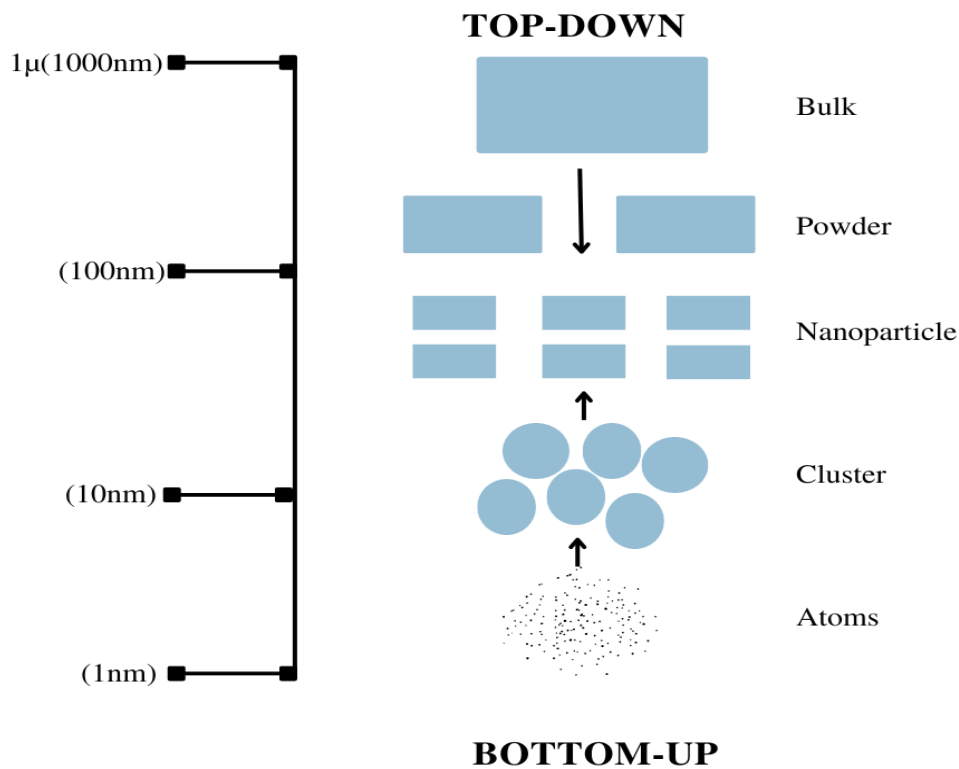


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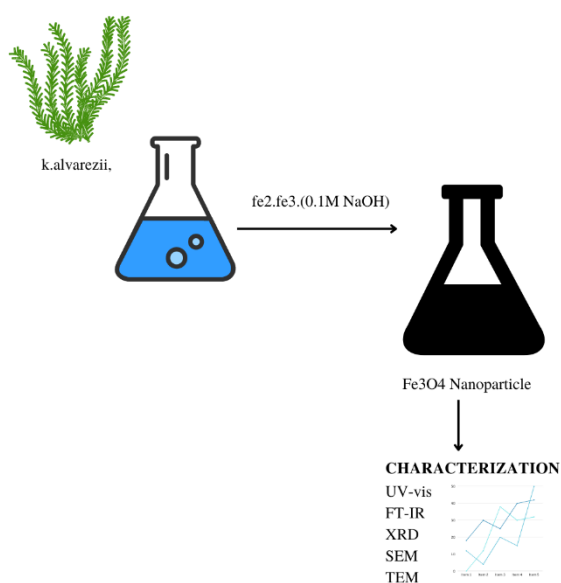


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susceptibility system composed of particle which does not interact. Considering the inversion of the magnetization orientation of each nanoparticle taken by time, each NP follows Arrhenius's behaviour. τ_0 and ΔE using the Arrhenius curve are used to evaluate the frequency of the imaginary component of ac susceptibility[22].

Diamagnetic, paramagnetic, and ferromagnetic materials are the three types of magnetic materials. When a diamagnetic substance is placed in magnetic field lines, the substance is compelled to avoid the magnetic field lines and does not attach to the external magnetic field. This is due to the non-uniform magnetic field of diamagnetic material, which transitions from a stronger to a weaker field, creating repulsion. Examples of diamagnetic materials include copper, gold, silver, mercury, argon, and so on. Paramagnetic materials are attracted to the external magnetic field. The substance is compelled to pass the magnetic field line. This is because the non-uniform magnetic field of paramagnetic material changes from a weaker to a stronger field with weak attraction. Aluminium, chromium, CuSO_4 , MnSO_4 , and alkaline earth metals are a few examples of paramagnetic materials. Ferromagnetic materials have a strong magnetic field attachment. In the specimen, the magnetic lines are compelled to condense. This is due to the non-uniform field of ferromagnetic material, which moves with tremendous attraction from a weaker to a stronger field. Examples of ferromagnetic materials include steel, iron, cobalt, nickel, and gadolinium.

3.2 SOME COMPATIBLE COATINGS OF MNPS

Surface coating is required for an MNP. In both in vitro and in vivo applications, it is crucial to decide. In the absence of the following coating characteristics, MNPs have a hydrophobic surface (higher surface area to volume ratio) and a propensity to assemble. The correct surface coating of MNPs and dispersed homogeneous ferrofluids can increase MNP stability. Organic surfactants like dodecyl amine and sodium oleate, Organic polymers like chitosan, polyethylene, dextran, polyaniline, polysorbate, and glycol, and Bioactive structures and molecules like ligands/receptors, liposomes, and peptides are just a few of the modifications that can be applied to an MNP. In organic metal coatings, including gold, in organic oxide coatings like silica and carbon.

Recent developments in inorganic coating (such as gold and silica) of magnetic core give promising results in exceptional stability in aqueous dispersions, and accessible surface modification allows in preparing the nonaqueous colloids. Shell thickness helps in

controlling inter-particle interaction in both structure and solution. Nature and synthetic polymers are the two main categories of polymer material. Natural polymer gives promising results in biocompatible and biomedical applications such as coating nanoparticles[8,31-33]. The coatings concept of MNPs is shown in the schematic diagram in **Fig 3** shows Thiolate PNA and gold shell-cobalt ferrite exhibit function for hybridization with complementary DNA target molecules.

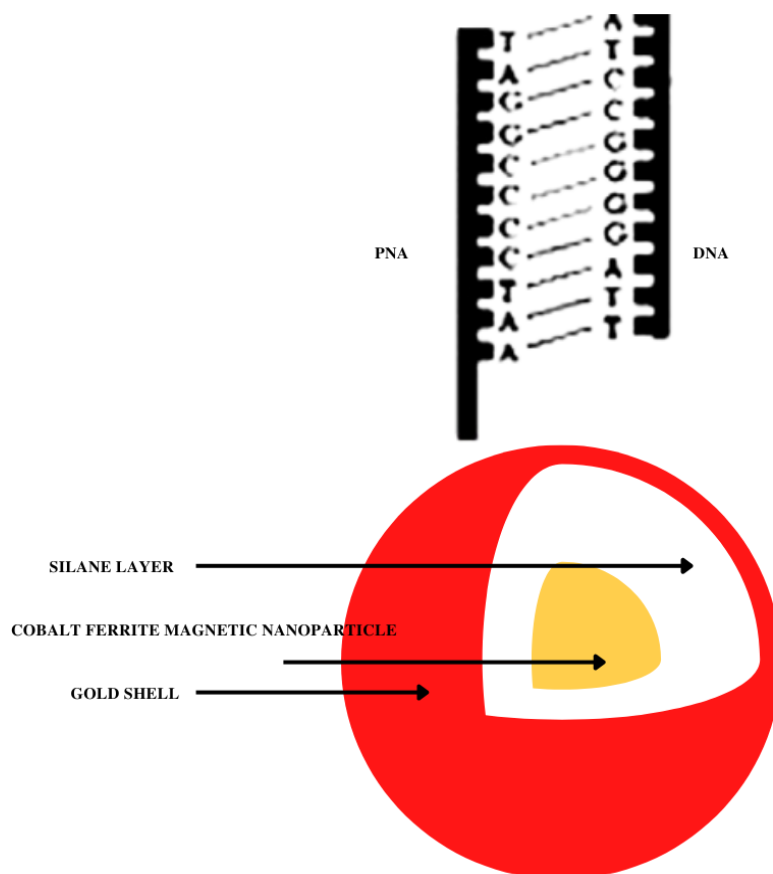


Fig 3 Coating of MNP

3.3 SPINTRONICS APPLICATION OF MNPS

A newly emerging field is known as spintronics. It combines three conventional carriers: electron charge, photon, and electron spin[24]. Electrons are essential in semiconductor-based devices; the electron spin decides the magnetic devices' performance. In the matrix of non-magnetic semiconductor, magnetic particle is introduced into it. Electrons, charge, and spin improve the device's performance. For example, ZnO as a multifunctional material is used in various applications like optoelectronics to biomedicine. After lots of research, the result shows that magnetic semiconductors have advantageous properties such as UV

protection, high exciton binding energy, photostability, antibacterial activity, and a low dielectric constant and thermal conductivity. Semiconductor properties based on shape and size exhibit ferromagnetic behaviour at room temperature when transitioning metal ions such as Fe, Ni, and Co-doped. For example, n-type conductivity is revealed in ZnO due to an intrinsic defect such as oxygen vacancies and zinc interstitials. ZnO can easily be manipulated by adding dopants. It has freedom of degree spin and charge. Spintronics field aiding in manufacturing the light-emitting diodes, laser diodes, sensors, spin-dependent transistors, and varistors. The absorbance property is modified in the broader bandgap (3.37 eV). It is used in photocatalytic applications. Magnetic semiconductors are converted by introducing oxygen into the metallic glass as demonstrated by W. Liu et al. same process is presented in non-magnetic to fabricate $\text{Co}_{28.6}\text{Fe}_{12.4}\text{Ta}_{4.3}\text{B}_{8.7}\text{O}_{48}$ magnetic semiconductors. The bandgap is modified to obtain desirable properties for a specific application. Transition metals known as Fe, Ni, and Co are doped, and the bandgap is narrowed down to a lower value. Spintronics device gives promising results in information processing and data storage, with high efficiency and low cost[35-42]. It plays a significant field in information technology. Ferromagnet-based memories process Magnetoelectronic and conventional semiconductor-based electronics are based on spin-polarized electronic transport providing promising results in faster progress in data storage. Metallic multilayer and spin-dependent electron transport are observed in GMR (GMR). The GMR effect is practically used in HDD (HDD). TMR (Tunneling Magnetoresistance) was discovered the impact was also a milestone. As early research shows, the GMR of the fact is not realized at room temperature. More studies have been undergone in the semiconductor community and diluted magnetic semiconductors (DMS). Novel characters are found in carrier-induced and photo-induced ferromagnetism after discovering the significant g factor[26] optical fibre used in Gbps data transfer in IT. The diode laser depends on the efficiency of the optical isolator; it is used in data transfer for faster operation. DMS consisted of an optical isolator used in a spintronics-based significant magneto-optical effect[27].

4. INTERACTIONS OF MNPS WITH BIOLOGICAL SYSTEM

MNPs play a vital role in clinical and biological applications; nano-magnetism is the most challenging research area. Cobalt ferrite is giving promising results in biomedical applications, for example, drug delivery, magnetic hyperthermia, biosensors, and MRI[45-47] in CNS diseases MNP, so promising results in target administration. For example, chemotherapy used in brain tumours is helpful for gene therapy, or malignant cells are

destroyed by mechanical force by nano surgeons[24,48]. IONPs are the ability to reach the brain through the olfactory neuronal pathway with the help of sensory nerve ending; translocation takes place in the axon bypassing the BBB or direct injection for hyperthermia treatment. IONPs are deposited in the olfactory bulb, striatum, and hippocampus [30]after intranasal instillation, which induces some oxidative stress in the striatum. Fluorescent IONPs is be used in intraperitoneal injection or inhalation and pass through BBB into the brain. It was documented in fluorescence microscopy and MRI[50,51]. A sensitive monoisotopic detection system gives promising results in the research area, clinical diagnostics, DNA sequencing, and fundamental molecular biology; it provides new opportunities in automated biological assays and ultrasensitive. Biological staining and diagnostic in fluorescent biosensing probes are other nanoparticle usage. The typical representation of MNP interactions with biological systems has been depicted in **Fig 4**.

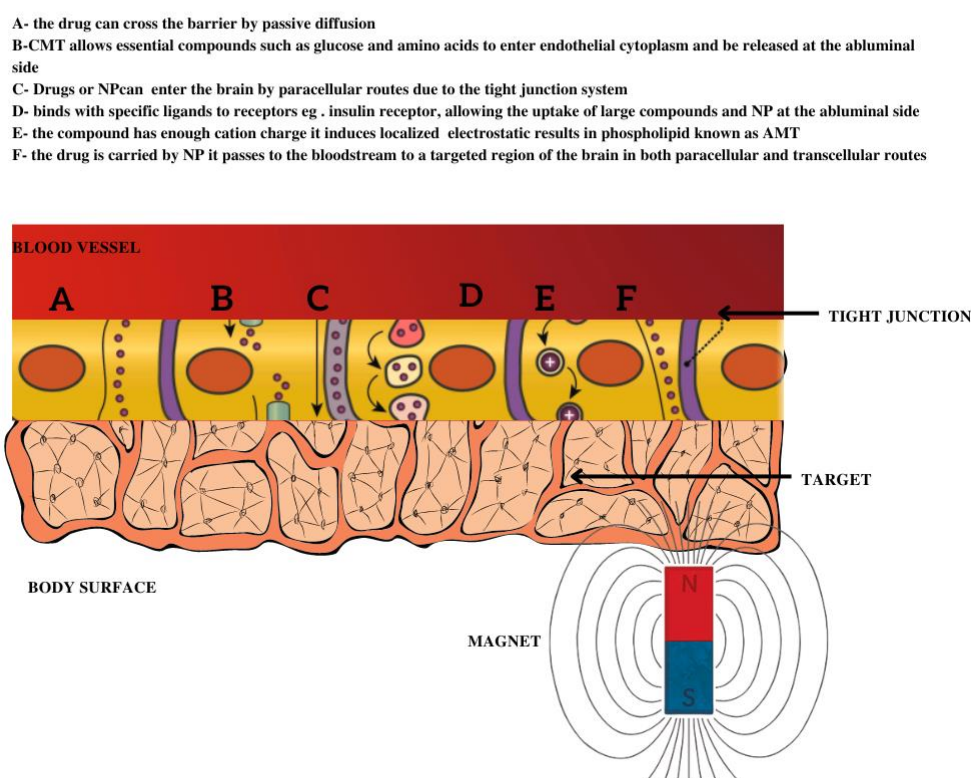


Fig 4 Interaction of MNP in a biological system

4.1 INTERACTION WITH MACROMOLECULES

It has two groups of specific interaction methods to conjugate the MNP with nucleic acids.

The first one is on binding DNA/ RNA nanoparticles recognized by molecular. The second one is on chemical reduction and electrostatic interaction, where the interaction is less specific in this type. Design MNPs are formed in the desired field of application. MNP-DNA interaction follows: nanoparticle surface in the DNA molecule is oriented, or aligned molecules have a mutual position of nanoparticles. Nature bonding between DNA and MNP results in a change in Ph value, the addition of ionic strength, or the addition of compound according to the desired field of application. DNA molecules bind nanoparticles through electrostatic interaction with the surface. Synthesis of the magnetic [32]nanoparticle surface properties is based on electrostatic interaction. Strong-oriented and particular binding biomolecules to nanoparticles can be achieved using surface-functionalized MNPs. Thiolated and aminated molecules are attained by covalent immobilization. These groups, such as sulfhydryl, carboxyl, amino, and azido, are formed in the surface nanoparticle. Traditional methods form covalent bonds, bioconjugation, and click chemistry applications involved in the thiol-disulphide exchange, carbodiimide activation, aldehyde-amine condensation, azide-alkyne cycloaddition, etc. Different manners blocked the unreacted functional groups in the immobilization of nucleic acids to avoid nonspecific interaction on the surface of MNPs. High-affinity noncovalent interaction biotin-streptavidin or protein aptamers are used to bind the specific MNPs to DNA. The surface of MNPs and nucleic acid needs preliminary modification with appropriate molecules. The complementary binding of oligonucleotides immobilizes MNPs and targets nucleic acid molecules; an operation principle underly certain diagnostic assays. Functionalization of the surface of nanoparticles with double-stranded DNA is intercalated by molecules, paving the way to achieve complex MNPs with DNA.[53,54]. Magnetism is used in emerging technology in magnetic separation to efficiently separate micrometre-sized Ferro and paramagnetic particles from biological or chemical suspension. Magnetic separation tech is used to purify natural compounds such as nucleic acids, proteins, and cell organelles, cells, and regrowing.

MNP can interact with DNA in a variety of ways, including electrostatic contact, covalent bonding, and assembly on DNA molecules. Numerous processes can result in the creation of MNP-DNA, including the electrostatic coating of the MNP surface by the nucleic acid, high-affinity noncovalent interactions between the nucleic acid and pre-functionalized surfaces, and complementary hydrogen bond formation between the oligonucleotide molecules (down) or long single-stranded nucleic acid molecules immobilized on MNPs (up)[32]

4.2 INTERACTION WITH *IN VITRO* CELL LINE

A suitable strategy is provided by MNPs for separating distinct sub-populations of cell suspensions, antibodies attached to nanoparticles, and directing the interaction of nanoparticles of the cell. Recent interaction of tumour cells and leukocytes was previously shown in carboxymethyl dextran-coated MNPs. 8 min exposure showed a result as tumour cells showed more rapid interaction than leukocytes. Adding 5% plasma can slightly enhance the pH level in the incubation solution. CMD-coated nanoparticles were used to incubate peripheral blood from various cell lines and leukocytes. Without carbohydrate shells in MNPs, so instantaneous interaction in most cell type results in more toxicity; carbohydrate coating show good biocompatibility[52,55]. MCF-7 is incubated individually with Fe₃O₄, which helps investigate the inhibition of cell growth rate using the MTT assay. Fe₃O₄ is coated with dextran; the cell viability was absorbed in 78-90% at a full range of 1–100 µg/ml for 24 h. Fe₃O₄ carries dextran, which has good biocompatibility due to its strong electrostatic attraction[35]. The plasma membrane interacts over the period with Fe₃O₄, which causes cell lysis to induce cell shortage[36]. QCMNPs display cell viability below 25% at the same concentration, comparatively higher than quercetin. DNA damage in breast cancer causes a toxicity effect due to the release of the drug by magnetic oxidation. MCF-7 cell binds with quercetin molecules, known as strong binding; Fe₃O₄ partially releases quercetin. Different pH conditions confirmed drug release. Drug quercetin shows a high therapeutic effect which enhances anticancer activity. More cells become round and shrunken, implying adherence between breast cancer cells and Fe₃O₄ nanoparticles. Drug quercetin promotes interaction and release, which causes a high reduction in cell count. QCMNPs treatment (73 µg/ml) more a survival rate than cells treated with 31µg/ml.[58,59].

5. TOXICITY OF MNPS IN THE LIVING SYSTEM

In the heart, the most common damage with cardiac arrhythmia. In the digestive system, the primary organ affected is the liver. Genotoxicity and hormone imbalance occur in WBCs. Dermal profile damage occurs due to long-time exposure to the nanoparticles. The effect is also observed in the reproductive system, eye, ear, pulmonary injury, and urinary profile. DNA damage in living systems is due to the indirect inducement of cell inflammation and activates oxidative stress known as MNPs. The physicochemical properties of MNPs induce toxicity when it interacts with biological systems. The principal reason for toxicity is the size of the MNPs. The study suggests liver and spleen are trapped via macrophage phagocytosis when MNPs are more extensive than 100nm. Compared to rod shape nanoparticles,

spherical-shaped nanoparticle shows less toxicity. Increasing intracellular reactive oxygen levels in the host cell is initiated by the toxicity of MPs—toxic effects MNPs such as DNA damage, oxidative stress, and apoptosis. Trapping of the renal spleen port during transportation is reduced by reducing the size of MNPs. Surface coating of nanoparticles plays a significant role in the toxicity MNPs coatings such as polymers, dextran, Pluronic acids, and PEG, resulting in reduced toxicity and improved stability and distribution of nanoparticles. Toxicity of MNPs based on dosage duration of stay in the organ [55,60,61].

5.1 STUDIES IN MICE

Fe₂O₃ is exposed in the olfactory bulb section in mice. It was observed in the axon. There is a slight change in the morphology of the olfactory bulb, like dilation in the rough endoplasmic reticulum, degeneration of neuro dendron, increased lysosome in a nerve cell, disruption of membrane structure it was exposed by 21nm Fe₂O₃ induced by intranasal, where Fe₂O₃ is ~280 nm is used in a group of mice showing slight swelling in mitochondria in nerve cells was analysed in the olfactory bulb. TEM image revealed some nanoparticles in mice of the hippocampus when it is instilled with 21nm Fe₂O₃, and the nanoparticle is located inside mitochondria and found in the surface, the lysosome. There is a slight alteration in nerve cells, such as dilation in the endoplasmic reticulum, and an increase in lysosome in the hippocampus was analysed in 21nm Fe₂O₃. It was treated in mice, in 280nm Fe₂O₃ is instilled in mice showing some vacuoles appearing in the hippocampus cytoplasm.

Iron oxide nanoparticles as the ability to travel through the brain via an olfactory route. Iron oxide nanoparticles' neurotoxicity is highly concern in the brain[39]. Fe₂O₃ initiates lung injury and causes oxidative stress in the lung. A high dosage in the lung by AMs known as phagocytosis of the nanoparticles. In alveolar epithelial non-phagocytosed particles were found, and nanoparticles were taken by epithelium cells. It is hard to clean from the lung; the long-term overloaded particles in epithelium cells cause pathological symptoms causing lung emphysema to develop into lung fibrosis. Microvascular permeability and cell lysis increase in lung epithelium by instilling Fe₂O₃ in the intra-tracheal affects the blood coagulation system[40].

Fe₃O₄ is used in liver cells in mice. The effect was evaluated in the histology property of liver tissue after exposure to different doses. Fe₃O₄ at 150 and 300 ug/gr dosage leads to histopathological effects in the liver, causing necrosis in hepatocytes and a plethora of Kupper cells. The significance shows an increase in Kupper cells and a decrease in count

per unit in hepatocytes. In mice, IONPs do not cause toxicity apparent *in vivo*; many studies are undergoing it, indicating that Fe₃O₄ causes lung injury and increases microvascular permeability and lysis of the epithelial cell in the lung. Fe₃O₄ induces several oxidative stress damages never cells in mice. The underlying positivity toxicity of IONP contributes to ROS in the intracellular system. IONPs cause toxicity in tissue and organs, whereas Fe₃O₄ acts as a low-risk dose in *in vivo* systemic delivery[41].

5.2 STUDIES IN ZEBRAFISH

A present study on zebra fish shows the toxicity of Fe₂O₃ by using two aquatic exposure systems such as static and semi-static. It is tested in exposure conditions; the condition depends on the hypothesis, embryotoxicity of citrate function of Fe₂O₃ in zebrafish; at an early stage of fish, it can analyse the exposure condition such as static and semi-static aquatic system on toxicity[42]. Iron nanoparticle shows a toxic effect on zebrafish, causing cardiotoxicity, there is no effect on static Fe₂O₃, and semi-static Fe₂O₃ shows a high frequency. Both forms of iron show reduced heart rate. A younger embryo also indicates the same heartbeat rate in semi-static iron ions. IONP shows various effects on embryonic development. Semi-static and static iron ions exposed at 0.3mg L⁻¹ confirm the cardiotoxic in zebrafish embryos. Fe₂O₃ shows are cardiotoxic in zebrafish embryos. It induces inflammation, lipid peroxidation, oxidative stress, apoptosis in tissue degeneration, and abnormal heartbeat.[43]. Oxidative stress was reported after exposure to Ag NPs in zebrafish. It indicates that nano-materials induce cardiotoxicity by ROS and oxidative stress. The present zebrafish study shows detailed information on IONP (Fe₂O₃) and their cardiotoxicity. It was studied mainly for medical applications [42].

5.3 STUDIES IN DROSOPHILA

The toxicity effect of CuO NP is observed in drosophila. At first, CuO NPs were given in uptake in the *in vivo* method in oral route administration and were given food in various concentrations from 0.05 to 0.1 mg/ml for F1(first filial or generation). CuO NP-fed instar larvae are analysed in (ICP-MS), and CuO NP shows indeed accumulating in the body. Five late-third larvae were collected from every group experiment, then acid digested and homogenized. At 0.05 mg/ml of CuO NP was observed, compared, and controlled. CuO NP at 0.1mg/ml shows a significant was reduced compared to 0.5mg/ml. CuO NP exposed in late third instar larvae at high dosage, such small at 0.1mg/ml that 0.5mg/ml. The dosage of CuO NP shows several disruptions in the metabolism of larvae, raising the possibility of

larvae exposed to the high dosage; CuO NP stopped ingestion of food in the early larvae stage. Larvae exposed to lower-level experience ingesting food throughout the entire larval stage. It is further noticed that ingestion of CuO NPs causes a dose-dependent decrease in the pupa. After monitoring, CuO NPs are exposed in eggs to adult survivorship. F1 generation shows dose-dependently decreased exposure successfully enclosed to adults. A high dosage of CuO NP (such as 0.15 mg/mL or 0.25 mg/mL) shows a significant reduction of survivorship compared to untreated control in flies. CuO NP treated with flies shows delayed development in the process of drosophila. CuO NP at high dosage in F1 flies shows drastically delayed enclosing, compared to 0.05mg/ml or control exposed. Research shows cytotoxicity in drosophila when treated in CuO NP and other models like a marine model, cultured cell lines, and *C.elegans*. genotoxic effect is detected in drosophila when it is exposed to CuO NP, and Cu^{2+} plays a vital role in physiochemical properties induce a toxic effect. Comprehensive analyses analyze the toxicity of CUONP or Cu^{2+} with developed techniques required for differentiation, which help in hypotheses and minimize the toxic effect of CuO NPs by modifying the CuO NPs physiochemical properties and selecting the proper route of administration and controlling can reduce the risk.[67-71]. The schematic representation of the toxicity of Nps on Drosophila was shown in **Fig 5**.

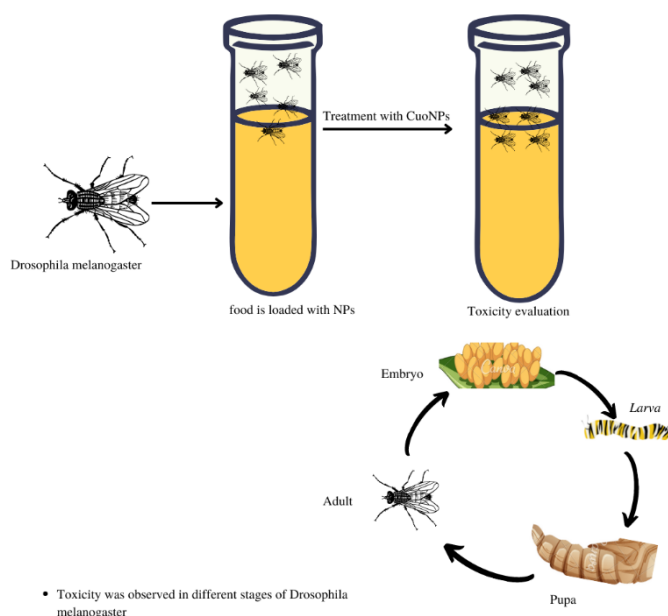


Fig 5 Toxicity evaluation of NPs on Drosophila

6. DRUG DELIVERY APPLICATION OF MNPS

Drug delivery is the modern technology for drug targeting; in recent years, drug targeting drastically increased by MNP application. External magnetic field or magnetizable combination with MNPs such as Co-ferrite implant allowed for delivery of the drug to the desired targeted area and fixed in the local site; when the medicine is realized from the local site, it activates the magnetic drug delivery. Eliminate the side effect and reduce the required dosage. It is possible by sending the drug to a specific area. Organic polymer and inorganic metals or oxides required in modifying the surface help in a biocompatible and suitable further attachment, such as attaching various bioactive molecules[12,72,73]. Complex interaction depends, it is the success of targeting and delivery. The property of complex interaction is applied to MNP and magnetic field, including hydrodynamic condition, MNP concentration, drug-particle binding, target site location, and depth and injection method. Biomedical applications aim to study mechanical and rheological cell properties and cellular function, and recent emerging relies on magnetic actuation of membrane and cellular components on molecular signal pathways[6,74].

6.1 TDD- TARGETTED DRUG DELIVERY

Physical targeting describes localized agents and target areas because the composition, size, or characteristics are not explicitly designed for biological receptors. Targeting, in many cases, is based on the nonspecific accumulation in some areas related to another site. Localized agents and areas externally can be targeted by physical targeting; specific external applications are ultrasound, heat magnetic field, etc., which help in drug-delivering systems or diseased tissue. It gives promising results in controlled action/ accumulation in the target area or delivering and releasing agents against the target cell or tissue. Site-specific prodrugs are involved in chemical targeting, the localized agent to the target area. The agents use enzymic or chemical reactions; they can direct the site to a chemical reaction. It leads to the agent's control, targeting release, and action. Biological targeting pays the way for an agent to target the area with the help of peptides, antibodies, other compounds, or proteins, as an affinity to organs, another biological target, or receptor sites in a specific manner. Tissue, cell, or another particular promoter in the vector are targeted to localized by gene expression[46]. Active and passive targets are the two types of targeted drug delivery. Aptamers, antibodies, and ligands are former contained it is functionalized or conjugated to the drug-loaded MNPs. The drug had nanoparticles guided to the target, which is the object of the site-specific. Through this passive target delivery, the retention effect and enhanced

permeability or localized body system are possible. Retention of the affected part and drug permeability is increased. It is the main advantage of passive drug delivery. Drug-loaded nanoparticle injection is directed to the disease target. This is possible in localized delivery. The motive of targeted drug delivery is a therapeutic technique and treatment. Chemotherapeutic agents in the human body behave like foreign agents, but they deliver the drug to the disease target in the circulatory system. Foreign objects enter living systems such as drug molecule systems, and the thing is removed through the mechanism and sent through the excretory system. The specific dose of the drug become difficult to reach the target disease site and cure the particular disease. A low-dose side effect is drug resistance. A nonspecific chemotherapeutic agent can cause severe damage to human tissue. Chemotherapeutics has several advantages in targeted drug delivery. MNPs are used, which helps in rectifying the issue because their external magnetic field gives the best results in specific drug delivery. Some advantages of MNP used in targeted drug delivery are target identification, drug delivery only to the affected area, and customizing the drug amount. The concentration of nanoparticles, blood flow rate, circulation time, drug delivery, and effective treatment are the various factor of contribution. MNPs can influence the external magnetic field. The drug is loaded with MNP and introduced into the circulatory system; the external magnetic field guides this to deliver the drug to a specific point. MNP is loaded with drugs or therapeutic agents according to their biocompatibility; it is used as a carrier or in MTC.

MTC is introduced in veins due to its colloidal suspension. The drug moves toward the target site due to the magnetic field gradient detached. Drug delivery is complex. It has to undergo certain factors such as biocompatibility, the physiochemical impact of drug loaded in MNP, size, target distance and dept, magnetic field strength, flow, and vascular blood supply. All factors influence targeted drug delivery. The great impact of targeted drug delivery can be achieved by optimization of the size and nature of MNPs. MNPs are linked or encapsulated with a conventional therapeutic agent like doxorubicin and methotrexate for effective treatment and delivery of prostate cancer, arthritis, and malignant breast. The typical description of Targeted Drug Delivery (TDD) has been shown in **Fig 6**. MRI is used to monitor drug delivery and targeted drug delivery and disease can be monitored by bio-imaging [33,76-79].

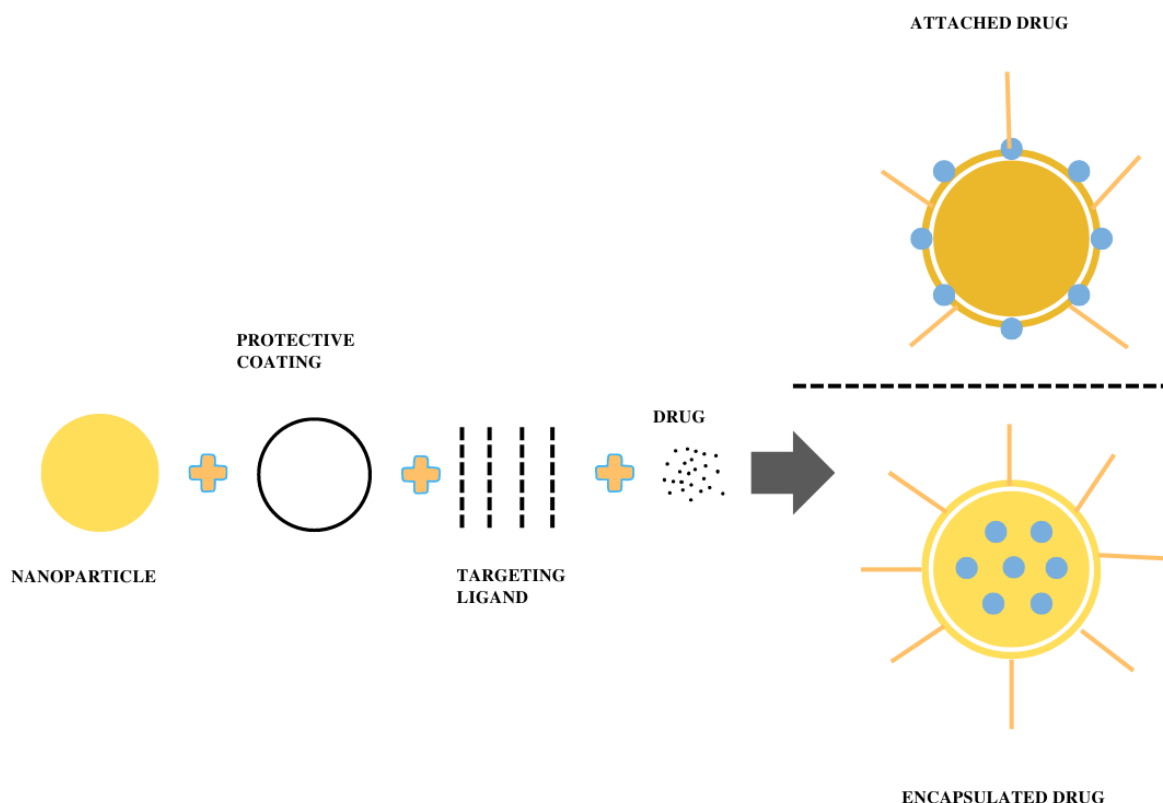


Fig 6 Targeted drug delivery

6.2 GENETICALLY ENGINEERED DRUG DELIVERY

Intensive research about polymer-based chemotherapeutic drugs or anti-cancer gene delivery systems shows various advantages. For example, polymeric nanoparticles can load the drug effectively and pack the gene to increase the cargo solubility and enhance the efficacy of cargo compared to free cargo, and the circulation half-life is prolonged. PDNA can protect against degradation, the extracellular milieu in numerous serum nucleases and the anionic plasma membrane, neutralization of negative charge can avoid repulsion, and there is the possibility of collapsing the nano-sized particle, enhancing the permeability and retention effect of the tumour via passive targeting. Strategies like the conjugation of PEG moieties can proceed in polymeric delivery systems. The drug delivery to cancer can be improved by targeting the group and stimuli-sensitive functional groups. Releasing of drug and gene cargo in the tumour tissue is triggered by particular stimuli-sensitive moieties. This strategy is more effective in effective drug delivery, anticancer drugs, and genes used in the therapy of drugs and genes.[80-84] Separating carriers get more advantage in single carrier offer for co-delivery of multiple therapeutic agents. Two therapeutic agents are synchronized

into a single carrier, and pharmacokinetics synchronization is ensured it allows to target cells. Have to be assured that PDNA and the drug work synergistically in the small cell to enhance the anticancer effect. For *in vivo* dose administration, the concentration of PDNA and drug can be done *in vitro*. Various carriers were used by co-delivery of small molecule anti-cancer genes and drugs such as inorganic nanoparticles, liposomes, polymeric nanoparticles, and peptides.[49].

6.3 BIOIMAGING OF MNMS

The researcher can understand the performance of therapeutic agents with the help of molecular imaging like stability, function, and principle. They are three parts of imaging known MRI, radionuclide imaging, and OI. The signal is given to separate the cell from the host tissue to label the compartment. To achieve the target contrast agent is used. Applied light wavelength penetrates the human body and restricts visual imaging style. The radionuclide imaging style belongs to the best sensitive imaging in medicine. Still, it has certain drawbacks, such as less anatomical information, the cell cannot be tracked in lengthways, and it endangers human health. MRI without invasive can track the therapeutic cells effectively in patients. MRI has a specific limitation in monitoring the cell. It can only help in understanding the distribution of therapeutic cells in organisms. The most crucial point is that after stem cells' engraftment, we cannot get any information about their destiny and function. To solve the problem of getting the correct information on position and location, a multifunctional platform has to be developed and executed in MRI with a sensor[50]. Various approaches of non-invasive OI, such as magnetic resonance (MR), computer tomography (CT), SPECT and OI. Complementary and competitive are general in different imaging techniques; image modification depends primarily on a specific question. Both *in vitro* and *in vivo* biological specimen is imaged as long relied on light microscopy, Current attraction of fluorescence, and luminescence imaging enhance the technology and capabilities. In medical diagnostics, technology is growing remarkable tools. MRI and OI can detect the multimodal nanoparticle, and fluorescence microscopy and paramagnetic ions generate luminescent cores incorporated in MRI signals into the same particle. Advanced high sensitivity is integrated with such particles, for example, optical method detection, e.g., fluorescence, with processed cellular resolution and potential of accurate 3D imaging of biological nanostructure. MRI has limitations due to its low sensitivity; OI provides anatomical information. These are a few of the numerous applications of bio-imaging[51].

7. BIOMEDICAL APPLICATIONS OF MNP

Therapeutic and diagnostic area biomedical applications play a paramount role. MNMs show a positive impact in biomedical applications due to their applied magnetic field. There is no limitation for penetration in real-time monitoring or gene or drug delivery. Biomolecules or hydrophilic macromolecules linked with several modified routes are developed to improve the biocompatibility and stability of MNMs. MNMs detected target biological entry by MRI, and in research, laboratories imaging-guided photothermal therapy and drug delivery were developed. In biomedical application, MNMs gives promising results in the research field. MRI is used in clinical applications in addition to chemotherapy and photothermal therapy providing agents. It serves nano-platforms to kill tumour cells[52]. This biomedical application can be used in both *in vitro* and *in vivo* conditions, such as injection enclosed with colloidal nanoparticles is introduced into the bloodstream it's delivered to the target site through magnetic field gradients. The biomedical applications of MNPs are shown schematically in **Fig 7**. Once it reaches the targeted area, it will change into a theragnostic agent. This can be used in diagnosis and therapy[53].

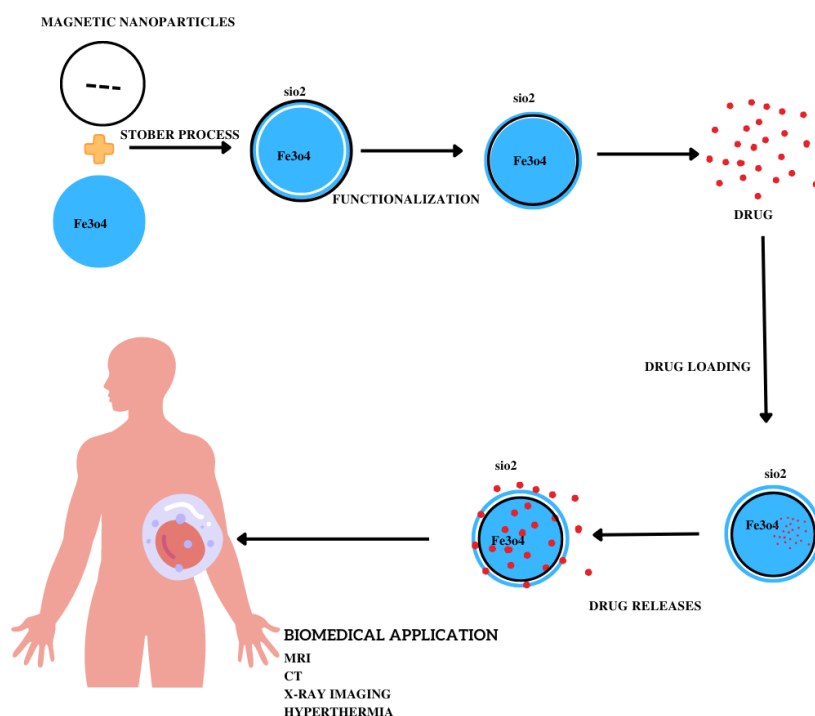


Fig 7 Biomedical application of MNP

7.1 MRI

The distinct characteristic of MRI is the imaging modality. It has excellent intrinsic in soft tissue contrast and radiation at a safer level. Hardware technology was recently developed, and a 10ms scan time can be achieved by sub-millimetre resolution new implementation in MRI. For tissue enhancement, the contrast was made using extraneous CA that shortened the longitudinal (T1) and transverse (T2) relaxation time. Unpaired electrons possessed by the element provide dipole-dipole interaction and magnetic moment; it is used in protons relaxation enhancement. Gadolinium superseded manganese which was used first. The significant relative magnetic moment of gadolinium is due to the several unpaired electrons. Larger molecules are shielding chelate the Gd from direct contact with water protons. Mobility was affected by increased molecule activity, and Gd magnetic moment in the vicinity the protons spend their time. Inner-sphere relaxation theory governs these factors, which will explain the T1 and T2 data in low concentrations of paramagnetic CA. Paramagnetic was surrounded by electronic spin relaxation and relative motion of proton water, the characteristic of time constant was given to each of its own, for dipole-dipole interaction the necessary fluctuation where provided. The more considerable magnetic moments material, such as ferromagnetic particles and iron oxide or ferrite substituted like Gd, Zn, Mn, etc., can enhance the efficiency of CA. Giant magnetic spin material is exposed when the volume is reduced to the nanoscale. Super para MNPs present the relaxation theory of protons; modifying the idea of paramagnetic CA can develop the superparamagnetic NP; two different media is taken into account by paramagnetic CA. It includes the size and dependence of magnetic moment applied on the magnetic field, magnetic anisotropy presence, the extent of distribution and particle size, and the shell and surface nature of the particle. It is strongly modified by the magnetic properties of the Np and hydrodynamic. An MNP is high in T2 relaxivity (concentration of MNPs per rate of relaxation) MNP. Its colloidal suspension is considered and used in MRI CA.[54] MRI imaging as a magnetic contrast agent consists of metal alloys and oxides. The specific targeting of MNPs ensured the desired tissue for the effective development of a contrast agent. RES eliminates the unwanted MNP structure, but significant challenges are posed by the non-specific accumulation of the spleen, lymph node, and liver. Surface function and target agent were involved in specific targeting. Iron oxide nanoparticle employs contrast agent with are without particular targeting. Tumour cells can be selectively imaged by precise targeting; for example, they are several targeted nanostructures, such as chlorotoxin conjugated PEG-

coated iron oxide particles, anti-carcino embryonic antigen conjugated iron oxide nanoparticle, which is used to enhance the contrast in magnetic imaging. MRI shows more sensitivity *in vivo* by using the MNP. It exhibits high magnetization. MFe_2O_4 is an iron oxide nanoparticle engineered with a series of metal-doped magnetism, where MFe_2O_4 (M^{+2}) cation of Fe, Co, Ni, or Mn. In mice, the susceptible target was exhibited by Herceptin-conjugated manganese doped magnetism-engineered iron oxide (MnME IO); it shows even the tiny tumour and sufficient sensitive image. Herceptin-conjugated Mn ME IO and CLIO (cross-linked iron oxide) were injected various times to get an MR image. As a result, Mn MEIO showed colour-changing contrast from red to blue, and time increased for advanced targeting. In CLIO, there is no change observed. High MNP and the targeting agent provide a powerful platform for ultrasensitive cancer detection. Multifunctional nanoparticles exhibit the impact of multifunctional merged with various metals like plasmonic and magnetic (quantum dots). For example, to employ magnetic or optical images, the dumbbell structure is connected with the plasmonic or MNP; the same approach was exhibited in nanostructure for the dual purpose of therapy and imaging[55].

7.2 CT SCAN

CT scan is a CT with excellent anatomical imaging technology but has the disadvantage of functional image. MRI/CT have almost similar functions, as dual-modal agent combines two anatomic images. MRI and CT are involved physically for image processing, and CT uses X-ray radiation to pass through the tissue. The recording of the image is based on attenuation characteristics and absorption of the contrast agents. In MRI, the idea is captured with the help of a magnetic field; the image is based on T1 and T2 to analyse the contrast enhancement induced by the nanoparticles. CT contrast agents are mostly gold nanoparticles, forming a hybrid they combined with SPION; the hybrid is known as gold iron oxide nanoparticles (GION). It can be used MRI/CT as a dual-contrast agent. In CT, gold acts as a contrast moiety compared to MRI, which provides less sensitivity and spatial resolution. In CT, the dual-contrast agent provides a synergistic effect with a layered gold shell and iron oxide core coated with PEG on the surface synthesized and produces a result. In water, PEG-coated GION is dispersed, and the average size of the hydrodynamic is approximately 47 nm, measured by TEM. PEG coating GION is greater than the iodine-based counterpart in the CT image even at the same concentration; the efficiency decreased in the T2 signal compared to oleyl amine/oleic acid-coated SPION. Au-oleylamine and Fe-oleate mixture of thermal decomposition synthesizes the hybrid nanoparticle; amphiphilic poly is coated by

nano-emulsion. As a result, water dispersion and biocompatibility are accepted by the nanoparticles. In in-vivo CT contrast, enhancement is improved (1.6-fold) 1h post-injection in the murine hepatoma model. Compared to resovist T2 relativity coefficient is greater. In vivo, hepatoma and normal tissue can be noticeable. Unlike iodine agents, hybrid GION provides a better effect and serves as a dual-contrast agent. In CT contrast enhancement, hybrid gold-SPION displace better, and the T2 relativity is higher in MRI. This case gives a strong example of developing and demonstrating a multimodal system[91,92].

8. CHALLENGES ASSOCIATED WITH MNMS

Nanotechnology offers promising results in medicine and environmental fields, but this technology has unintended effects on the environment in humans and the natural ecosystem. The advantage of new technology was brought up in health, sustainability benefits, and environments but must be examined before implementation. The technology should undergo specific medical, ethical, legal, and applicable criteria to the climate; MNM monitors and analyses the quality. MNPs play a vital role in biomedical applications, magnetic energy storage, and spintronics application[57]. The challenge to be overcome is a large-scale development and issues related to toxicity because be a threat to living systems because they can act as a contaminated carrier and interact with living organisms that can cause a hazardous reaction. Reducing the toxicity is possible by modifying it into biodegradable and biocompatible material. The current challenge of handling MNPs should be considered. The economic issues of MNP are that the adsorption stage is low, and the pollutant removal of contact time is short compared to conventional absorbents; in this case, it is profitable, but MNPs will perform with other materials, such as adsorbents, at low cost. Here, the financial barrier appears. Higher cost of absorbent is used in agriculture and industrial waste. MNPs give promising results in removing hazardous contaminants, and MNPs are environmentally and economically beneficial[58].

8.1 ENVIRONMENTAL TOXICITY

The potential risk of nanoparticles attracted national and international attention. The major drawback of nanomaterial is the analysis method of nanoparticles, nanomaterial vary in size, shape, and factors determine toxicity. Characterizing of nanomaterial is lack of information and the process of making it so that it defects to detect nanomaterials in air and environment, and nanomaterial chemical structure is critical to analyse the factor of toxicity, a slight

change in chemical cause a dramatic change in property. Nanoparticles should be evaluated in each stage to avoid the risk factor on the safety basis of human health and the environment. The risk factors to be concluded include toxicological analysis, probability of exposure, persistence risk, transformation risk, ability to recycle, and transport risk. Environmental impact can be predicted by life cycle risk assessment—nanoparticle products are designed to reduce waste products. The nanoparticle can interact in various environmental forms, such as bio-uptake or organic compounds. The nanoparticle can be divided into colloidal nanoparticles by aggregation, which allows for conventional transport to a sensitive environment. Specific ways nanoparticles and nanomaterial can become toxic to the environment are hydrophobic nanoparticles, mobility of contaminants, solubility, and disposal. Hydrophobic nanoparticle, TiO₂ powder currently nanocoating researchers are working on it. As a result, it reduces the weather effect, such as salt rain degradation on composite material. Still, it has a certain drawback TiO₂ is leaked in the environment. Mobility of contaminants in atmosphere nanoparticles can be emitted in two ways. Primary emission is where nanoparticle is directly talked to the air source; it is the primary source of emission where the secondary particles, such as (homogeneous nucleation with sulfuric acid and ammonia) are emitted naturally. Solubility, invention, and development of nanoparticles result in toxicity. Many nanoparticles are dissolved in water, so it is hard to separate them from water causing toxic effects in the water. Disposal of nanomaterial being disposed of properly may cause environmental impacts. Specific adverse effects such as the high energy needed to synthesize the nanoparticle, lower recycling rate, recovery, and dissemination of toxic nanosubstance can cause environmental impacts, and implication nanomaterial stage in the environment is not cleared[57].

8.2 STABILITY AND FUNCTION OF MNPS

Special requirement is needed in biomedical applications to control the MNPs interfaces, tailoring the property by functionalization of MNP surface. The functionalization process enhances colloidal stability in a biologically complex environment; molecular recognition is affected. Aggregation is generally prevented by stabilizers such as surfactants (sodium carboxymethylcellulose, sodium oleate) or synthetic polymers such as poly (lactic-co-glycolic acid), poly (ethylene-vinyl acetate), poly(ethylene-imine), poly(vinylpyrrolidone) to nature such as dextran, chitosan, and gelatin. The hydrocarbon layer covers the MNP, enhancing the non-aqueous solvent's stabilization. MNP used to consist of hydrophilic and biocompatible properties required in biomedical applications. Functionalization can be

described by three methods they are hydrophilic, ligand addition, and ligand exchange. Stabilization can be increased by organic and inorganic coating and resistance through oxidation in humid air or water. For example, On an MNPs surface, different molecules can bind. This process can be explained as hydroxyl groups of nanoparticles, and co-precipitation is obtained. The pH of the media determines the positive and negative charge. When OH-ligands are free and pH at 7.5, non-peptizable particles can be synthesized; when the pH range at 6- 10, ligands are still attached to MNP. So free hydroxyl group at the particle's surface can attach biomolecules further. This hydrophilic particle can be used in biomedical applications, represented by targeted drug delivery. In MNPs, surface modification is significant to carry the drug due to the fast clearance and low drug loading capacity from blood fluids. There are three approaches to overcome this drawback: surface modification with various polymer conjugation of MNP with the drug. The following two are based on a change of MNP with pro-drug-polymer composite or MNP modification of polymer, adsorption drug on MNP. PEG is a frequently used polymer with two ends of hydroxyl functional groups. This polymer is used in drug conjugation, enhanced drug solubility, and bioavailability. PEG gives promising results in drug carrier biocompatible anti-tumor medicine like doxorubicin and curcumin. Dextran is used in the modification of MNPs, which can entrap indomethacin. An MNP is based on magnetite; according to the acidic pH (<4), it is easily oxidized. And it is coated by inert oxides such as silica and alumina, which can improve the magnetism stability and suitability for surface functionalization. SiOH can prevent degradation, mainly used for drug carriers and in other fields such as electronics, catalysis, and paint. Noble metal such as gold or silver is decorated with MNP, which gives promising results in enhancing biocompatibility, chemical and physical properties, and bio affinity[95-98].

8.3 SAFETY AND ENVIRONMENTAL IMPLICATION

The recent study is on the safety implication of nanoparticles, but there needs to be more information or result obtained. Radial trouble, which notes is mainly caused by particulate matter. The matter increases by decreasing the diameter of nanoparticles. For example, a dust explosion is caused by a fine particle that causes high surface reactivity. An excellent particle is known as a nanoparticle. The metal's low boiling point can easily be exposed to a dust explosion. In this case, the particle is noted, but in every case, the particle won't tell independently in the form of a single particle. In a dust explosion, particle size increase won't become the solution. Extremely high agglomeration can be caused by fine particles

such as 1 μ m or smaller nanoparticles. In some cases, a large particle can cause behaviour conversely. This point is to be taken into consideration when it comes to the implementation of the nanoparticle. The performance of nanoparticles gives promising results in applications like biomedical, information technology, environmental science, energy production, and material. It offers opportunities for new materials to be designed, fabricated, and discarded. But we must consider safety measurements of production, process, and discard. To know the toxicity of the nanoparticle, we have conducted experiments to analyse the toxicity of the particle in a minimal concentration, with threshold concentration to be standardized in each material. If it is not fixed, it should not be used at any concentration, meaning the engineering process should not be carried out[99-102].

9. DISCUSSION

There are numerous uses for MNPs, particularly in biomedical fields. MNP uses in biomedicine have improved diagnostic and therapeutic approaches. Cobalt, iron, and nickel are three commonly utilized MNPs made of magnetic components. MNPs are essential for drug delivery and cell targeting in biomedical applications. MNPs are employed as drug carriers because they are biocompatible. It improves drug distribution to the intended place and regulates toxicity. With the help of this technology, we can treat the affected area of the living system and analyse how toxicity interacts with it. Due to the contrast agent MNP's ability to provide high-quality pictures where even microscopic tumours may be identified, MRI in combination with MNPs produces excellent results in the interaction and analysis of targeted medication delivery. MNPs exhibit lower toxicity in a biological organism than other nanoparticles; they are frequently utilized in chemotherapy and drug delivery.

10. CONCLUSION

In terms of biomedical use and research, MNP yields encouraging outcomes. The application provides fundamental understanding and aids in the development of biomedical applications because of its surface effect and anisotropy. Due to surface functionalization, MNP exhibits distinctive and important characteristics, particularly in terms of stability. This application, which is employed in many different domains of diagnosis and treatment, makes them less hazardous and gives them good biocompatibility with the biological system.

coated iron oxide particles, anti-carcino embryonic antigen conjugated iron oxide nanoparticle, which is used to enhance the contrast in magnetic imaging. MRI shows more sensitivity *in vivo* by using the MNP. It exhibits high magnetization. MFe_2O_4 is an iron oxide nanoparticle engineered with a series of metal-doped magnetism, where MFe_2O_4 (M^{+2}) cation of Fe, Co, Ni, or Mn. In mice, the susceptible target was exhibited by Herceptin-conjugated manganese doped magnetism-engineered iron oxide (MnME IO); it shows even the tiny tumour and sufficient sensitive image. Herceptin-conjugated Mn ME IO and CLIO (cross-linked iron oxide) were injected various times to get an MR image. As a result, Mn MEIO showed colour-changing contrast from red to blue, and time increased for advanced targeting. In CLIO, there is no change observed. High MNP and the targeting agent provide a powerful platform for ultrasensitive cancer detection. Multifunctional nanoparticles exhibit the impact of multifunctional merged with various metals like plasmonic and magnetic (quantum dots). For example, to employ magnetic or optical images, the dumbbell structure is connected with the plasmonic or MNP; the same approach was exhibited in nanostructure for the dual purpose of therapy and imaging[55].

7.2 CT SCAN

CT scan is a CT with excellent anatomical imaging technology but has the disadvantage of functional image. MRI/CT have almost similar functions, as dual-modal agent combines two anatomic images. MRI and CT are involved physically for image processing, and CT uses X-ray radiation to pass through the tissue. The recording of the image is based on attenuation characteristics and absorption of the contrast agents. In MRI, the idea is captured with the help of a magnetic field; the image is based on T1 and T2 to analyse the contrast enhancement induced by the nanoparticles. CT contrast agents are mostly gold nanoparticles, forming a hybrid they combined with SPION; the hybrid is known as gold iron oxide nanoparticles (GION). It can be used MRI/CT as a dual-contrast agent. In CT, gold acts as a contrast moiety compared to MRI, which provides less sensitivity and spatial resolution. In CT, the dual-contrast agent provides a synergistic effect with a layered gold shell and iron oxide core coated with PEG on the surface synthesized and produces a result. In water, PEG-coated GION is dispersed, and the average size of the hydrodynamic is approximately 47 nm, measured by TEM. PEG coating GION is greater than the iodine-based counterpart in the CT image even at the same concentration; the efficiency decreased in the T2 signal compared to oleyl amine/oleic acid-coated SPION. Au-oleylamine and Fe-oleate mixture of thermal decomposition synthesizes the hybrid nanoparticle; amphiphilic poly is coated by

nano-emulsion. As a result, water dispersion and biocompatibility are accepted by the nanoparticles. In in-vivo CT contrast, enhancement is improved (1.6-fold) 1h post-injection in the murine hepatoma model. Compared to resovist T2 relativity coefficient is greater. In vivo, hepatoma and normal tissue can be noticeable. Unlike iodine agents, hybrid GION provides a better effect and serves as a dual-contrast agent. In CT contrast enhancement, hybrid gold-SPION displace better, and the T2 relativity is higher in MRI. This case gives a strong example of developing and demonstrating a multimodal system[91,92].

8. CHALLENGES ASSOCIATED WITH MNMS

Nanotechnology offers promising results in medicine and environmental fields, but this technology has unintended effects on the environment in humans and the natural ecosystem. The advantage of new technology was brought up in health, sustainability benefits, and environments but must be examined before implementation. The technology should undergo specific medical, ethical, legal, and applicable criteria to the climate; MNM monitors and analyses the quality. MNPs play a vital role in biomedical applications, magnetic energy storage, and spintronics application[57]. The challenge to be overcome is a large-scale development and issues related to toxicity because be a threat to living systems because they can act as a contaminated carrier and interact with living organisms that can cause a hazardous reaction. Reducing the toxicity is possible by modifying it into biodegradable and biocompatible material. The current challenge of handling MNPs should be considered. The economic issues of MNP are that the adsorption stage is low, and the pollutant removal of contact time is short compared to conventional absorbents; in this case, it is profitable, but MNPs will perform with other materials, such as adsorbents, at low cost. Here, the financial barrier appears. Higher cost of absorbent is used in agriculture and industrial waste. MNPs give promising results in removing hazardous contaminants, and MNPs are environmentally and economically beneficial[58].

8.1 ENVIRONMENTAL TOXICITY

The potential risk of nanoparticles attracted national and international attention. The major drawback of nanomaterial is the analysis method of nanoparticles, nanomaterial vary in size, shape, and factors determine toxicity. Characterizing of nanomaterial is lack of information and the process of making it so that it defects to detect nanomaterials in air and environment, and nanomaterial chemical structure is critical to analyse the factor of toxicity, a slight

change in chemical cause a dramatic change in property. Nanoparticles should be evaluated in each stage to avoid the risk factor on the safety basis of human health and the environment. The risk factors to be concluded include toxicological analysis, probability of exposure, persistence risk, transformation risk, ability to recycle, and transport risk. Environmental impact can be predicted by life cycle risk assessment—nanoparticle products are designed to reduce waste products. The nanoparticle can interact in various environmental forms, such as bio-uptake or organic compounds. The nanoparticle can be divided into colloidal nanoparticles by aggregation, which allows for conventional transport to a sensitive environment. Specific ways nanoparticles and nanomaterial can become toxic to the environment are hydrophobic nanoparticles, mobility of contaminants, solubility, and disposal. Hydrophobic nanoparticle, TiO₂ powder currently nanocoating researchers are working on it. As a result, it reduces the weather effect, such as salt rain degradation on composite material. Still, it has a certain drawback TiO₂ is leaked in the environment. Mobility of contaminants in atmosphere nanoparticles can be emitted in two ways. Primary emission is where nanoparticle is directly talked to the air source; it is the primary source of emission where the secondary particles, such as (homogeneous nucleation with sulfuric acid and ammonia) are emitted naturally. Solubility, invention, and development of nanoparticles result in toxicity. Many nanoparticles are dissolved in water, so it is hard to separate them from water causing toxic effects in the water. Disposal of nanomaterial being disposed of properly may cause environmental impacts. Specific adverse effects such as the high energy needed to synthesize the nanoparticle, lower recycling rate, recovery, and dissemination of toxic nanosubstance can cause environmental impacts, and implication nanomaterial stage in the environment is not cleared[57].

8.2 STABILITY AND FUNCTION OF MNPS

Special requirement is needed in biomedical applications to control the MNPs interfaces, tailoring the property by functionalization of MNP surface. The functionalization process enhances colloidal stability in a biologically complex environment; molecular recognition is affected. Aggregation is generally prevented by stabilizers such as surfactants (sodium carboxymethylcellulose, sodium oleate) or synthetic polymers such as poly (lactic-co-glycolic acid), poly (ethylene-vinyl acetate), poly(ethylene-imine), poly(vinylpyrrolidone) to nature such as dextran, chitosan, and gelatin. The hydrocarbon layer covers the MNP, enhancing the non-aqueous solvent's stabilization. MNP used to consist of hydrophilic and biocompatible properties required in biomedical applications. Functionalization can be

described by three methods they are hydrophilic, ligand addition, and ligand exchange. Stabilization can be increased by organic and inorganic coating and resistance through oxidation in humid air or water. For example, On an MNPs surface, different molecules can bind. This process can be explained as hydroxyl groups of nanoparticles, and co-precipitation is obtained. The pH of the media determines the positive and negative charge. When OH-ligands are free and pH at 7.5, non-peptizable particles can be synthesized; when the pH range at 6- 10, ligands are still attached to MNP. So free hydroxyl group at the particle's surface can attach biomolecules further. This hydrophilic particle can be used in biomedical applications, represented by targeted drug delivery. In MNPs, surface modification is significant to carry the drug due to the fast clearance and low drug loading capacity from blood fluids. There are three approaches to overcome this drawback: surface modification with various polymer conjugation of MNP with the drug. The following two are based on a change of MNP with pro-drug-polymer composite or MNP modification of polymer, adsorption drug on MNP. PEG is a frequently used polymer with two ends of hydroxyl functional groups. This polymer is used in drug conjugation, enhanced drug solubility, and bioavailability. PEG gives promising results in drug carrier biocompatible anti-tumor medicine like doxorubicin and curcumin. Dextran is used in the modification of MNPs, which can entrap indomethacin. An MNP is based on magnetite; according to the acidic pH (<4), it is easily oxidized. And it is coated by inert oxides such as silica and alumina, which can improve the magnetism stability and suitability for surface functionalization. SiOH can prevent degradation, mainly used for drug carriers and in other fields such as electronics, catalysis, and paint. Noble metal such as gold or silver is decorated with MNP, which gives promising results in enhancing biocompatibility, chemical and physical properties, and bio affinity[95-98].

8.3 SAFETY AND ENVIRONMENTAL IMPLICATION

The recent study is on the safety implication of nanoparticles, but there needs to be more information or result obtained. Radial trouble, which notes is mainly caused by particulate matter. The matter increases by decreasing the diameter of nanoparticles. For example, a dust explosion is caused by a fine particle that causes high surface reactivity. An excellent particle is known as a nanoparticle. The metal's low boiling point can easily be exposed to a dust explosion. In this case, the particle is noted, but in every case, the particle won't tell independently in the form of a single particle. In a dust explosion, particle size increase won't become the solution. Extremely high agglomeration can be caused by fine particles

such as 1 μ m or smaller nanoparticles. In some cases, a large particle can cause behaviour conversely. This point is to be taken into consideration when it comes to the implementation of the nanoparticle. The performance of nanoparticles gives promising results in applications like biomedical, information technology, environmental science, energy production, and material. It offers opportunities for new materials to be designed, fabricated, and discarded. But we must consider safety measurements of production, process, and discard. To know the toxicity of the nanoparticle, we have conducted experiments to analyse the toxicity of the particle in a minimal concentration, with threshold concentration to be standardized in each material. If it is not fixed, it should not be used at any concentration, meaning the engineering process should not be carried out[99-102].

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This review compiles nanoparticle applications and methods used in biomedicine. There is a thorough explanation of the nanoparticle synthesis process. In this review, the interaction of MNP with the biological system is discussed. This review included a toxicology investigation. Applications for medication distribution were thoroughly explained. The review enclosed safety evaluation and nanoparticle environment implications.

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