



Green Biosynthesis of Drug Therapy Magnetite Fe₃O₄ Nanoparticles in Targeted Modulatory Delivery System: A Review

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Abstract

This review examines the green biosynthesis of magnetite nanoparticles (Fe₃O₄ NPs) and their biomedical applications, particularly in targeted anticancer drug delivery. Due to their "superparamagnetic" properties, Fe₃O₄ NPs have shown versatility in various applications. In targeted delivery, drug-loaded Fe₃O₄ NPs can accumulate at tumor sites using an external magnetic field, enhancing effectiveness and minimizing harm to healthy cells. For safe human application, Fe₃O₄ NPs must be biocompatible and biodegradable, minimizing toxicity. Green biosynthesis ensures safety, utilizing biological materials like plant extracts and natural polymers. Plant extract biosynthesis is popular due to its simplicity, economy, and eco-friendliness. This review explores Fe₃O₄ NP applications, focusing on anticancer drug delivery, discussing criteria for optimal drug delivery vehicles. Research demonstrates promising results in treating cancer cells in vitro. This review summarizes key points and proposes future study directions.

Keyword- Green biosynthesis, Superparamagnetic, Magnetite nanoparticle, Anticancer, Targeted drug delivery

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1. Introduction

Nanotechnology has emerged as a transformative field with vast potential, increasingly gaining recognition for its benefits and applications across various industries. Initially applied in the materials industry, nanotechnology's influence has expanded exponentially into fields such as environmental science, food production, and notably, biomedical research. Despite its growing significance, there remains a knowledge gap regarding the green synthesis of iron oxide nanoparticles (Fe₃O₄-NPs) via biological methods and their applications in biomedicine. This review aims to bridge that gap by exploring the green biosynthesis of Fe₃O₄-NPs using plant extracts and other biological materials. Furthermore, it will delve into the biomedical applications of Fe₃O₄-NPs, focusing on drug delivery systems, to illuminate their role in modern technological advancements.

1.1. Superparamagnetic iron oxide nanoparticles (magnetite nanoparticles)

Iron oxides, naturally abundant in various forms, include iron (III) oxide (FeO), hematite (α -Fe₂O₃), and maghemite (γ -Fe₂O₃). Among these, magnetite (Fe₃O₄) stands out as the most valuable and widely applied iron oxide. As a naturally occurring mineral, magnetite's crystal structure exhibits an inverse spinel arrangement, characterized by alternating tetrahedral and octahedral layers. Notably, Fe²⁺ ions occupy half of the octahedral sites due to increased crystal field stabilization energy (CFSE), while Fe³⁺ ions occupy the remaining octahedral and all tetrahedral sites. This unique configuration contributes to magnetite's remarkable magnetic properties, making it an essential material in various applications, including data storage, biomedical devices, and environmental remediation.

Magnetite's exceptional magnetic properties have captivated global researchers, earning its reputation as Earth's strongest natural magnetic mineral. Notably, magnetite exhibits ferromagnetism at room temperature, with a Curie temperature of 850K. However, the magnetic

behaviour of Fe₃O₄ nanoparticles (NPs) is highly synthesis-method-dependent. Furthermore, the size and morphology of magnetite crystals significantly impact their magnetic properties. To unlock optimal applications, precise control over Fe₃O₄-NPs' parameters is crucial. Researchers must carefully calibrate synthesis conditions to achieve desired magnetic characteristics, paving the way for innovative applications in fields like data storage, biomedical devices, and magnetic resonance imaging.

Superparamagnetic nanoparticles, including iron oxide nanoparticles (Fe₃O₄-NPs), have garnered significant attention due to their unique properties. When exposed to an external magnetic field, these nanoparticles rapidly magnetize to saturation, only to lose their magnetic interaction once the field is removed. Notably, Fe₃O₄-NPs exhibit this superparamagnetic behavior, making them ideal for biomedical applications. Furthermore, Fe₃O₄-NPs boast exceptional biocompatibility, biodegradability, and minimal toxicity, positioning them as a promising tool for future medical advancements.

Iron oxide nanoparticles (Fe₃O₄-NPs) offer numerous benefits, but their diverse applications require tailored properties. Specifically, 50nm diameter nanoparticles are optimal for efficient endocytosis in drug delivery. Various synthesis methods have been developed, including co-precipitation, sol-gel, hydrothermal, solid-state, flame spray, thermal decomposition, and solvothermal approaches. However, these conventional physical and chemical methods pose significant challenges, such as high production costs, toxic chemical usage, and hazardous by-product generation. Recently, green synthesis methods have emerged as a sustainable alternative to mitigate these issues.

Fe₃O₄-NPs' exceptional properties superparamagnetic, biocompatibility, and biodegradability have sparked widespread research to unlock their full potential across various applications. Notably, investigations into Fe₃O₄ nano-fluids' thermal conductivity and viscosity, influenced by external magnetic fields and electric currents, have gained traction in heat transfer applications.

Green biosynthesis

Green synthesis in nanotechnology is an environmentally friendly approach that eliminates hazardous chemicals and toxic by-products. This eco-friendly method prioritizes human health and environmental sustainability. Conventional methods, however, involve high-cost production, complex procedures, and excessive waste. In contrast, green synthesis offers numerous benefits. These benefits include simple manufacturing processes, rapid production, economic viability, and minimized waste generation. Green synthesis provides a sustainable and efficient alternative to conventional chemical and physical methods. This approach aligns with the growing demand for environmentally responsible nanotechnology.

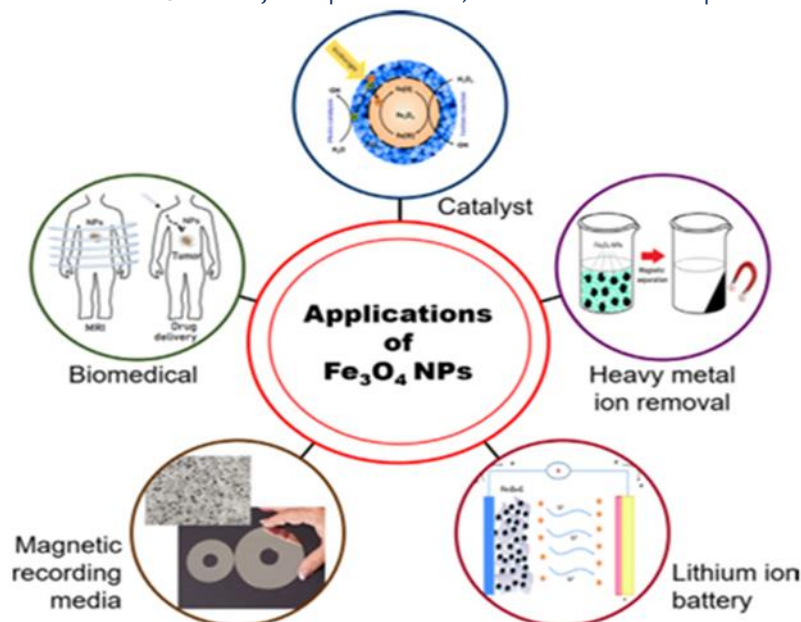


Fig.1 Applications of magnetite nanoparticles (Fe_3O_4 -NPs).

The green biosynthesis of nanoparticles utilizes a bottom-up methodology, where metal atoms coalesce into clusters, forming nanoparticles. Green materials' biological compounds serve as dual-function reducing and capping agents, stabilizing nanoparticles during synthesis. This controls nanoparticle size and shape, enabling tailored applications. Nanoparticle synthesis requires only two materials: metal salt precursors and green substrates. To achieve desired properties, various synthesis parameters can be adjusted, including metal salt concentration, green substrate concentration, reaction time, temperature, and solution pH.

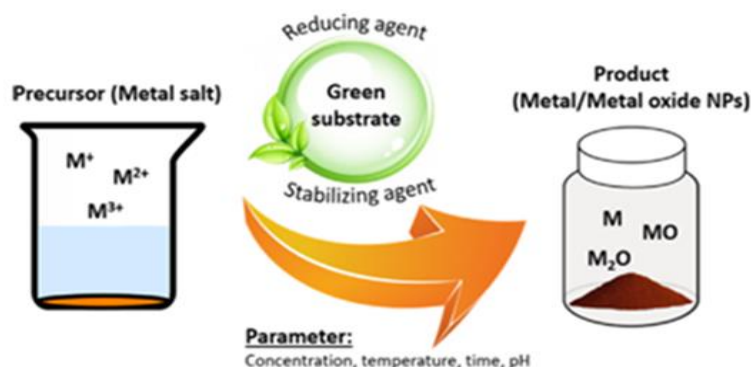


Fig.2 Nanoparticles synthesis process.

Green biosynthesized Fe_3O_4 -NPs offer superior biocompatibility and biodegradability. Their special surface coating, derived from non-toxic green materials, enables targeted drug delivery and minimized toxicity. This makes them suitable for biomedical applications. Besides, Fe_3O_4 -NPs can conjugate with drugs, enzymes or proteins which can be directed to targeted tissue, organ or tumor with the aid of external magnetic field, or can be heated in alternating magnetic fields for hyperthermia treatment.

2.Plant extract

This review focuses on the green biosynthesis of Fe_3O_4 -NPs. Figure 3 illustrates the various green materials employed by researchers in synthesizing Fe_3O_4 -NPs, which will be discussed in detail. Numerous successful studies have utilized different biological routes to synthesize Fe_3O_4 -NPs, with plant extracts being the most extensively used due to their ease of obtainment,

scalability, cost-effectiveness, and environmental benignity. Plant extracts serve as reducing and stabilizing agents in nanoparticle synthesis, attributed to the presence of phytochemicals.

2.1. Plant

The physical characteristics of Fe₃O₄-NPs, such as size, shape, and magnetic properties, can be tailored by using different plant types, enabling effective applications. Research by Ngermpimai et al. and Phumying et al. demonstrated the synthesis of Fe₃O₄-NPs using Aloe vera, albeit under varying conditions. Ngermpimai et al.'s study employed serial centrifugation steps, yielding spherical nanoparticles with decreasing size as centrifugation increased. In contrast, Phumying et al.'s investigation utilized varied reaction times and temperatures, resulting in smaller, irregularly-shaped nanoparticles.

2.2. Marine plant

Seaweed, also known as marine plants or algae, can be utilized to synthesize Fe₃O₄ nanoparticles (NPs). Seaweed is vital to marine life, providing food and habitats in many South-East Asian countries. It's a rich source of lipids, minerals, vitamins, and bioactive substances like proteins, polyphenols, and polysaccharides, which have potential medical applications. Marine algae are divided into microalgae and macroalgae, with macroalgae (seaweed) being plant-like organisms commonly used in nanoparticle synthesis. The phytochemicals in seaweed act as metal-reducing and capping agents, providing a robust coating on metal NPs in a single step synthesized Fe₃O₄ NPs using brown seaweed (*Sargassum muticum*) through a simple mixing process of FeCl₃ solution with seaweed extract. The reduction process yielded Fe₃O₄ NPs immediately. Key components in seaweed, such as sulfate, hydroxyl, and aldehyde groups, facilitate the reduction of Fe³⁺ and stabilize nanoparticles. The pH decrease during synthesis indicates hydroxyl group participation. Alternatively, sulfate groups may reduce metal ions through aldehyde group oxidation to carboxylic acids.

2.3. Leaf

Researchers have extensively utilized leaves for the green synthesis of Fe₃O₄ nanoparticles (NPs). Rajendran et al. prepared Fe₃O₄ NPs using *Sesbania grandiflora* leaf extract as a photocatalyst for chemical oxygen demand (COD) removal. They added ferric chloride (FeCl₃) to heated leaf extract, stirred, and calcinated the paste at 500°C for 2 hours to remove impurities. The authors found that increasing leaf extract concentration enhanced reduction rates and nanoparticle formation. A 20% leaf extract concentration was optimal, as analyzed by UV-visible spectroscopy. Similarly, Andean blackberry (*Rubus glaucus* Benth.) leaves were used by Kumar et al. to synthesize Fe₃O₄ NPs. They adjusted the pH of the mixed solution to 10-11 with sodium hydroxide (NaOH) and stirred at 75-80°C until a black solution formed.

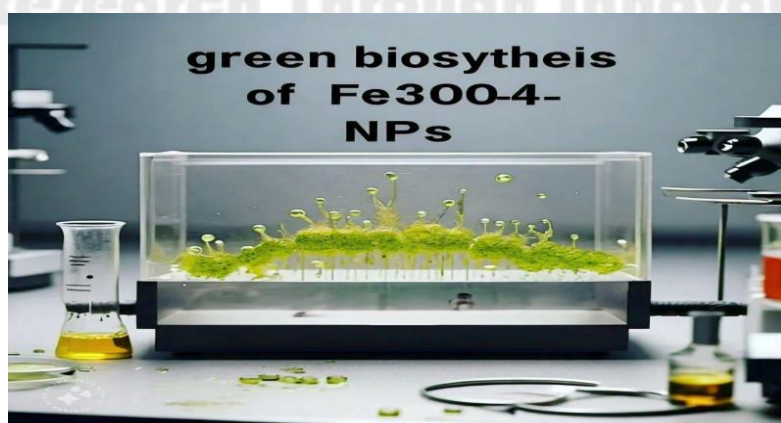


Fig.3 Materials of green biosynthesis of Fe₃O₄-NPs.

2.4. Fruit peel

Fruit peels, the protective skin of fruits, are too thick for human consumption and can serve as natural fertilizers. Researchers have leveraged fruit peels to synthesize nanoparticles, utilizing fruit rind extracts to produce Fe₃O₄ nanoparticles (NPs). Venkateswarlu et al. investigated pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*) peel extracts for Fe₃O₄ NP synthesis. Both experiments yielded spherical NPs with an average size of 17 nm.

The synthesized Fe₃O₄ NPs exhibited magnetic properties, with saturation magnetization values of 21.7 emu/g (pineapple) and 28.4 emu/g (watermelon). Successful surface functionalization with ligands enabled heavy metal removal, targeting cadmium (II) for pineapple and mercury (II) for watermelon. Compared to other studies, these surface-modified Fe₃O₄ NPs demonstrated exceptional adsorption capacity. The ferromagnetic properties of Fe₃O₄ NPs allow for simple separation from large-volume samples using an external magnetic field, eliminating filtration or centrifugation. This enables rapid and easy isolation, and the nanoparticles remain recyclable without significant loss of heavy metal removal efficiency. This sustainable approach showcases the potential of fruit peel-derived Fe₃O₄ NPs in environmental remediation.

Table1 Fe₃O₄-NPs synthesized using different part of plants.

Part	Name	Size range	Morphology	Saturation magnetization (Ms) value (emu/g)
Plant	Soyabean sprouts	8nm	Spherical	37.1 at 300K
	Aloe vera	93–227nm	Spherical	74.1–75.9
Marine plant	Sargassum muticum	18±4nm	Cubic	22.1
	Padina pavonica	10–19.5nm	Spherical	–
Seed	Grape Seed Proanthocyanidin (GSP)	30nm	Irregular shape	–
Leaf	Carob	4–8nm	Well monodisperse	–
	Tridax procumbens	–	Irregular shape-rough surfaces	–
Fruit peel	Plantain peel	30–50nm	Spherical	15.8 at r.t
	Rambutan	100–200nm	Agglomerated, spinel	–
Fruit	Passiflora tripartita	18.2-24.7nm	Spherical	13.2
	Lemon	14–17nm	Spherical	31.4–61.8at r.t

Root	Mimosa pudica	60–80 nm	Agglomerated rough spherical	55.4 at r.t.
Stolon	Potato	40 ±2.2nm	Cubic	28.8
Waste	Tea residue	5–25 nm	Cuboid/pyramid	6.9 at r.t.
	Rice straw	9.9 ±2.4 nm	Aggregated spherical	–

2.5. Seed

Fruit seeds, a type of fruit waste, can serve as green solvents, reducing, and capping agents in synthesizing Fe₃O₄ nanoparticles (NPs). A study by Venkateswarlu et al. utilized *Syzygium cumini* (*S. cumini*) seeds to produce Fe₃O₄ NPs. During green synthesis, *S. cumini* extract acted as a reducer due to its carbohydrates and polyphenols, reducing Fe salt to Fe₃O₄ through a simple reduction reaction. To characterize the NPs, X-ray diffraction (XRD) confirmed crystallinity and purity, while Raman spectroscopy verified Fe₃O₄ formation without impurities. Significant peaks at 670 cm⁻¹ (As modes) and 538 cm⁻¹ and 430 cm⁻¹ (T_{2g} modes) were characteristic of Fe₃O₄. Brunauer-Emmett-Teller (BET) surface area analysis revealed a surface area of 35.17 m²/g. Pore size distribution showed majority mesoporous sizes approximating 2 nm. These green-synthesized mesoporous Fe₃O₄ NPs demonstrate potential in various applications.

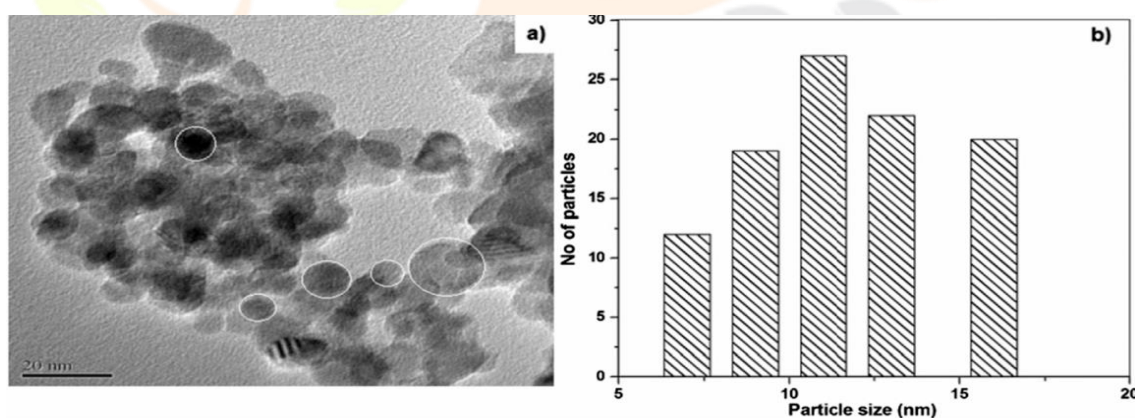


Fig.4 TEM image of (a) DHPCT@Fe₃O₄MNPs and (b) the particle size histogram. Reproduced with permission.

2.6. Fruit

Bahadur et al. conducted a study on synthesizing Fe₃O₄ nanoparticles (NPs) using lemon juice as a reducing agent and surface capping source. The modified precipitation technique produced water-dispersible Fe₃O₄ NPs, essential for biomedical applications. By adjusting the reducing agent amount, the researchers controlled the NP size, fabricating 11 nm and 15 nm Fe₃O₄ NPs. X-ray diffraction (XRD) results revealed seven significant peaks at 30.07°, 35.31°, 43.33°, 46.58°, 57.18°, 62.88°, and 74.02°, confirming the purity of synthesized Fe₃O₄ NPs. UV-vis spectrometry analyzed the optical properties, showing particle-size-dependent energy band gaps: 2.6 eV (15 nm) to 2.8 eV (11 nm) for direct transitions and 1.7 eV (15 nm) to 1.82 eV (11 nm) for indirect transitions. These results classify Fe₃O₄ NPs as semiconductors, with direct and indirect energy band gap values within the ranges of 2.6-2.8 eV and 1.7-1.82 eV,

respectively. The particle-size-dependent band gap highlights the potential for tailored applications.

2.7. Stolon and root

Recent studies have explored novel plant parts for Fe₃O₄ nanoparticle (NP) synthesis, including potato stolons and *Mimosa pudica* roots. Gum et al. investigated potato petate, rich in carbohydrates, as a capping and reducing agent. The starch-rich potato extract played a crucial role in Fe₃O₄ NP formation. The reaction involved adding NaOH, oxidizing starch in alkaline solution, and producing electrons that reduced Fe³⁺ ions to Fe NPs. Primary hydroxyl groups were oxidized to carboxyl groups. Potato extract prevented NP aggregation, enhancing dispersion and steric protection. Niraimathee et al. synthesized Fe₃O₄ NPs using *Mimosa pudica* root extract. UV-vis analysis confirmed iron oxide presence at 294 nm. Magnetic properties were enhanced by controlling solution pH with NaOH addition. Vibrating Sample Magnetometry (VSM) results showed a saturation magnetization (M_s) value of 55.40 emu/g, comparable to other studies. Magnetization decreased to zero when the magnetic field was removed, indicating superparamagnetic behavior. This correlated with single-crystal domain Fe₃O₄ NPs, exhibiting only one magnetic moment orientation.

2.8. Gum

Alternatively, utilizing gum Arabic (GA) has proven successful in producing Fe₃O₄ nanoparticles (NPs). Horst et al. explored the possible mechanism of FeO NP formation, involving two types of interactions between GA polysaccharides and iron oxide nucleus: electrostatic and/or hydrophobic interactions. Initially, the synthesis process is acidic due to iron salt precursors interacting with polymeric matrices. Adding NH₄OH increases pH, producing the first FeO nucleus. Under these conditions, Fe₃O₄ and GA exhibit opposite surface charges, facilitating electrostatic interactions. FTIR data confirmed hydrogen bonding between GA's carboxylic groups and Fe₃O₄'s hydroxyl groups. At higher pH, both polymer and iron oxide possess negative charges, leading to steric interactions responsible for GA binding. Steric interactions are crucial in illustrating the stabilization mechanism of Fe₃O₄ NPs by polymeric matrices. GA's hydrophilic nature enables Fe₃O₄ NPs to remain stable, as charged (mostly negative) functional groups on GA chains remain surface-exposed, causing electrostatic repulsion between nanoparticles. This occurs when not all functional groups of the polymer interact with the surrounding Fe₃O₄, leaving some groups available for repulsion.

2.9. Plant waste

The applications of Fe₃O₄ nanoparticles (NPs) can also depend on the substrate used. Surprisingly, natural waste materials like rice straw, fruit peels, and coffee waste hydrochar, often considered worthless, can be valuable in synthesizing Fe₃O₄ NPs. Research has shown that Fe₃O₄ NPs prepared from tea residue, coffee waste hydrochar, and corn (*Zea mays*) can be used for arsenic removal, Acid Red 17 (azo dye) removal, and drug delivery applications, respectively. These studies demonstrate that Fe₃O₄ NPs capped with green substrates have immense potential in various applications, including environmental remediation, textile waste treatment, and pharmaceutical delivery. The use of eco-friendly substrates in Fe₃O₄ NP synthesis opens up promising avenues for sustainable solutions in diverse fields.

3. Other green materials

Green biosynthesis extends beyond plant-based nanoparticle synthesis, incorporating other eco-friendly materials such as natural polymers, amino acids, vitamins, enzymes, and fungi in

Fe₃O₄ nanoparticle (NP) production. Besides plants, these alternative green substrates also facilitate the synthesis of Fe₃O₄ NPs. Table 2 illustrates the diversity of green substrates utilized in synthesizing Fe₃O₄ NPs of various sizes and shapes.

3.1. Glucose

As expected, the shape, particle size, and magnetic properties of synthesized Fe₃O₄ nanoparticles (NPs) vary depending on the type of green material used. These differences are attributed to distinct synthesis conditions and inherent properties of the green substrates. Notably, glucose is revealed as the most popular choice for synthesizing Fe₃O₄ NPs. Research by Denir et al. investigated the impact of five saccharides: mannose, maltose, lactose, and galactose on Fe₃O₄ NP characteristics, demonstrating their potential as bifunctional agents, serving as both reducing agents and precursors, to tailor Fe₃O₄ NP properties.

3.2. Polysaccharides

On the other hand, Chang et al. synthesized superparamagnetic Fe₃O₄ nanoparticles (NPs) using polysaccharides, including soluble starch, carboxy methyl cellulose (CMC), and agar. These polysaccharides acted as stabilizers, enhancing stability, biocompatibility, and biodegradability. TEM images revealed approximately 10 nm spherical Fe₃O₄ NPs capped by polysaccharides. Polysaccharides hybridize with metal ions through coordinating functional groups (hydroxyl and glucoside groups), associating closely with iron ions. Nucleation and initial crystal growth of Fe₃O₄ NPs occur preferentially on polysaccharides. Dynamic supramolecular associations, facilitated by inter- and intra-molecular hydrogen bonding, serve as templates for nanoparticle growth. The size of Fe₃O₄ NPs synthesized using soluble starch was less than 10 nm, whereas CMC and agar produced larger sizes. This disparity may be attributed to polysaccharide structure. Soluble starch, composed mainly of branched amylopectin, interacts more with iron ions than CMC and agar, which have linear polysaccharide structures, restricting Fe₃O₄ NP growth. Fe₃O₄ magnetization is sensitive to microstructure. Particles smaller than the critical size are single-domain particles, exhibiting superparamagnetic attributes. As particle size decreases below this critical size, magnetizations become randomly oriented, leading to superparamagnetic.

Table. 2 Fe₃O₄-NPs synthesized by other green substrates.

Green substrate	Name	Size range/ Average size	Morphology	Saturation magnetization (Ms) value (emu/g)
Glucose	a-D-glucose	12.5nm	Roughly spherical shape	71.3 at 5K
Vitamin	Nicotinic acid (N. acid)	0g N. acid	Nano rod	0g=55.0
Enzyme	Urease	19±5 nm	60C=nanosphere	52.6
Fungi	Yeast	16nm	Wormhole-like	22.1 at r.t.
Natural polymer	Sodium alginate	27.2nm	Uniform and spherical	61.2
Polysaccharides	Starch	Less than 10nm	Spherical	36.2 at 300K

Amino acid	Arginine	18–26nm	Spherical	51.7 at 300K
Organic acid	L-(+)-Tartaric acid	19.5±4.2nm	Spherical	60 at r.t
Clay	Perlite(soil)	Less than 100nm	Irregular	14.7at r.t.

3.3. Clay

Clay is a natural, versatile rock material commonly used in making pottery and construction products like bricks and tiles. However, clay can also serve as a supporting material for nanoparticle synthesis. Kalantari et al. synthesized Fe₃O₄ nanoparticles (NPs) using montmorillonite (MMT) as a solid support. The shape and size of Fe₃O₄ NPs can be controlled by altering the amount of NaOH (1.50-12.50 mL) as a reducing agent in the medium. Fe₃O₄ NPs were prepared through coprecipitation by adding base to Fe²⁺ and Fe³⁺ solutions. The chemical reaction equation is: Fe²⁺/Fe³⁺/MMT + 8OH⁻ → Fe₃O₄/MMT + 4H₂O. XRD analysis confirmed the presence of Fe₃O₄. The basal spacing expanded from 1.47 to 2.85 Å as the Fe₃O₄ content in MMT increased from 1.0 to 12.0 wt%. The XRD patterns shifted from 8.75° to 7.46°. This shift indicates iron ions penetrate the MMT interlayer space via ion-exchange and are reduced to Fe₃O₄ by NaOH addition. The interlayer space acts as a microreactor and size controller. The XRD peak intensities decreased, and the highly ordered parallel lamellar structure of MMT was disrupted by Fe₃O₄ NP formation. TEM results showed Fe₃O₄ NPs in the interlayer space or on the MMT surface. The NP size decreased with increasing NaOH amount. The charged Fe₃O₄ NPs are electrostatically bound to MMT due to its high ion-exchange site density. Fe₃O₄ NP aggregation in MMT correlates with smaller primary nanoparticle dimensions. The magnetic properties of Fe₃O₄/MMT nanocomposites increased from 12.1 to 32.4 emu/g with Fe₃O₄ content. LAPONITE, a nano clay smectite, was used to synthesize LAPONITE@Fe₃O₄ NPs (LAP-Fe₃O₄ NPs) for in vivo magnetic resonance imaging of tumors. LAP-Fe₃O₄ NPs exhibited good colloidal stability and enhanced T₂ relaxivity. XRD patterns and FTIR spectra confirmed the composition of LAP and Fe₃O₄ NPs. The peaks at 586-598 cm⁻¹ corresponded to Fe-O vibrations, and Si-O stretching vibrations were located at 1016-1019 cm⁻¹.

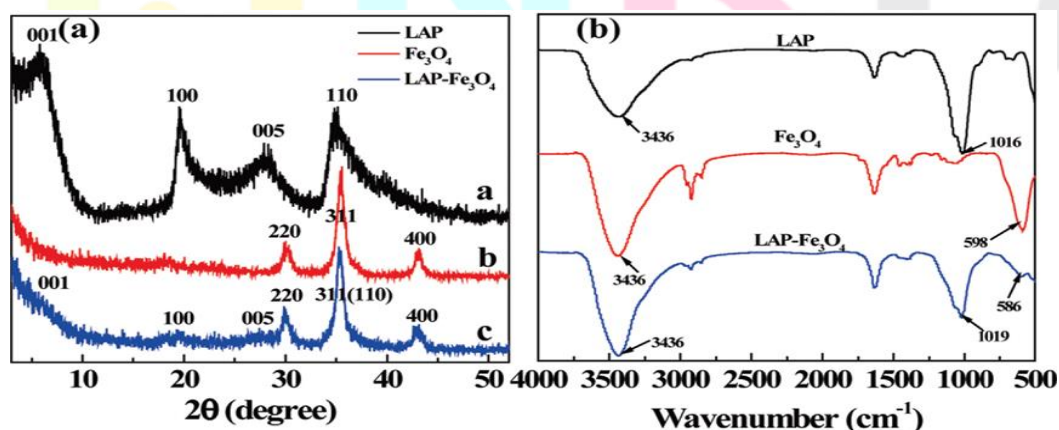


Fig.5 XRD pattern (a) and FTIR spectroscopy (b) of LAP, Fe₃O₄, and LAP- Fe₃O₄-NPs. Reproduced with permission

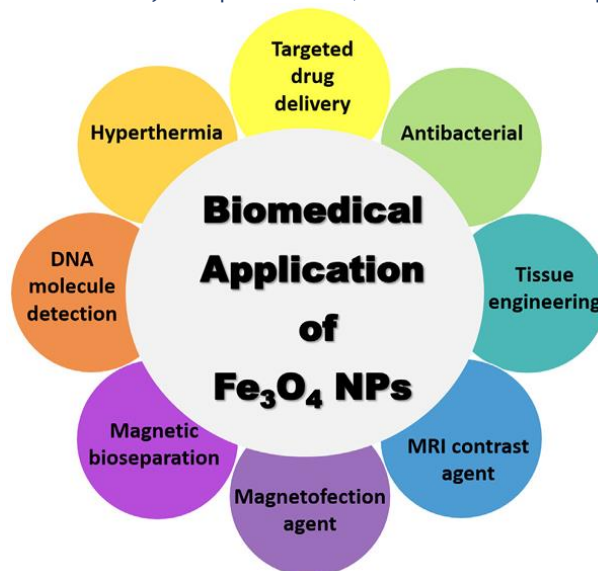


Fig.6 Examples of biomedical applications of Fe₃O₄-NPs.

4. Biomedical applications of Fe₃O₄-NPs

Fe₃O₄ nanoparticles (NPs) have shown antibacterial properties, making them a potential alternative to silver nanoparticles. Patra et al. studied green-synthesized Fe₃O₄ NPs using corn ear leaves, demonstrating antibacterial activity against five foodborne bacteria when combined with standard antibiotics. The combination of Fe₃O₄ NPs with kanamycin exhibited synergistic effects, reducing antibiotic doses and bacterial resistance. Additionally, Fe₃O₄ NPs combined with rifampicin showed antibacterial activity against *Staphylococcus aureus*. Fe₃O₄ NPs are also suitable for hyperthermia treatment due to their superparamagnetic properties. Horst et al. characterized Gum Arabic-synthesized Fe₃O₄ NPs for magnetic hyperthermia therapy, achieving a specific absorption rate of 218 W/g Fe. Tissue engineering is another important application, where Fe₃O₄ NPs facilitate cell sheet construction using magnetic forces. Demonstrated the formation of sheet-like structures without artificial polymer scaffolds. Fe₃O₄ NPs enable efficient magnetic bio separation, particularly in cell separation. Lu et al. synthesized PEI-coated Fe₃O₄ NPs for lung cancer cell separation from sputum samples, increasing positive cell detection from 6.3% to 38.5%. Fe₃O₄ NPs can also serve as magnetofection agents, enhancing transfection efficiency. Developed DMSPION-G6/DNA/PEI ternary magnetoflexes for in vitro gene delivery, achieving high-level transgene performance. Functionalized Fe₃O₄ NPs with plasmid DNA for nucleic acid therapy, demonstrating effective gene delivery. Fe₃O₄ NPs are applicable in DNA molecule detection. Sun et al. fabricated a chemiluminescence biosensor for ultrasensitive DNA determination using Fe₃O₄@SiO₂@GO polymers. Lastly, Fe₃O₄ NPs have potential in targeted drug delivery systems, integrating anticancer and anti-inflammatory drugs. Conjugated indomethacin with Fe₃O₄ NPs incorporated into electro spun nanofiber composites.

5. Utility of Fe₃O₄-based nanoparticles as drug delivery vehicles

Recently, human health has been confronted with a multitude of diseases, necessitating the development of novel drugs to address this critical issue. Consequently, Fe₃O₄ nanoparticles (NPs) have gained popularity in drug delivery systems due to their exceptional magnetic properties, specifically superparamagnetic. Furthermore, to effectively utilize Fe₃O₄ NPs as drug delivery vehicles, they must exhibit several crucial properties that cannot be overlooked. These significant properties will be discussed in this section.

5.1. Criteria of Fe₃O₄-based nanoparticles to be used in drug Delivery

5.1.1. Superparamagnetic

The fundamental requirements for Fe₃O₄-based nanoparticles in drug delivery applications encompass magnetic properties, shape, size, and surface characteristics, which are crucial aspects to be addressed. Initially, Fe₃O₄-based nanoparticles are renowned for their magnetic properties, enabling precise guidance to specific sites using an external magnetic field, thereby enhancing local concentration. This advantage significantly boosts drug delivery efficacy.

5.1.2. Shape of nanoparticles

In drug delivery, the shape of Fe₃O₄-based nanoparticles is a crucial factor to consider. Researchers aim to prolong nanoparticle retention at targeted sites and enhance cellular cytotoxicity. However, blood circulation time, cellular uptake, and biodistribution vary depending on nanoparticle shape. Numerous studies have investigated nanoparticle shape, particularly for anticancer drug delivery. Filamentous micelles, for instance, demonstrate higher anticancer drug encapsulation capacity and apoptotic efficiency compared to spherical micelles. Research has shown that filamentous DOX-loaded micelles exhibit superior DOX loading capacity, encapsulation efficiency, safety, and therapeutic effects on artificial solid tumors. Various nanoparticle shapes, such as rod, worm, and bead, have been synthesized for drug delivery studies. Non-spherical and rod-shaped nanoparticles typically have longer blood circulation times due to lower phagocytic activity. However, spherical nanoparticles (sub-100 nm) offer advantages over rods, including even surface coating, ligand conjugation, and increased drug loading for targeted release. Most synthesized nanoparticles are spherical for in vitro studies targeting cancerous cells, demonstrating significant cytotoxicity and effectiveness in inhibiting cancer cell growth. These findings highlight the promising potential of nanoparticles in drug delivery applications.

5.1.3. Size of nanoparticles

The dimensions of nanomaterials play a crucial role in determining total cell uptake in drug delivery systems. To maximize cell uptake rate and intracellular concentration in mammalian cells, the optimal size of nanodrug carriers must be determined. Typically, a diameter of 50 nm is considered optimal, but this varies depending on cell type. Larger nanoparticles (>50 nm) may bind to numerous receptors, limiting additional nanoparticle binding. In contrast, 40-50 nm nanoparticles can assemble and bind sufficient receptors to facilitate membrane wrapping. However, cell uptake effectiveness depends on cell type, as each cell has a distinct phenotype. Nanoparticle size also influences blood circulation half-life. Particles >200 nm accumulate in the spleen or are taken up by phagocytic cells, while those <10 nm are removed through renal clearance. Optimal nanoparticles (10-100 nm) evade the reticuloendothelial system, penetrate small capillaries, and exhibit longer circulation times. Superparamagnetic nanoparticles in this size range enhance permeability and retention effects, maximizing accumulation at targeted sites. However, nanoparticles <2 nm are not advisable for medical use due to potential diffusion through cell membranes, causing intracellular organelle damage and toxicity. Different shapes and sizes of nanoparticles should be avoided to prevent agglomeration and clogging in the blood system. Therefore, controlling nanoparticle size during preparation is crucial for effective drug delivery.

5.1.4. Surface modification and stability

Surface properties significantly influence the performance of nanoparticles in drug delivery. Ideally, nanoparticles should possess good antifouling properties to prevent nonspecific adsorption of proteins or biological macromolecules. This property helps prolong blood circulation time through adequate surface functionalization. Most synthesized Fe₃O₄ nanoparticles undergo surface modification before drug loading, as it's essential for their role as drug carriers. Polymers are commonly used to modify nanoparticle surfaces through coating, enhancing colloidal stability and dispersity. Coated Fe₃O₄ nanoparticles resist oxidation and agglomeration, unlike uncoated ones. Surface-engineered nanoparticles provide a platform for linking drug molecules and targeting ligands, increasing blood circulation time and biocompatibility while reducing toxicity. In biomedical applications, surface coating improves the stability of Fe₃O₄ nanoparticles. However, coating methods can be complicated, time-consuming, and energy-intensive. Therefore, proper coating techniques are crucial to maintaining desirable properties. Positively charged Fe₃O₄ nanoparticles exhibit better properties than neutral or negatively charged ones. The cell membrane's slight negative charge enables electrostatic attraction-driven cell uptake. Positively charged nanoparticles are taken up faster, but intake depends on cell type.

5.1.5. Drug loading and release

Drug loading onto nanoparticles must preserve the drug's functionality. Additionally, drug-loaded nanoparticles should release the drug at targeted sites at an optimal rate without harming healthy cells. There are two primary methods for loading drugs onto nanoparticles: conjugation and encapsulation. Conjugation involves linking drug molecules to the nanoparticle surface via cleavable covalent linkages or physical interactions. Covalent linkage combines drug molecules with functional groups on polymer-coated nanoparticles, using linkers to enhance drug loading capacity and preserve functionality. Physical interactions, such as electrostatic, hydrophobic, and hydrophilic interactions, also facilitate conjugation. For instance, cationic polymer-coated Fe₃O₄ nanoparticles interact electrostatically with negatively charged DNA. Lipophilic drugs bind to hydrophobic FeO-NPs, enhancing drug release as the coating degrades. Ideal drug delivery systems should release their payload in response to specific conditions. However, drawbacks include rapid release upon injection (burst effect) and low entrapment efficiency, reducing effectiveness. Highly stable conjugations may also prevent drug release at targeted sites. Researchers must address these issues to effectively eradicate tumors. Fe₃O₄ nanoparticles should be designed to release chemotherapeutic drugs while ensuring non-toxicity, biodegradability, and sterility for safe use in drug delivery systems.

5.2. Targeted anticancer drug delivery

A targeted drug delivery system is illustrated in Fig. [insert figure number]. The drug-loaded Fe₃O₄ nanoparticles (NPs) are administered parenterally, entering the human body. Upon injection, the drug-loaded Fe₃O₄ NPs are transported into the blood capillary and guided to the targeted site – cancer cells or tumors – with the aid of an external magnetic field. This targeted approach enables the accumulation and controlled release of the drug at the desired site, enhancing efficacy in treating cancer cells while minimizing harm to neighboring healthy cells. This precise delivery mechanism optimizes therapeutic outcomes and reduces side effects associated with traditional chemotherapy. Targeted drug delivery systems utilizing drug-loaded Fe₃O₄ nanoparticles (NPs) offer enhanced therapeutic efficacy and reduced side effects. These biocompatible and non-toxic NPs can carry high drug payloads and be guided precisely to

targeted sites, such as tumors, using external magnetic fields. Upon accumulation, the NPs release drugs in a controlled manner, maximizing effectiveness while minimizing harm to healthy cells. Applications include cancer treatment, gene therapy, neurological disorders, and infectious diseases.

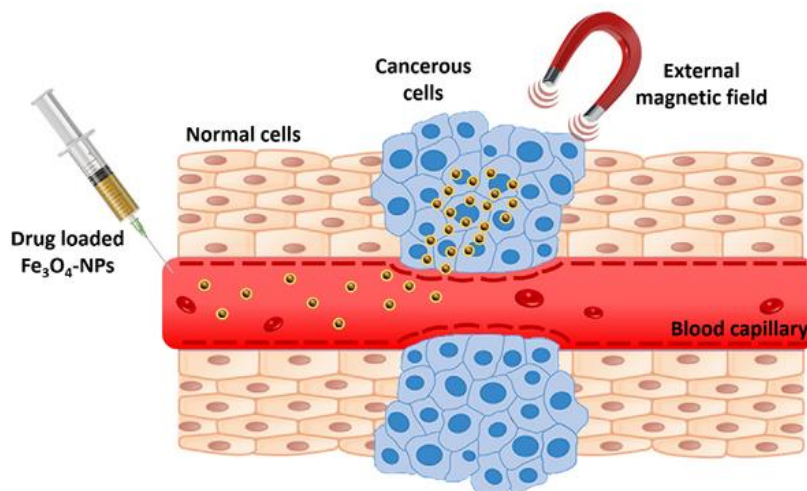


Fig. 8 Targeted drug delivery system using drug loaded Fe₃O₄ NPs.

6. Conclusion and future perspective

The pharmaceutical field has rapidly evolved in recent decades, introducing numerous novel drug delivery systems, including Fe₃O₄ nanoparticles (NPs). Despite their potential, most Fe₃O₄ NP-based systems remain in the early stages of development. The use of external magnetic fields to guide Fe₃O₄ NPs to specific regions shows promising applications in targeted drug delivery and various biomedical fields. However, no Fe₃O₄-based nanoparticle drug delivery products are currently available on the market, highlighting the need for intensive research to commercialize these nanoparticles. Before launching Fe₃O₄ NPs as medical products, several limitations must be overcome, including improving preparation methodologies and characterization. Critical factors to consider when selecting Fe₃O₄ NPs for drug delivery include saturation magnetization, size, shape, surface charge, colloidal stability, drug loading capacity, release behaviour, biocompatibility, and toxicity. Additionally, the fate of Fe₃O₄ NPs in the body after drug delivery is crucial, as safe elimination is essential to prevent exposure and potential neurological disorders. Careful selection of Fe₃O₄ NPs and conjugation mechanisms between polymers, drug molecules, and NPs is necessary to avoid burst effects, which can produce toxic chemicals harmful to the body.

6.1. Future works

Future studies should investigate the *in vivo* efficacy of Fe₃O₄ nanoparticles (NPs) in clinical settings to thoroughly understand their behavior in the body. These studies will provide valuable insights into the desirable characteristics of Fe₃O₄ NPs for targeted drug delivery. Researchers should also examine the interaction between drug-loaded Fe₃O₄ NPs and cells to elucidate the cell uptake mechanism, potentially using molecular docking simulations. To evaluate nanomedicine efficacy, preclinical research is necessary to generate data on tumoral accumulation, intra tumoral distribution, tumoral retention, and peripheral pharmacokinetics. Key parameters affecting nanomedicine efficacy, such as intra-tumoral carrier retention, treatment efficacy in small tumors, and optimal dosing schedules, must be confirmed through preclinical testing. Ongoing research aims to overcome targeted drug delivery challenges in cancer treatment, with promising outcomes indicating Fe₃O₄ NPs' potential. These findings

suggest that Fe₃O₄-based nanoparticles may soon transition from research to clinical applications, offering a potential cure for diseases.

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