LITERATURE REVIEW ON BIO-MEDICAL APPLICATIONS OF IRON OXIDE NANOPARTICLES

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CERTIFICATE

This is to certify that the dissertation entitled, "**LITERATURE REVIEW ON BIO-MEDICAL APPLICATIONS OF IRON OXIDE NANOPARTICLES"** submitted to Goa University in partial fulfilment of the award of the degree in Masters of Science in Chemistry, is a work done by **Mr. AESTRONY HERBERT FRANCO COSTA** during the year 2021-2022 under the supervision and guidance of **Mr. Vishnu Chari.**

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DECLARATION

I hereby declare that the work embodied in this report entitled "**LITERATURE REVIEW ON BIO-MEDICAL APPLICATIONS OF IRON OXIDE NANOPARTICLES"** was carried out by me during the year 2021-2022 under the guidance of **Mr. Vishu Chari.** In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

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1. **Introduction**

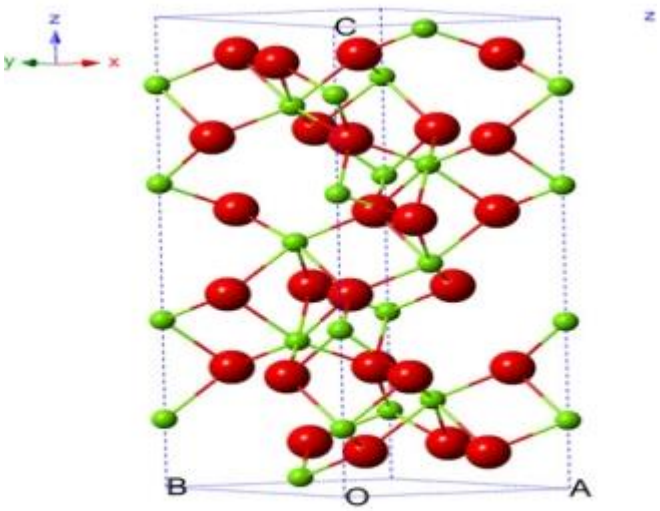
Compounds of Iron oxides are common, and they are widely spread in nature and can be readily synthesized in the laboratory. The earth's crust is made up of 6.3% of iron. It is the fourth most abundant element in the earth's crust. Nanoparticles are at the forefront of rapid development when it comes to the field of nanotechnology. Their exclusive size dependent properties make these materials indispensable and superior in many areas of human activities.[1]Iron is the most current transition element in the earth's crust and it stands as a backbone of current infrastructure. However, in comparison to the group elements such as cobalt, nickel, gold and platinum, iron oxides are somewhat neglected. Iron and oxygen chemically combine to form iron oxide. And there are approximately 16 identified iron oxides[2]. In nature, iron (III) oxide is found in the form of rust.[3] Generally iron oxides are prevalent and widely used as they are inexpensive and play an imperative role in many biological and geological processes. Iron oxides are extensively used by humans. They found their use as iron ores in thermite processes, as catalysts, as durable pigments (coatings, paints, and coloured concretes). Iron is also found in the biological system i.e., in the haemoglobin. The three most important forms of iron oxides commonly found in nature are magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and hematite (α-Fe_2O_3). These oxides are of utmost importance in the field of science and technology and are therefore the subject of study.[4]

1.1 Iron oxides

Hematite (α-Fe_2O_3), magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are very promising and popular candidates among the iron oxides because of their polymorphism involving temperature-induced phase transition. Each of these three iron oxides has unique biochemical, magnetic, catalytic, and other properties which provide suitability for specific technical and biomedical applications[5].

A. Hematite (α-Fe_2O_3)

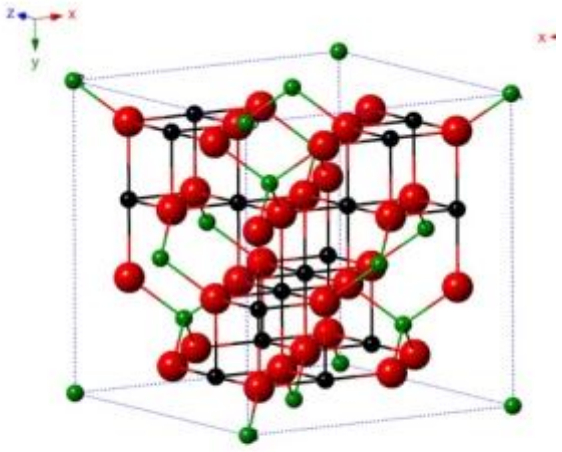
It is the most stable iron oxide. It is widely used as an n-type semiconductor under ambient conditions. Haematite is widely used in catalysts, pigments and gas sensors due to its low cost and high resistance to corrosion. It can also be used as a starting material for the synthesis of magnetite and maghemite which have been intensively pursued for both scientific interests and technological applications in the last few decades[6]. Hematite is an n-type semiconductor with a band gap of 2.3 eV, where the conduction band (CB) is composed of empty d-orbitals of Fe^{3+} and the valence band (VB) consists of occupied 3d crystal field orbitals of Fe^{3+} with some admixture from the O 2p non-bonding orbitals[7]. As shown in figure (a) Fe^{3+} ions occupy two-thirds of the octahedral sites that are confined by the nearly ideal hexagonal close-packed O lattice.



(a) Hematite
Rhombohedral, $R\bar{3}c$ [4]

B. **<u>Magnetite (Fe_3O_4)</u>**

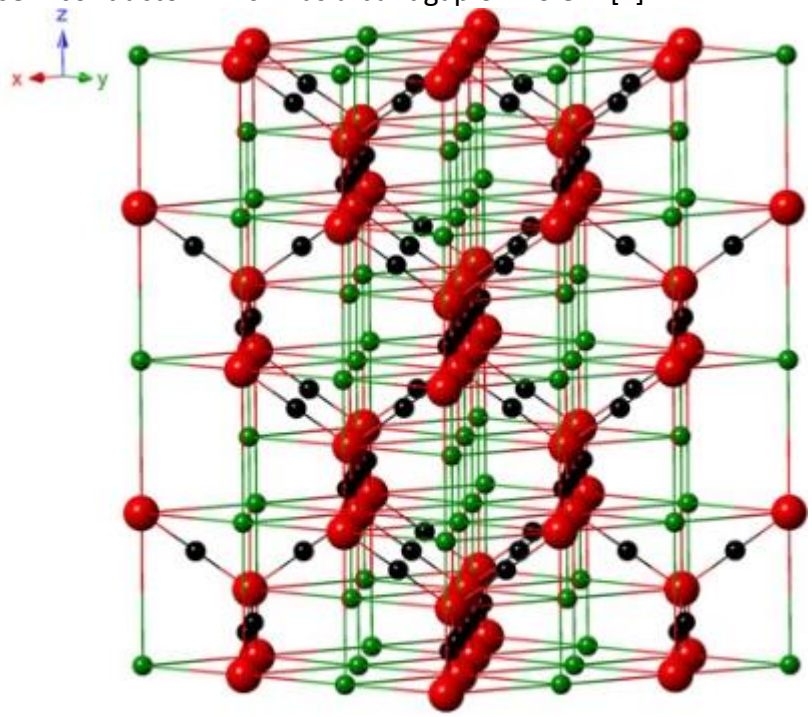
As shown in figure (b) Fe_3O_4 has the face centred cubic spinel structure, based on 32 O^{2-} ions and close-packed along the [111] direction. Fe_3O_4 differs from most other iron oxides in that it contains both divalent and trivalent iron. Fe_3O_4 has a cubic inverse spinel structure that consists of a cubic close packed array of oxide ions, where all of the Fe^{2+} ions occupy half of the octahedral sites and the Fe^{3+} are split evenly across the remaining octahedral sites and the tetrahedral sites. In magnetite Fe (II)/Fe (III) = 1/2, and the Fe (II) ions can be partly or fully replaced by other divalent ions such as Co, Mn, Zn, etc. Thus, Fe_3O_4 can act as both an n- and p-type semiconductor. However, Fe_3O_4 has the lowest resistivity among iron oxides due to its small bandgap (0.1 eV) [8].



(b) Magnetite
cubic, Fd3m [4]

C. <u>Maghemite (γ-Fe_2O_3)</u>

As shown in figure I, the structure of γ-Fe_2O_3 is cubic; each unit of maghemite contains 32 O^{2-} ions, 21⅓ Fe^{3+} ions and 2⅓ vacancies. Oxygen anions present in maghemite can give rise to a cubic close-packed array. The ferric ions are found to be distributed over tetrahedral sites i.e., 8 Fe ions per unit cell and octahedral sites i.e., the remaining Fe ions and vacancies. Because of this, the maghemite can be considered as a fully oxidized magnetite. It is a well-known n-type semiconductor which has a bandgap of 2.0 eV. [4]



(c) Maghemite

Cubic, $P4_332$/Tetragonal, $P4_12_12$ [4]

Nano particles composed of ferromagnetic substances like iron oxide and with the size <10-20 nm showcase a peculiar type of magnetism. This is known as superparamagnetic. Ferromagnetic materials are substances like elemental metals, alloys, oxides and other chemical compounds that are magnetized by an external magnetic field. This is an important phenomenon showcased by nanoparticle systems. Due to their low toxicity, superparamagnetic properties, such as surface area and volume ratio, and simple separation methodology, magnetic iron oxide (Fe_3O_4 and γ-Fe_2O_3) nanoparticles have attracted much attention and are especially interesting in biomedical applications such as diagnostic magnetic resonance imaging (MRI), thermal therapy, and drug delivery. Iron's reactivity is important in macroscopic applications (particularly rusting), but is a dominant concern at the nanoscale.[9]

2. ***Method of* synthesis *of iron oxide nanoparticles***

Iron oxide nano particles can be prepared by employing various methods which can be classified as wet chemical methods, dry methods or microbiological techniques. Briefly, these methods of synthesis can be further classified into three categories:

a. Physical methods: These methods are elaborate methods and they suffer from problems like control of particle size in the nanometre range. [10]

b. Chemical preparation methods: these methods are simple and efficient. The size of the nanoparticles, their composition and even the shape can be controlled. The size, shape, and composition of iron oxide nanoparticles synthesized through chemical methods totally depends on the type of salt used, Fe^{2+} and Fe^{3+} ratio, pH and ionic strength.

c. Biological methods: These methods are not very well developed and hence a handful of them are reported. [11]

Chemical-based methods of synthesis are one of the most adopted among the other methods due to their low production cost and higher yield.

The physical and chemical properties of nanoparticles (NPs) vary depending upon the conditions. In order to prevent iron NPs from getting oxidised and agglomerated, Fe_3O_4 NPs are coated with organic or inorganic molecules. However, it is a necessary requirement to synthesize magnetic iron oxide NPs in oxygen-free environment. This can be most preferably done in the presence of N_2 gas. It is known in the literature work that bubbling nitrogen gas protects NP from oxidation and also reduces the size of the synthesized nanoparticles[10].

Physical methods are easy to perform but controlling the size is an issue that has been faced by many researchers across the world. This problem is rectified in wet chemical methods as it is possible to have a control on the size of the nanoparticles. The chemical methods usually include electrochemical method, sol–gel method, supercritical fluid method, hydrothermal method, chemical coprecipitation method, sonochemical decomposition method, flow injection method etc.[12] All these techniques utilize aqueous medium as a most efficient pathway to obtain iron magnetic nano particles. It has been demonstrated that the particle size as well as the polydispersity of the nano particles can be tailored by changing the associated factors such as Fe^{2+}/ Fe^{3+} ratio, base (NaOH, ammonium hydroxide, and CH_3NH_2), and ionic strength ($N(CH_3)_4^+$, CH_3 NH_3^+, NH_4^+, Na^+, Li^+ , and K^+)[13]. Some other factors which also have an influence on the size of the NPs are as follows: an increase in mixing rate, temperature, inlet of nitrogen gas, agitation, pH, and reactants ratio. A handful of microbial methods which are known ensure low cost, reproducibility, high yield, and scalability[11]. The major drawback of microbial methods is that they are quite time-consuming and hence not utilized in the field of industry.

3. Techniques for the synthesis of magnetic nano particles

Several synthesis routes to achieve shape, size, crystallinity, dispersity, and magnetic behaviour of iron oxide nano particles have been developed.

A. Liquid phase methods

These are the simplest methods as they allow the preparation of magnetic NPs with rigorous control of size and shape. Homogeneous precipitation reactions are usually utilized in order to synthesize uniform sizes of particular nano particles. This involves the separation of the nucleation step and growth of the nuclei step. One of the classic models for synthesis is proposed by LaMer and Dinegar.[14] According to their method the nuclei are allowed to slowly diffuse which results in growth, until the final size is attained. Nucleation should be avoided during the period of growth in order to attain monodisparity. One of the most frequently used methods is coprecipitation from aqueous solutions. The reaction of Fe (II) salt, in aqueous solution, to a base in the presence of mild oxidant synthesizes spherical NP of 30–100 nm.[15] The factors on which the phase and size of the particles depend are the concentration of cations, the presence of counter ions, and pH of the solution. Change in pH and ionic strength play a vital role in controlling the mean size of the particles (from 15 nm to 2 nm).[16] aggregation of nanoparticles is seen due to large surface-area-to-volume ratio and to reduce surface energy. Anionic surfactants are added in order to stabilize the nanoparticles and to avoid agglomeration. Stabilization is also achieved by coating the surface with proteins, starches, non-ionic detergents, or polyelectrolytes. Adsorption of such substances stabilizes the electrolyte concentrations of particles that would otherwise be high enough. In this regard the first controlled preparation that used alkaline precipitation of $FeCl_3$ and $FeCl_2$ of superparamagnetic iron oxide particles was performed by Massart.[17] Originally, synthesized magnetite (Fe_3O_4) particles were roughly spherical, and their diameter measured by X-ray diffraction analysis was 8 nm.[17] Liquid phase methods demonstrate the influence of base (ammonia, CH_3NH_2 , and NaOH), added cations ($N(CH3)_4^+$, K^+ , $CH_3\ NH_3^+$, Li^+ , Na^+ , and NH_4^+), pH and Fe^{3+} /Fe^{2+} ratio on the diameter and polydispersity of the synthesized NPs and the yield of this reaction and. Adjusting the above parameters it is easily possible to obtain particles with a size ranging from 16.6 nm to 4.2 nm.[15]

B. Sol–gel method

This method consists of two main steps; hydroxylation and condensation of molecular precursors in solution. The “sol” obtained from the nanometric particles is then dried or ‘‘gelled’’ either by removal of the solvent or by chemical reaction to obtain three-dimensional metal oxide network. The solvent normally used is water, but the precursors can be hydrolysed either by using an acid or a base. It has been found in the literature that basic catalysis yields a colloidal gel

and acid catalysis generates a polymeric gel.[18] These reactions are usually performed at room temperature; but heat treatment is required to some extent to obtain the final crystalline state.

The important factors influencing synthesis of nanoparticles by this method are pH, nature, and concentration of salt precursor, kinetics, temperature, agitation, and properties of gel being formed. The advantages involving this method include synthesis of materials with a structure that is predetermined, good control of microstructure, pure amorphous phase, monodispersity, excellent control over the particle size, homogeneity of the formed products, and chances to generate embed molecules, which maintain their stability and properties within the matrix.[12] This is one of the easiest methods for the production of metal oxides from salts predetermined conditions. Iron oxide–silica aerogel is being prepared using this method and they are found to be more reactive than conventional iron oxide. Commercial precursors such as tetraethyl orthosilicate and Fe (III) solutions are made to dissolve in alcoholic aqueous medium. The gels formed from them are heated to generate the final materials. Large surface area of iron oxide nanoparticles generated by this method are responsible for their high reactivity. [19]

C. Gas/aerosol phase methods

Spray and laser pyrolysis is an efficient technique for high, continuous, and direct production of well-defined magnetic nano particles. In this method a solution of ferric salts is sprayed in the reactor. This is done in the the presence of reducing agent. Condensation of the solute takes place while the solvent undergoes evaporation. The dried residue is obtained at a later stage which has a size same as the original ones. Maghemite particles from 5 nm to 60 nm with diverse shapes have been generated using different iron precursors.[19] Laser pyrolysis of organometallic precursors depends on the resonant interaction, reactant, and/or sensitizer; however, one should be in gaseous phase. In this type of methods, a sensitizer is excited by combing it with a CO_2 laser radiation. This transfers energy absorbed from the laser to the reactants. The energy is continuously provided by heating the mixture of gases by CO_2 laser. The chemical reaction keeps on continuing until the desired threshold level of nuclei is obtained. This is followed by the nucleation of the particles. These nucleated particles produced during the reaction are carried away by the gas stream and they are gathered at the exit of the reactor chamber. Hasany et al[20] have studied the production of iron oxide by using gas phase, laminar diffusion flame methodology for the synthesis of reduced iron oxide NPs. Gas/aerosol method is known to produce very high-quality products. The only disadvantage being that the product yield is quite low. Decreasing gas impurities, control on the heating time and gas concentrations can lead to the production of highly pure products. However, this method is quite expensive and this remains its greatest drawback.

D. Hydrothermal reaction methods

The hydrothermal reactions are carried out in a reactor or autoclave which contains aqueous media. The pressure requirements are quite high and may go up to >2,000 psi. temperature of >200°C are to be maintained for the smooth functioning of the autoclave. The dehydration of metal salts and low solubility of oxides in aqueous phase supersaturate the medium. Hao and Teja[21] performed a thorough investigation in order to study the effects of temperature, precursor, and the time on morphology and particle size of the nanoparticles of iron oxide produced by this method. It was found that the precursor concentration increases the particle size, while residence time has more effect than concentration. Short residence time facilitate the production of monodispersed particles. The effect of changing the concentration of the precursor (e.g., ferric nitrate) while keeping all other variables constant was widely studied in various experiments. The transmission electron microscopy (TEM) images of particles obtained showed that the particles possessed a spherical shape with an average particle radius of 15.6±4.0 nm. In some other experiments where the precursor concentration was changed a few larger rhombic particles with an average particle size of 27.4±7.0 nm was obtained. [12]

E. Microwave irradiation

Microwave chemistry has gained momentum in the past few years, as it has been utilized in material synthesis and preparative chemistry since 1986. Although this method gained interest it does suffer many disadvantages. Time for crystallization is short and there is homogenous nucleation due to the application of uniform heat of microwave oven. In spite of its disadvantages this method has been successfully utilized to generate iron oxide nanoparticles. It was reported by Kijima et al [22] that the synthesis of ultrafine α-Fe_2O_3 nano particles by microwave heating generated nano particles with significantly high electrochemical performance. This was attributed to their uniformity and size. Most of these nano particles had ellipsoid shapes and they were found connected to each other. The average diameter of these primary particles was <10 nm. In another study by Parsons et al [23] it was reported that the synthesis of iron oxide/oxyhydroxide nano particles was possible by microwave method.

4. **Surface coatings of nanoparticles**

Iron oxide nanoparticles with bare surface cannot be left just like that as they tend to agglomerate. Agglomeration is caused by strong magnetic attraction among nano particles, van der Waals forces, and high surface energy. It has been found that the reticuloendothelial system; system of cells responsible for clearance of particles and substances in circulation and tissues, eliminates the agglomerated iron oxide particles.[24] High concentration of local Fe ions is known to be toxic to organisms due to Fe dissolution. Agglomeration of iron oxide nanoparticles and the ill effects caused by free existing nano particles can be avoided by coating a shell on the iron oxide nano particles surface which makes

them compatible to bioenvironments, hydrophilic, and functionalized. The appropriate surface coating allows a targetable delivery with particle localization in a specific area. It is also considered to be nontoxic and biocompatible.

It is noted that a lot of work and investigation is going on in order to improve the biocompatibility of surface coating material. But not much of scientific investigation is carried out with regards to the improvement in the quality of magnetic particles, their size distribution, shape, and surface. The nature of surface coating on the nano particles and their geometric arrangement determines the overall size of the colloidal particles. It also plays a role in biokinetics and biodistribution of nano particles in the body. Surface coatings of nanoparticles depends on their application and is aimed at either inflammation response or anticancer agents. Entities such as antibodies, proteins, drugs, enzymes, or nucleotides can bind to magnetic nano particles and can be easily adsorbed at a specific site in the human body using a magnetic field. They can also be heated in alternating magnetic fields for use in hyperthermia briefly discussed in the bio-medical applications of iron oxide nanoparticles section. Iron oxide nano particles synthesis coated with biological molecules, e.g., gluconic acid, lactobionic acid, or polyacrylic acid, through the most effective methods such as coprecipitation are reported in the literature.[25] This type of nano particles has a very narrow size distribution and are highly soluble in water. They potential has been envisaged in numerous biomedical applications including tissue engineering due to the biological coatings such as liposome coating.
In the absence of any surface coating material, magnetic iron oxide particles possess hydrophobic surfaces having large surface-area-to-volume ratio. The hydrophobic interactions between the particles causes nano particles to agglomerate and form large clusters hence resulting in an increased particle size. These clusters show ferromagnetic behaviour as they exhibit strong magnetic dipole–dipole attractions between them. When two large-particle clusters approach one another each of them comes into the magnetic field of their neighbour.[26] As each particle is in the magnetic field of their neighbour, they receive further magnetization. As this mutual magnetization takes place there is adherence of these remnant magnetic particles which further results in increase in aggregation properties. These particles cannot be subjected to any surface modification as they are attracted magnetically, in addition to the usual flocculation due to van der Waals forces. Separating these nano particles becomes a cumbersome business. Therefore, it is necessary to coat the iron oxide nano particles with a high-density coating in order to stabilize them. To prevent the aggregation of nanoparticles during the time of their preparation, a surfactant or a polymer is usually added. Most of these polymers adhere to surfaces in a substrate-specific manner. Table 1 shows different surface modifications and strategies for the fabrication of magnetic iron oxide NPs. [27] This table is directly taken from a research review article.

Table 1: Approaches for the preparation and surface modification of magnetic iron oxide NPs [27]

Diameter (nm)	**Approaches for the preparation and surface modification of magnetic iron oxide NPs**
1–20	Precipitation of magnetic iron oxide NPs in either solution or aqueous core of water in oil microemulsions. Oxygen-free and lower temperature environment provide the SPIONs with high magnetization values
20–30 (up to 50)	Precipitation of magnetic iron oxide NPs in the presence of polymers or surfactants. NPs obtained are monodispersed and fairly stable in solution
50–100	Surface coating of magnetic iron oxide NPs with surfactants or polymers (core–shell structure). Ferrofluids obtained are stable under in vivo and in vitro conditions, and also the particles could be derivatized with bioactive molecules.

Materials possessing polymeric coating are classified as either synthetic or natural. Some examples of distinctive synthetic polymeric systems are: glycol, poly(vinyl-pyrrolidone), poly (lactic-*co*-glycolic acid), polyethylene poly (ethylene-*co*-vinyl acetate), poly (vinyl alcohol), etc. on the other hand natural polymer systems include the use of chitosan, gelatine, dextran, etc. coating material are not easily dispersed in aqueous medium. Therefore, various surfactants, such as sodium carboxymethyl cellulose, sodium oleate, dodecyl amine, are usually used.[28] Utmost care and precautions needs to be taken while choosing the coating materials for the nano particles. Table 2 shows the various summarized coating methods and materials. There are coating techniques which are specifically designed to protect iron oxide cores from corrosion. Some other designs are also possible which are made specifically to enhance the chemical and physical functions for specific applications.[26]

Table 2 Different coating molecules/polymers for magnetic NPs to stabilize ferrofluids.

Molecules/polymers	Benefits	References
PEG	Improves biocompatibility by noncovalent immobilization of PEG on the surface, internalization efficiency of the NPs, and blood circulation time.	[29]
Dextran	Stabilizes the colloidal solution and increases the blood circulation time.	[30]
PVP	Stabilizes the colloidal solution and enhances the blood circulation time.	[31]
Fatty acids	Terminal functional carboxyl groups and colloidal stability.	[32]
PVA	Gives rise to monodisperse particles and prevents coagulation of particles.	[33]

5. Characterization of magnetic nanoparticles

In order to have a better understanding of surface properties, surface morphology, chemical composition and distribution of functional groups in space many characterization techniques are utilized. Fundamental techniques used to characterize iron oxide nano particles include: TEM, SEM, atomic force microscopy, X-ray diffraction analysis, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, vibrating sample magnetometry, and thermal gravimetric analysis. Other characterization techniques include ion–particle probe, thermodynamic, NP tracking analysis, tilted laser microscopy, zeta-potential measurements, isopycnic centrifugation, hydrophobic interaction chromatography, field flow fractionation, electrophoresis, and turbidimetry.[34]

6. Literature review on biomedical applications of iron oxide nanoparticles

Biomedical applications involve use of iron oxide nanoparticles in biological system. With the advancement in technology various methods have been laid down in this aspect. Bio-compatibility and toxicity of iron oxide nanoparticles are the important criteria to be accounted for in this regard. Magnetic iron oxide nanoparticles with long blood retention time, biodegradability and low toxicity have emerged as the primary nanoparticles for in vivo and in vitro biomedical applications.[24] In vivo

applications involve use of iron oxide nanoparticles within the biological system. Targeted drug delivery and magnetic contrast agents in MRI have emerged as the two most promising fields in this regard.

6.1. Targeted drug delivery

Traditionally drugs were introduced into the biological system either by oral ingestion or by intravenous injection. The circulatory system of the body would further distribute the drug throughout the system. However, for most therapeutic agents only a limited concentration of the drug would reach the targeted site or the affected organ. It was also found that there was reduced drug diffusion through biological barriers causing a high incidence of adverse effects. Both these drawbacks of traditional methods are addressed by modern targeted drug delivery methods. Increasing the concentration of the drugs in the targeted organ of interest while maintaining a relatively low concentration of the said drug in the other organ or tissues is one of the aims of targeted drug delivery.[35] It also aims at crossing the biological barriers via active accumulation. Functionalized iron oxide nanoparticles as a carrier can deliver a wide range of drugs to all the areas in the body. Magnetic iron oxide nanoparticles act as core and bio-compatible components acts as a functionalized shell to form the core shell structure for targeted drug delivery carriers. The drugs are bound or encapsulated in the polymer matrix. Many bio-compatible materials like bio-compatible organic polymers (PEG, chitosan, dextran), liposomes, silica and bio ceramics have been used to functionalise iron oxide nanoparticles for targeted drug delivery. [26] Magnetic iron oxide-based drug targeting has emerged as one of the promising methods for cancer treatment.

6.1.1. Iron oxide nano particle-based targeted drug delivery for cancer treatment

Cancers are defined as malignant diseases in which the uncontrolled development of abnormal cells leads to the formation of cancerous tumour having a potential to spread throughout the biological system. It represents the main health issue worldwide. The main treatment methods for treating this type of pathology are radiotherapy, surgery and chemotherapy.[36] While radiotherapy and surgery are rather local treatments for localized cancer treatment, chemotherapy suffers a lack of specificity thus giving rise to adverse side effects due to simultaneous and uncontrolled destruction of cancer and healthy cells. The anticancer activity of chemotherapeutic drugs is due to their ability to target and kill a cancer cell in a particular stage of development of the cell life cycle. Targeted magnetic nano particles can provide a better tumour selectivity. Carriers comprising magnetic iron oxide nano particles loaded with an anti-cancer drug are injected in the patient's body via human circulatory system. [37]An external magnetic field is applied to

localize the drug loaded carriers to the cancerous tumour site where the drug can be released either by enzymatic activity or changes in physiological conditions such as pH, osmolality and temperature to be taken up by the targeted cells.

In a study performed Zhang et al. they reported the development of a magnetic drug carrier composed of doxorubicin (DOX)-conjugated Fe_3O_4 nanoparticle cores and a PEG-functionalized porous silica shell (Fe_3O_4–DOX/pSiO_2– PEG). The DOX loading capacity of the porous drug carrier system was found to be 16.3 μg mg^{-1}.Fe_3O_4–DOX/pSiO_2–PEG nanoparticles were internalized by cells through an endocytosis process, and was easily functionalized with a targeting ligand via a silicone coupling agent for increased and specific uptake of the drug carrier in tumor cells over-expressing the folate receptor, such as MCF-7 and HeLa cells [35]

In a study performed by s kayal et al, they synthesised iron oxide nano particles for targeted drug delivery for cancer treatment. The iron oxide synthesized was Fe_3O_4 with average size of 10nm. Iron oxide nano particles were synthesized using co-precipitation methods. Synthesized Iron oxide nano particles were coated with poly vinyl alcohol. This was done by preparing an aqueous solution of poly vinyl alcohol. Iron oxide nano particles were mixed with the poly vinyl alcohol solution and stirred for 12 hours. Th coated nanoparticles were separated by using permanent magnets. They were dried and characterised. 5 different concentrations of poly vinyl alcohol solutions were prepared. The iron oxide nanoparticles were characterised by using x-ray diffraction, TEM, TGA, FTIR. The amount of poly vinyl alcohol attached to the iron oxide nano particles was estimated by using TGA and the attachment of PVA to the metal oxide core was confirmed by FTIR analysis. The iron oxide nano particles prepared in this manner were used to load a versatile anti-cancer drug doxorubicin (DOX). An aqueous solution of the drug was prepared. The dox loading and release studies showed that initially there is rapid adsorption of dox, then the adsorption rate slows down and finally reaches saturation. It was found that higher ploy vinyl alcohol content showed higher levels of adsorption of drug. 35 μg, 41 μg, 47 μg, and 58 μg of DOX per mg of carrier was loaded in 26 h with 0.5%, 1%, 2%, and 5% PVA respectively. The DOX release profiles from magnetic carriers coated with 0.5%, 1%, 2% and 5% PVA showed that initially there is a rapid release until 6 hours after which release slows down. A maximum of 45%, 33%, 25% and 17% of adsorbed drugs were released in 80 hours from carriers coated with 0.5%,1%, 2%, and 5% PVA respectively. [36]

In a study by Jnanranjan Panda et al, biocompatible nanodrug delivery formulation based on poly (D, L lactide-co-glycolic) acid (PLGA), polyethylene glycol (PEG) and superparamagnetic iron oxide nanoparticles was developed and evaluated for the enhanced delivery of docetaxel (DTX) drug to breast cancer cells. Docetaxel is a chemotherapy medication that has been used to

treat a number of types of cancers. The method used to synthesize iron oxide nanoparticles was hydrothermal methods. Iron oxide nano particles were encapsulated along with the DTX drug in a PLGA-PEG coating using a modified emulsion evaporation method. The characterization of the iron oxide nanoparticles was done by XRD, FE-SEM, TEM, DLS, and FTIR. The X-ray diffraction and analysis showed good crystallinity of the nanoparticles. It was revealed by the magnetization versus field (M-H) curve the superparamagnetic behaviour of the synthesized IONPs at room temperature with a saturation magnetization of 71.9 emu. g^{-1}. Iron oxide nanoparticles loaded with DTX (DIONP) showed a spherical shape and uniform size distribution in the range of 160-220 nm. DIONP showed higher internalization efficiency and moderate cytotoxicity in MCF-7 cells as found from the confocal microscopy and MTT assay. Further, in vivo plasma pharmacokinetic (PK) study was carried out and they showed improved values of important PK parameters such as area under curve, mean residence time, the volume of distribution, etc. for DIONP as compared to pure DTX. The drug loading capacity of DIONP was found to be 12% with a sustained drug release over the experimental time-period. The sustained release, higher saturation magnetization, predominant cancer cell uptake, controllable size, satisfactory drug loading, effective cytotoxicity along with improved PK profile of DIONP are the potentials that can make DIONP an outstanding drug delivery strategy for breast cancer therapy. [38]

Paclitaxel (PTX) is one of the most potent drugs used in the treatment of some advanced cancers. However, its clinical use is limited due to its poor water solubility. Although it is possible to use nanoparticles to deliver hydrophobic PTX, the greatest challenged faced was achieving sufficient drug loading capacity and maintaining small particle size and stability in biological media. In a study by Mike Jeon et al, they synthesized a PTX nanoparticle (NP) possessing a very small size and great stability; thus, overcoming the above-mentioned challenges. These PTX nano particles targeted breast cancer cells and enabled controlled release of PTX. This nanoparticle formulation (NP-PTX-FA) were made up of a superparamagnetic iron oxide core coated with short- and long-chain polyethylene glycol. The short-polyethylene glycol was meant for the conjugation of hydrophobic PTX and folic acid (FA). The long polyethylene glycol was meant to be an outer layer for improved hydrophilicity. The NP-PTX-FA synthesized in this work was found to possess a uniform size distribution (averaging 28.2 ± 0.64 nm) and high drug loading capacity of 22.8 wt % (vs <10 wt % normally). NP-PTX-FA released the drug under conditions mimicking the acidic intracellular pH of breast cancer cells and the FA conjugation led to higher NP uptake by target cells, enhancing the cytotoxicity to target cells compared to free PTX. It has been contemplated that this nanoparticle formulation holds a potential to improve breast cancer therapy. Its potential can also be explored for the delivery of other hydrophobic drugs meant for cancer treatment. [39]

Nanotheranostics is a new platform in nanomedicine aimed at developing both imaging and therapeutic functions in a single entity. A study performed by Qimeng Quan et al, previously had developed a human serum albumin (HSA) coated iron oxide nanoparticle (HINP) formula and used multiple imaging modalities to validate its tumour targeting attributes. This same group of enthusiasts in a later study, sought to load doxorubicin (Dox) onto the HINPs and to assess the potential of the conjugates as theranostic agents. They were able to find that about 0.5 mg of Dox and 1 mg of iron oxide nanoparticles (IONPs, Fe content) could be loaded into 10 mg of HSA matrices. The resulting D-HINPs (Dox loaded HINPs) possessed a size of 50 nm. It was also noted that they were able to release Dox in a sustained fashion. The HINPs were able to guide the translocation of Dox across the cell membrane and even its accumulation in the nucleus of the cell. It was also found that, In vivo, D-HINPs were able to retain a tumour targeting capability of HINPs. This was confirmed by in vivo MRI. Therapeutic study of these entities was carried out on a 4T1 murine breast cancer xenograft model. D-HINPs showcased a striking tumour suppression effect that greatly outperformed free Dox. Their potential can be further exploited to load other similar drugs or molecules that can further bring improvements in cancer therapy. [40]

Triple negative breast cancer (TNBC) is a type of breast cancer which is known to be very difficult to treat effectively, as it is highly aggressive and poses very high drug resistance. In a study performed by Qingxin Mu et al, they developed a multifunctional nanoparticle formulation encompassing an iron oxide core that has the ability to deliver doxorubicin; a cytotoxic agent, and polyinosinic: polycytidylic acid (Poly IC); a TLR3 agonist, in a targeted and simultaneous fashion to both breast cancer and dendritic cells. Endoglin-binding peptide (EBP); an important protein for tumour growth was used to target both TNBC cells and vasculature endothelium. The nanoparticle showcased physicochemical properties and also emerged as a tumour-specific targeting profile. The nanoparticle was able to induced apoptosis or cell death of the tumours via multiple mechanisms. Some of the mechanisms including direct tumour cell killing. The nanoparticle evidently was able to inhibit the growth of tumour and metastasis. It was observed that these nanoparticles were able to survive in an aggressive and drug-resistant metastatic mouse model of TNBC. This study showcased that a highly improved therapeutically efficient method for the treatment of metastatic TNBC can be developed. [41]

In a study by Pramod Kumar et al, were successful in developing a novel, low cost and easy synthesis of iron oxide nano particles. Iron-oxide nanoparticles synthesis was carried out through room-temperature reduction of a mixture of ferric and ferrous salts. Citric acid was used as a capping agent. These nanoparticles were electrostatically conjugated with an anticancer drug doxorubicin (Dox). Characterization of the resulting drug-nanoconjugates were carried out for their size, crystallinity, functionality, composition, along with their magnetic and optical behaviour. To probe their non-toxicity and biocompatibility these drug-nanoconjugates were treated with cultured lung carcinoma cell lines (A 549).

Their uptake in cells in culture was studied by optical bioimaging. *In vitro* studies carried out showed that these nanoparticles are nontoxic to cells in culture.[42]

Cancer therapy has widened its horizons and have tried to develop therapeutic cancer vaccines. Nanoparticles are obtaining more attention in the field of development of these cancer vaccines. Nanoparticles-based vaccine delivery platform possesses a very high potential for improving the immunogenicity of vaccine. In a study by Yi Zhao et al they explored a new technique in which they made use of iron oxide nanoparticles (superparamagnetic Fe_3O_4 nanoparticles) as a vaccine delivery platform and immune potentiator. They investigated how the potential of this formulation affected cytokine expression in macrophages and dendritic cells (DCs) in vitro and tumour growth in vivo. They were able to find significant differences in immune responses and tumour inhibition induced by OVA formulated with iron oxide nanoparticles. It was noted that Iron oxide nanoparticles were versatile in promoting the activation of immune cells and cytokine production. These results suggested that this nanoparticle-based delivery system has strong potential to be utilized as a general platform for cancer vaccines. Hence, they concluded that iron oxide nanoparticles have a great potential to be developed as cancer-vaccines. [43]

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of pancreatic cancer. Classical chemotherapy provides a limited treatment option. This cancer is projected to be the second leading cause of cancer related death by 2030. Therapeutic options remain temporary and ineffective in spite of extensive knowledge and insights into biological properties and genetic abnormalities of PDAC. One important explanation for the unsuccessful response to therapy is an inadequate and non-specific delivery of anticancer drugs to the tumour site. In a study performed by Ujwal Mahajan et al, an attempt was made to design Superparamagnetic iron oxide nanoparticles (SPIONs) coupled with siRNA directed against the cell cycle-specific serine-threonine-kinase, Polo-like kinase-1 (siPLK1-StAv-SPIONs), that could serve a dual purpose for delivery of siPLK1 to the tumour and for non-invasive assessment of efficiency of delivery in vivo by imaging the tumour response. Plk-1 plays a role in cell multiplication in tumours. siPLK1-StAv-SPIONs were designed and synthesised as theranostics. They were designed to function via a membrane translocation peptide with added advantage of driving endosomal escape for mediating transportation to the cytoplasm (myristoylated polyarginine peptides) as well as a tumour-selective peptide (EPPT1) to increase intracellular delivery and tumour specificity. Endogenous cancer model was treated biweekly with siPLK1-StAv-SPIONs and tumour growth was monitored by small animal MRI. Tumour-specific silencing of PLK1 was observed which halted tumour growth, marked by a decrease in tumour cell proliferation and an increase in apoptosis (cell death).[44]

In a study by Roberto Gonzalez-Rodriguez et al, superparamagnetic iron oxide nanoparticles (Fe_3O_4 NPs) were used to improve the functionality of graphene oxide (GO). GO were combined with iron oxide nano particles. These molecules served as a biocompatible magnetic drug delivery addends and magnetic resonance contrast agent for MRI. Synthesized GO-Fe_3O_4 conjugates were reported to have an average size of 260 nm. Compared to that of GO these entities showed low cytotoxicity. Fe_3O_4 nanoparticles improved superparamagnetic properties for magnetic targeted drug delivery hence allowing simple manipulation by the magnetic field and magnetic resonance imaging with high r_2/r_1 relaxivity ratios of ~10.7. It was found that GO-Fe_3O_4 retained pH-sensing capabilities of GO, which were used in their work to detect cancer versus healthy environments in vitro. They exhibited fluorescence in the visible for bioimaging. As a drug delivery platform GO-Fe_3O_4 showed successful fluorescence-tracked transport of hydrophobic doxorubicin non-covalently conjugated to GO with substantial loading and 2.5-fold improved efficacy. Hence GO-Fe_3O_4 nanoparticles can be proposed as a novel multifunctional magnetic targeted platform for high efficacy drug delivery. They can be traced in vitro by GO fluorescence and in vivo via MRI thus capable of optical cancer detection. [45]

A research study performed by Subrata Das et al, was aimed at showcasing the bactericidal activity and cytotoxic effect of iron oxide nanoparticles (IONPs). IONPs were successfully invented through thermal decomposition of a diiron (III) complex precursor. The morphology of the synthesized nanoparticles was characterised by the following methods. Scanning and transmission electron microscopy (FE-SEM and HR-TEM) analyses were able to estimate the cross-linked porous structure of IONPs with an average size ~97 nm. Dynamic light scattering (DLS) study of IONPs was carried out and it helped in determining the hydrodynamic diameter of the nano particles which was found to be 104 nm. In order to study the cytotoxic behaviour of these iron oxide nanoparticles a human lung cancer cell line (A549) through different fluorescence staining studies was carried out. They showcased the apoptosis or cell death of these type of cells. These iron oxide nanoparticles were also used to study their bactericidal properties. Measurement of reactive oxygen species was carried out and it suggested the destruction of mitochondrial membrane of *Staphylococcus aureus*. Hence these nanoparticles can be used against cancer and as antibacterial agents. [46]

6.2. Iron oxide nanoparticles as magnetic hypothermia agents.

Magnetic hypothermia (MH) is a procedure that is aimed at producing local heating. This is achieved by magnetically heating of low-frequency electromagnetic waves, through the power absorption by magnetic nanoparticle. Heating of magnetic nano particles inside the living tissues has been one of the most efficient methods known to causes apoptosis of tumour cells. It is necessary to improve upon the biocompatibility, cellular uptake, and magnetic heating performance of ferromagnetic iron-oxide magnetic nanoparticles (F-MNPs).[37] In a study by Christian et al, F-MNPs were coated with silica layers of different thicknesses. This was carried out using a reverse microemulsion method. It was found that the

presence of a SiO_2 layer was able to significantly increase the colloidal stability of F-MNPs. This stability was able to enhance the heating performance of these particles in water with almost 1000 W/g_{Fe} as compared to bare F-MNPs. The exhibited biocompatibility of up to 250 $\mu g/cm^2$ was observed in silica-coated F-MNPs. This was assessed by Alamar Blues and Neutral Red assays on two cancer cell lines and one normal cell line. It was found that the cancer cells were able to internalize a higher quantity of silica-coated F-MNPs, in places like large endosomes. Due to this internalization silica-coated F-MNPs e were more sensitive to in vitro MH treatment compared to the normal ones. More than 50% of the malignant cells showcased cellular death starting at a dose of 31.25 $\mu g/cm^2$ and an amplitude of alternating magnetic field of 30 kA/m at 355 kHz.[47]

The greatest challenge posed in magnetic hypothermia treatment is the generation of high therapeutic temperatures selectively in the whole tumour region. In similar lines a study by Susanne kossatz et al, aimed at improving magnetic hyperthermia of breast cancer. They tried to develop nanoparticles which displayed a high heating potential. They synthesized superparamagnetic iron oxide nanoparticles (MF66) and electrostatically functionalized them with either Nucant multivalent pseudopeptide (N6L; MF66-N6L), doxorubicin (DOX; MF66-DOX) or both (MF66-N6LDOX), as ligands. The cytotoxic potential of these nanoparticles was assessed in a breast adenocarcinoma cell line MDA-MB-231. An excellent heating potential around 500 W/g_{Fe} in the alternating magnetic field (AMF, conditions: H = 15.4 kA/m, f = 435 kHz) was shown by all nanoparticle variants. It was observed that the nanoparticle uptake in cells was increased by the N6L functionalization. It was found that MF66-DOX and MF66-N6LDOX along with hyperthermia displayed higher cytotoxicity to breast cancer cells as compared to the respective free ligands. After intratumoral injection of the nanoparticles in vivo it was observed that there was extensive tumour growth inhibition (in some cases up to 40% and in other cases complete tumour regression). The remaining tumour remnants showed lower activity as though they were immobilised. It can be concluded that the therapeutic effects of breast cancer magnetic hyperthermia could be strongly enhanced by the combination of MF66 functionalized with N6L and DOX and magnetic hyperthermia. The major advantage of this approach is that it was able to combine two well established pathways to kill tumour cells; magnetic hyperthermia and chemotherapy. [48]

6.3. **Iron oxide nanoparticles as MRI agents**

Magnetic resonance imaging (MRI) is critical for visualizing soft tissue and organs. Over 60 million MRI procedures are performed each year worldwide.
Magnetic resonance imaging (MRI) is a technique used for imaging very high-quality images of the organs inside the human body. This technique is based on the principles of nuclear magnetic resonance (NMR).[49] A powerful and uniform external

magnetic field is applied to align the protons that are randomly oriented within the water nuclei of the tissue being examined. This alignment is further perturbed by introducing an external radio frequency energy. Now the nuclei return to their resting alignment and doing so emit radio frequency energy. These emitted signals are measured frequency information that is contained in each signal from the location in the imaged plane is converted to corresponding intensity levels by Fourier transformation. These are than displayed as shades of grey in a matrix arrangement of pixels. Sequence of radio frequency pulses applied and collected can be varied in order to obtain different types of images. Repetition time (TR) is known to be the amount of time between successive pulse sequences applied to the same slice. Time of Echo (TE) is the measured time between the delivery of the RF pulse and reception of the echo signal.[49]

Tissues are usually characterised by two different relaxation times known as T1 and T2. T1 (longitudinal relaxation time) is the time measure taken for the spinning protons to realign with the external magnetic field. T2 (transverse relaxation time) is the time constant that determines the rate at which excited protons reach equilibrium or go out of phase with each other. MRI sequences that are most common are T1-weighted and T2-weighted scans. The T1-weighted images are usually produced by using short TE and TR times. The contrast and brightness of the image are predominantly determined by T1 properties of the tissues. On the other hand, T2-weighted are produced by using longer TE and TR times. The contrast and brightness in these images are determined by T2 properties of the tissues.[50]

Ultra-small and related functionalized IONPs possess unique superparamagnetic properties, which generate significant susceptibility effects resulting in strong T2 (spin–spin relaxation process) and T2* contrast, as well as T1 effects (spin–lattice relaxation process) at very low concentrations for MRI, and are widely used for clinical oncology imaging as contrast agents. [37] A major problem when it comes to the application of nanotechnology in practical application is the difficulty faced to deliver nanoparticles to intracranial positions. This difficulty can be overcome by iron oxide nano particles which show promising results in delivering nano particles to targeted sites by applying external magnetic field. Antibodies can be coupled onto the surface of iron oxide nanoparticles. These nanoparticles can carry them to the tumour site where the antibodies can bind to the tumour cells. This can facilitate the viewing of the affected area via MRI. [49] Here iron oxide nanoparticles act as MRI contrast agents. This is the very reason why MRI is one of the most promising applications for magnetic IONPs.
Gadolinium-based contrast agents (GBCAs) are the mainstream MRI contrast agents widely used in the clinics. It has been found that about one-third of these procedures are contrast-enhanced MRI. Most of the GBCAs in use are very safe. Yet some of them have showcased a risk of adverse effects. Nephrogenic systemic fibrosis (NSF), is one of the known untreatable condition attributed gadolinium exposures during MRI with contrast. NSF is a condition which occurs in patients with

renal impairment. It has also been reported that there are cases of Gadolinium deposition in human brain which are now under investigation by the US Food and Drug Administration (FDA). Hence there was a need to come up with a Gadolinium free contrast agents with pharmacokinetic and imaging properties comparable to GBCAs. He Wei et al, reported design and development of zwitterion coated exceedingly small superparamagnetic iron oxide nanoparticles (ZES-SPIONs) consisting of ~3-nm inorganic cores and ~1-nm ultrathin hydrophilic shell. These ZES-SPIONs were found to be free from Gadolinium and showed a very high T1 contrast power. They were able to demonstrate the potential of ZES-SPIONs in pre-clinical MRI and magnetic resonance angiography.[50]

In a study performed by Marashdeh M. W. et al, iron oxide (ɣ -Fe_2O_3) nanoparticles were successfully synthesized. The method of synthesis employed was sol-gel method. The synthesized nanoparticles were characterized by XRD and VSM. The nanoparticles had a different size range (22 nm and 30 nm) whose potential was investigated for being magnetic resonance imaging (MRI) contrast agents. The relaxation time (T2) of the iron oxide nanoparticles was measured at room temperature and concentration range of 9-84 µg/ml using fast spin echo sequence with six echoes. It was found that the size of the nanoparticles greatly affected the contrast enhancement of the MRI image. The T2 for 22 nm sized ɣ-Fe_2O_3 nanoparticles exhibited a shorter dephasing as compared to the 30 nm sized ɣ-Fe_2O_3 nanoparticles. There was a decrease in the T2 relaxivity with increase in the concentration (9-84 µg/ml) of the ɣ-Fe_2O_3 nanoparticles. According to the T2-weighted analysis it was found that a better signal (i.e., brighter image) achievement was possible for the 30 nm sized ɣ-Fe_2O_3 nanoparticles. Hence from this study it could be concluded that the iron oxide nanoparticles used as MRI contrast agents is solely dependent on the size and concentration of the nanoparticles. ɣ-Fe_2O_3 nanoparticles synthesized in this work are promising materials for use as MRI contrast agents.[51]

Exceedingly small magnetic iron oxide nanoparticles (ES-MIONs) (< 5 nm) have recently emerged as one of the most promising T1-weighted contrast agents for magnetic resonance imaging (MRI). They are known for their better biocompatibility as compared to the Gd-chelates. Gd-chelates are widely used as MRI contrast agents but suffer from many drawbacks. Although ES-MIONs have gained momentum as emerging MRI contrasting agents, the best particle size of ES-MIONs for T1 imaging is still unknown. In a study performed by Shen Zheyu et al, an attempt was made to synthesize ES-MIONs with 7 different sizes below 5 nm. In their study they found that 3.6 nm is the best particle size for ES-MIONs which can be utilized as T1-weighted MR contrast agent.[52] In order to avoid uptake of these nanoparticles by healthy cells and to enhance tumour targetability of theranostic nanoparticles, a drug delivery system based on the 3.6 nm ES-MIONs for T1-weighted tumour imaging and chemotherapy was constructed. This study aimed achieving two goals at the same time; MRI of the tumour cells and targeted drug delivery. The MR imaging and chemotherapy results on the cancer cells and tumour-bearing mice reinforced that their DOX@ES-MION3@RGD2@mPEG3 nanoparticles are promising

to be used for high resolution T1-weighted MR imaging and precise chemotherapy of tumours.[52]

In another study by Bae Hongsub et al, coprecipitated ferrite nanoparticles were synthesized and coated with carbon via hydrothermal method. Transmission electron microscopy pictures showcased that the coated iron oxide nanoparticles had a spherical shape and an average diameter of about 90 nm. The strong bonding of carbon with the nanoparticle surfaces was confirmed by noting the C = O and C = C vibrations in Fourier transform infrared spectra. The spin-lattice relaxation process (T1) and spin-spin relaxation process (T2) relaxivities of hydrogen protons in the aqueous solution of coated nanoparticles were determined to be 1.139 $(mM \cdot s)^{-1}$ and 1.115 $(mM \cdot s)^{-1}$, respectively. It was concluded from this study that the carbon coated iron oxide nanoparticles can be utilized as both T1 and T2 contrast agents in magnetic resonance imaging.[53]

In a study by Maximilian O. Besenhard et al, small iron oxide nanoparticles (IONPs) were synthesised in water via co-precipitation method. The particle growth was quenched after the desired magnetic iron oxide phase was formed. In order to achieve this a milli fluidic multi stage flow reactor was used and acidic solution was added at a precisely determined timing. Iron oxide nanoparticles of a size ≤5 nm was obtained and stabilised. This size is found suitable for positive T1 magnetic resonance imaging (MRI) contrast agents. The novel approach of this method is the use of flow chemistry. This method entails reproducible and scalable production of iron oxide nanoparticles as contrast agents and replace currently used Gd complexes. Utmost care must be taken to time the addition of acid because the inverse spinel structure is formed within seconds after the initiation of the co-precipitation method. It was observed that late quenching allowed iron oxide nanoparticles to grow larger than 5 nm. It was also observed that premature addition of acid gave rise to undesired oxide phases. Use of a flow reactor successfully synthesised monodisperse and non-agglomerated small iron oxide nanoparticles. The effect of temperature and dextran present during co-precipitation on the final particle size was also investigated. This method is at an advantage over syntheses in batch utilising growth inhibitors as such methods likely led to impurities. another advantage of this method is the lost cost involved due to continuous synthesis and large-scale production of highly stable small iron oxide nanoparticles without the use of toxic reagents. The flow-synthesised small IONPs showed high T1 contrast enhancement and hence can be utilised as efficient contrast agents. [54]

In another study performed by Byung Hyo Kim et al; an attempt was made to synthesize uniform and extremely small-sized iron oxide nanoparticles (ESIONs) of <4 nm. Their synthesis was carried out via thermal decomposition of iron oleate complex in the presence of oleyl alcohol. Oleyl alcohol acted as a temperature lowering agent by reducing iron oleate complex thus resulting in the production of small-sized nanoparticles. Maghemite crystal structure was reported upon XRD

studies of 3 nm-sized nanoparticles. These nanoparticles are hydrophobic and were easily transformed to water-dispersible nanoparticles by capping with the poly (ethylene glycol)-derivatized phosphine oxide (PO-PEG) ligands. It was noted that the 3 nm-sized nanoparticles exhibited a high r_1 relaxivity of 4.78 mM^{-1} s^{-1} and low r_2/r_1 ratio of 6.12, thus demonstrating that ESIONs can be efficient T_1 contrast agents. The large number of surfaces Fe^{3+} ions with 5 unpaired valence electrons were said to be responsible for high r_1 relaxivities of ESIONs. ESIONs showcased longer circulation time than the clinically used gadolinium complex-based contrast agent in T_1-weighted magnetic resonance imaging (MRI) thus enabling high-resolution imaging. High-resolution blood pool MR imaging using ESIONs allowed very clear and precise observation of various blood vessels with sizes down to 0.2 mm. hence it was concluded that ESIONs possess a wide scope as T_1 MRI contrast agents in clinical settings.[55]

Although spherical superparamagnetic iron oxide nanoparticles have been developed as T_2-negative contrast agents for magnetic resonance imaging in clinical they exhibit relatively low transverse relaxivity. In a study by Zhenghuan Zhao et al, aimed at addressing this drawback. They tried to achieve this by controlling the morphology of iron oxide nanoparticles. They were very efficiently able to fabricate size-controllable octapod iron oxide nanoparticles by introducing chloride anions. The octapod iron oxide nanoparticles possessed an edge length of 30 nm and exhibited an ultrahigh transverse relaxivity value (679.3±30 mM^{-1} s^{-1}). Thus, it can be indicated that these octapod iron oxide nanoparticles are very much effective T_2 contrast agents for in vivo imaging and small tumour detection as compared to the conventional iron oxide nanoparticles. Hence these nanoparticles can be efficiently utilized in MRI as contrasting agents.[56]

7. **Conclusion**

Iron oxide nanoparticles carry the potential to revolutionize the various research fields in which they are utilized. As seen in the literature review the studies carried out in this field are yet at their conception. A lot more has to be done in order to explore the horizons of research and development. Use of iron oxide nanoparticles in the medical field shines a ray of hope towards development of better prospects in improving the quality of life. Untreatable medical conditions may finally find a cure thus ringing an end to the misery and pain alarmed by them. Researchers and chemists need to work hand in hand to explore the ability of nanoparticles to penetrate each and every research area.

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