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Mini Review on Green Synthesis, Properties and Potential Biomedical Applications of Iron Oxide Nanoparticles (Fe_3O_4-NPs) from Neem Leaves Extracts

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Nanotechnology is a recent advancement in the fields of science and technology. It has contributed significantly to numerous uses of metal nanoparticles in different sectors, mainly in research institutions, science, and industries. This review paper focuses on the green route method synthesis, properties, and potential biomedical applications of iron oxide nanoparticles (Fe_3O_4-NPs) from *Azadirachta indica* (neem) leaf extracts. Iron oxide nanoparticles have piqued the interest of researchers due to their diverse characteristics and various application fields, particularly biomedical applications. Iron oxide nanoparticles have been produced using various methods, including chemical, physical, and biological procedures. These methods, however, are costly, time-consuming, and could be hazardous to individuals and the environment. Conversely, the green method is a promising method for synthesizing nanoparticles. The green synthesis of different types of nanoparticles using plant extracts has also received a lot of attention over the last decade. In addition, its biomedical applications, such as targeted drug administration, magnetic resonance imaging (MRI), treatment of magnetic hyperthermia, and cell separation, are also discussed below.

Keywords: Neem leaf; *Azadirachta indica*; iron oxide nanoparticles; green synthesis; superparamagnetism

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Nanoparticles have been widely used in the past few years in a variety of industries including food preservation, cosmetics, and pharmaceuticals. Due to their superparamagnetism, biocompatibility, low toxicity, and high saturation magnetization, iron oxide nanoparticles (Fe_3O_4 -NPs) are frequently used in a variety of applications, including magnetically targeted medication delivery and magnetic resonance imaging (MRI) [1]. However, their usage in the medical and biomedical disciplines is prohibited due to hazardous chemicals, non-polar solvents, industrial compounds, or capsizing agents. There are three ways to synthesize nanoparticles, namely chemical approach, biological approach, and physical approach (co-precipitation, sol-gel process). However, chemical and biological approaches have a number of drawbacks, whilst the physical technique is expensive and time-consuming. In addition, toxic chemicals such as lead oxide and sodium citrate are formed through chemical processes that may harm humans and the environment. As a result, researchers are turning to bioprocesses and "green" chemistry to develop a method for synthesizing nanoparticles that is secure, effective, benign, environmentally friendly, and biocompatible. Nowadays, producing nanoparticles using environmentally friendly techniques is the most important and effective strategy. These one-step, eco-friendly, cost-effective, non-toxic, repeatable, and more stable green synthesis techniques are superior to competing techniques in several other ways as well [2]. Due to these benefits, green synthesis has become extremely important in many disciplines focusing on a greener environment.

Neem, or the scientific name *Azadirachta indica*, leaf is a naturally available material. Neem leaf was employed in sodium hydroxide (NaOH) solution in the earlier study by Zambri and coworkers (2019) [3] to reduce aqueous Fe^{2+} and Fe^{3+} ions. Terpenoids and flavanones, two phytochemicals found in neem leaf, are responsible for this reduction. These plant compounds serve as reducing and capping agents, bond to metals and support the stabilization of nanoparticles [4]. Neem leaf also offers several therapeutic benefits, including antibacterial, anthelmintic, and antifungal qualities [5].

This article highlights the synthesis of environmentally friendly green methods as well as the challenges or problems that affect the synthesis of iron oxide nanoparticles, such as engineering issues, financial limitations, and environmental considerations. These constraints are fully discussed to give a complete view of the synthesis outcomes. In addition, the possible uses of iron oxide nanoparticles in biomedicine are also covered in this review paper.

Nanoparticles

Nanotechnology has recently arisen as a multi-functional technology dealing with nanoscale innovations with real-world applications [6], involving nanomaterials with at least one dimension between 1 and 100 nm [7]. Nanotechnology research began in the 1980s and has persisted as a modern marvel of scientific discovery. Nanomaterials are prevalent in our daily lives and are ushering in a new age for the entire human race [8]. Nanotechnology offers many opportunities and benefits for global industry and technology since it can be employed globally for various applications, including health-care, electronics, cosmetics, chemicals, energy, and composites [9, 10]. Nanomaterials also have prospective uses in biomarkers, diagnostics, antimicrobial agents, and cell labeling for biological imaging and drug delivery systems; nanodrugs for the treatment of various diseases, and the decontamination of drinking water [11].

Most researchers are primarily concerned with nanomaterials involving nanoparticles (NPs). Nanoparticles (NPs) are advanced materials in nanotechnology which modify matter using physical and chemical techniques to create materials with specific characteristics and properties that are helpful in various applications. NPs have distinct properties and functions than bulk materials, including thermal, electrical, chemical, optical. medical, agriculture technology, information, and communication [12].

Iron Oxide Nanoparticles (Fe_3O_4-NPs) Properties

Chemical compounds of iron and oxygen are known as iron oxides [13]. Research on iron oxide nanoparticles has received numerous recognitions over the last decade due to their unique characteristics, such as small size, high magnetism, least toxicity, and biocompatibility, which significantly impact the characteristics of the nanoparticles [1]. Fe_3O_4-NPs with a size smaller than 20 nm, thus exhibit superparamagnetic, have been useful in various biomedical applications such as magnetic resonance imaging (MRI), drug delivery, diagnosis, detection, and treatment of illnesses like cancer and cardiovascular diseases. These properties also make iron oxide nanoparticles fit in other applications in environmental remediation [14] and water treatment because of their high surface area and unique adsorption [15]. Iron oxides are also used because they are inexpensive and prevalent [16], which exist in maghemite, hematite, and magnetite formed through chemical, biological, and physical approaches [17]. Hence, among various magnetic nanoparticles, iron oxide nanoparticles are the only ones validated by the Food and Drug Administration (FDA) for clinical use [1].

Shape and Size

Nanoparticles' shape and size have a significant impact on the characteristics of nanoparticles. For instance, tiny size stables nanoparticles and can readily pass numerous barriers, allowing nanoparticles to be employed for in-vivo experiments. Furthermore, superparamagnetic iron oxide nanoparticles (SPIONs) with very tiny sizes can quickly enter tumor areas and generate therapeutic effects [18]. It is worth mentioning that several SPIONs' characteristics are affected by their shape and size. Only particles with a uniform surface and size smaller than 20 nm exhibit superparamagnetic behavior, so they become persistently magnetized when an external magnetic field is introduced. Wu and co-workers investigated the magnetic characteristics of iron oxide nanoparticles (IONPs) ranging in size from 6 to 18 nm [19]. The results indicated that magnetic disorder was most noticeable for 13 to 18 nm IONPs, leading to a significant loss in hyperthermia performance. However, Talbot et al.'s [20] reported agglomeration is also a big issue among iron oxide NPs. As a result, several tactics or approaches should be used to prevent nanoparticles from agglomerating and keep them in a stable monodispersed state. The functionalization of these nanoparticles promotes monodispersing and aids in connecting with other metals, drugs, or molecules of interest. The addition of different biomolecules is able to increase nanoparticles' properties and allow them to be employed as a multifunctional entity.

Iron Oxide Structures

Maghemite (γ-Fe_2O_3), magnetite (Fe_3O_4), and hematite (α-Fe_2O_3) are the three most frequent iron oxides which may be found in contaminated air, soil, and others. According to Kralji et al. [21], iron oxide may be found in numerous forms in nature, the most common of which are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3). Meanwhile, physical, chemical, and biological techniques create hematite [16]. Iron oxides' crystal structure may be defined mainly in terms of iron cations and oxygen anions in octahedral or tetrahedral interstitial spaces, as shown in Figure 1. From Figure 1, the black ball represents Fe^{2+}, the green ball represents Fe^{3+}, and the ball in red color represents O_2. Oxygen ions are arranged hexagonally close-packed in hematite, with Fe(III) ions occupying octahedral positions [18].

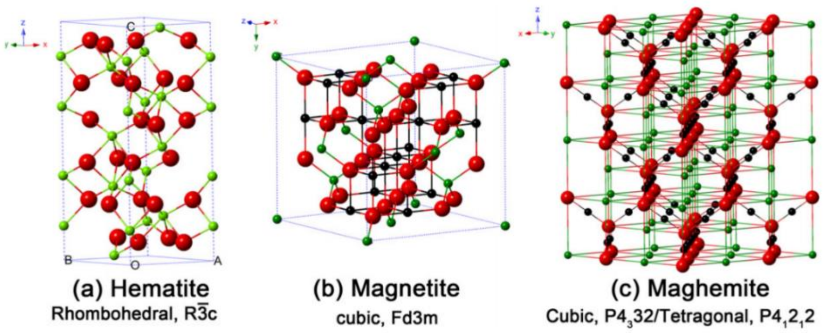

Figure 1. Hematite, magnetite, and maghemite crystal structures and crystallographic data [19]

Meanwhile, the oxygen ions in magnetite and maghemite are organized in a cubic, close-packed arrangement. Because of the similarities between magnetite and maghemite, it is difficult to identify them using traditional spectroscopy like XRD, and additional spectroscopy techniques such as Mossbauer spectroscopy can be helpful. In addition, according to Sangaiya & Jayaprakash (2018) [22], one of the most common, natural, and ecologically friendly types of nanoparticles is hematite (iron oxide). It plays a crucial role in iron biogeochemical cycles in the environment. Hematite is thought to have a more stable n-type semiconductor behavior. Because of its low cost and non-toxicity, researchers are increasingly interested in stable hematite iron oxide for biomedical applications. Furthermore, the bandgap of this hematite iron oxide nanoparticle is 2.1–2.2 eV.

Magnetic Properties

Magnetism is a phenomenon associated with the effects of magnetic fields and the motion of electrical charges that can produce attractive or repulsive forces between objects. The orbital and spin motions of electrons and how electrons in an object interact with each other determine its magnetic nature [23]. Materials can be classified based on their magnetic susceptibility (χ), which defines the degree to which a material is magnetized (M) in response to an applied magnetic field (H) [23] and is defined as

$$\chi = \frac{M}{H} \tag{1}$$

There are five main classes of magnetic materials, each classified by its response to an external magnetic field. These are diamagnetic, paramagnetic, ferromagnetic, ferrimagnetic, and antiferromagnetic (Figure 2) [24, 25].

i. Diamagnetism

Atoms or ions that have all their orbital shells filled and no unpaired electrons make up diamagnetic materials. The individual electrons are cancelled by their pair; therefore, they have no net magnetic moment [23]. All materials, including water, wood, and gold, exhibit diamagnetism, with the magnetic susceptibility being negative ($\chi < 0$), as the magnetization is negative when exposed to a field. This sort of magnetism is independent of temperature and magnetic field.

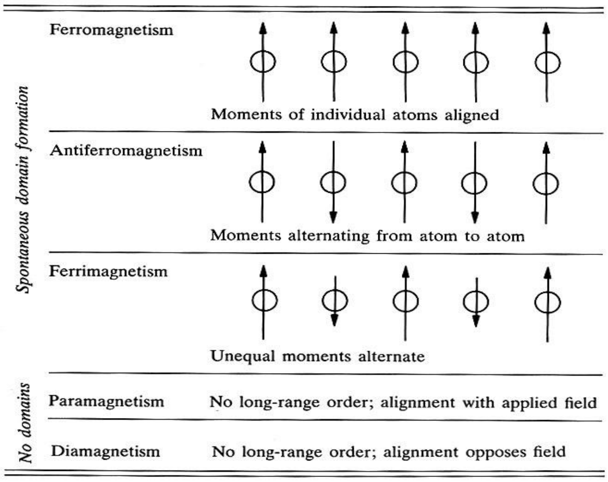

Figure 2. Different types of magnetic behavior. Figure reproduced from reference [26].

ii. Paramagnetism

Unpaired electrons in partially filled orbitals provide paramagnetic ions, such as chromium (Cr^{3+}) or iron (Fe^{2+} and Fe^{3+}), with a net magnetic moment. When exposed to a field, atomic magnetic moments partially align in the field's direction, resulting in a positive magnetization and temperature dependent susceptibility ($\chi > 0$). At lower temperatures, the alignment of magnetic dipole moments is more influenced by an applied magnetic field. Overall, this means that the susceptibility of paramagnetic materials is inversely proportional to temperature.

$$\chi \propto \frac{1}{T} \tag{2}$$

The magnetic moments, however, are arbitrarily aligned in the absence of a magnetic field, resulting in a net magnetic moment of zero.

iii. Ferromagnetism

Ferromagnetic and antiferromagnetic materials have a critical temperature θ, above which the temperature dependent susceptibility is

$$\chi \propto \frac{1}{T-\theta} \tag{3}$$

Even when the magnetic field is removed, ferromagnetic materials show the parallel alignment of moments, leading to significant net magnetization. The parallel or antiparallel alignment of the magnetic moment is a result of the electronic exchange forces between the moments [27].

This type of magnetism has positive values of θ known as the Curie temperature. Until temperature nears $T = \theta$, where susceptibility approaches to infinity, a material reacts similarly to paramagnetic materials. The implication is that in the absence of a magnetic field below the Curie temperature, the spins in a ferromagnetic material spontaneously align, resulting in a net magnetic moment. Common ferromagnetic materials are iron (Fe), cobalt (Co), and nickel (Ni).

iv. Superparamagnetism

Multiple domains make up the bulk of a ferromagnetic crystal. Each domain has magnetic spins oriented in one direction. The magnetic moments of the different domains can be aligned in random directions. Under these conditions, the total magnetic moment of a ferromagnetic crystal cancels out, and the crystal has zero net magnetization. When a magnetic field is applied, the magnetic moments of the dipoles of the domains align in the field's direction. The magnetization increases slowly at first, then more rapidly as the magnetic field increases until it reaches the saturation point at high magnetic fields where all magnetic domains are aligned and above which no further increase in magnetization is possible. As seen in Figure 3, when the magnetic field decreases, the new curve does not retrace the original curve, creating a loop. Ferromagnetic and ferrimagnetic materials will display remanence magnetization and coercivity characteristics. When the magnetic field is withdrawn from ferromagnetic single domain nanoparticles, the crystal keeps its magnetization due to the particles' single domain structure. The nanoparticles' magnetic properties change when the particle size reduces.

As the particle size decreases, multiple domains of the crystal are reduced to a single domain. For superparamagnetic nanoparticles below a threshold size (usually <15-20 nm) [28, 29], the net magnetization of a single domain is zero because the particle's magnetic moment flips continuously from 0^o to 180^o of the easy axis of magnetization. When a magnetic field is applied to a collection of particles, the magnetic moments of the dipoles align in the direction of the magnetic field, increasing the magnetization of the nanoparticles. The net magnetization returns to zero without remanence when the field is removed.

A single domain nanoparticle has an anisotropy energy barrier that hinders the magnetic moment from flipping from one direction to the opposite. Anisotropy energy (E_a) is an important parameter to characterize ferrimagnetic or ferromagnetic materials:

$$E_a = K_a V \tag{4}$$

where K_a is the anisotropy constant and V the volume of the particle.

Anisotropy energy is the variation amplitude of magnetic energy of a nanomagnet dependent on the direction of its magnetization vector relative to the crystal axes [30]. The recovery of the magnetization for ferromagnetic crystals to equilibrium is regulated by Néel relaxation and Brownian relaxation. Néel relaxation involves the rotation of the magnetization of the particle with respect to the crystal axes of the particle. In contrast, Brownian relaxation involves the rotation of the crystal structure of the particle, bringing magnetization with it. Magnetic materials composed of magnetic particles are superparamagnetic when the magnetization curve is entirely reversible due to thermodynamic equilibrium [31].

Figure 3 depicts the magnetization process of superparamagnetic particles. The magnetization of the superparamagnetic sample increases rapidly as the magnetic field increases until it reaches the saturation point. In addition, when the externally supplied magnetic field reduces to zero, the magnetization of superparamagnetic materials (materials containing superparamagnetic particles) decreases to zero. Since they do not retain a significant amount of remnant magnetization during the hysteresis loop, superparamagnetic materials will not display remanence magnetization or coercivity.

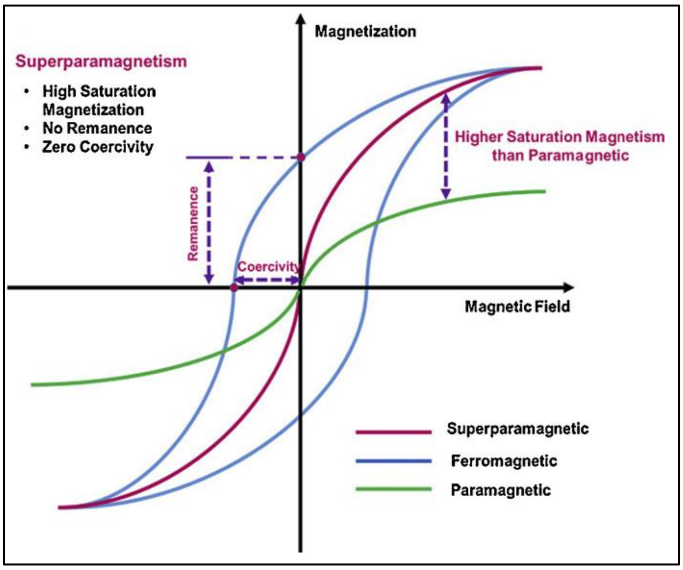

Figure 3. Hysteresis loops characteristic of ferromagnetic and superparamagnetic nanoparticles.

The difference between ferromagnetism and superparamagnetism is depicted in Figure 3. When magnetic particles' anisotropy energy is greater than their thermal energy, k_BT, where k_B is the Boltzmann constant and T the absolute temperature, the magnetic moment maintains a direction that is extremely close to that of the anisotropy axes. In small crystals, the anisotropy energy is similar to the thermal energy $(E_a \leq k_BT)$, allowing superparamagnetic relaxation. The magnetic moment is no longer fixed along the easy directions. This will transform ferromagnetic to superparamagnetic behavior.

v. Ferrimagnetism

Ferrimagnetic materials are composed of two magnetic sub-lattices often separated by oxygen ions. Super-exchange or indirect oxygen ion interaction causes anti-parallel spin alignment between two lattices. The crystal structure of ionic substances produces this sort of magnetism. It is similar to ferromagnetism, except the net spin in these materials aligns with the direction of the magnetic field (see Figure 3).

vi. Antiferromagnetism

Antiferromagnetic materials occur among oxides such as nickel oxide (NiO). Antiferromagnetic materials do not retain magnetism in the absence of an external magnetic field due to their opposing magnetic moments. This type of magnetism has equal but opposite sub-lattice moments; hence, the net magnetic moment is zero in a zero-applied field (see Figure 3). The absolute value of θ is known as the Néel temperature [32], with $\theta < 0$.

Conventional Approach in Synthesizing Iron Oxide Nanoparticles

Various chemical, physical and biological methods have been used to synthesize nanoparticles of different sizes and shapes, structures, and magnetic properties in this modern world. The techniques are divided into two main groups: "bottom-up" and “top-down” routes. The top-down method is a process in which nano-particles are created by crushing rigid materials into small parts with external forces such as grinding, machining, etc. [33]. In contrast, the bottom-up method combines atoms and molecules from small blocks to form a large structure of new nuclei [34], as shown in Figure 4. However, the top-down approach is not comparable to the bottom-up approach. The bottom-up is the most admissible and constructive for the preparation of nanoparticles because nanoparticles are produced from simple molecules called precursors [14]. The following are several synthesis methods:

Physical Method

It is known as a top-down approach which begins in the form of a solid mass. The mass is reduced to smaller nanosized particles using any mechanical process, for example, mechanical grinding, before the particles are stabilized to the appropriate size. Unfortunately, this process makes it difficult to reach the desired thin dimensions [35]. Besides, gas-phase deposition, combustion, electron beam lithography, laser-induced pyrolysis, and power ball milling are often associated with a physical method. Samrot et al. [18] created single-crystal α-Fe_2O_3 nanorings, which were then transformed into Fe_3O_4 and γ-Fe_2O_3 using

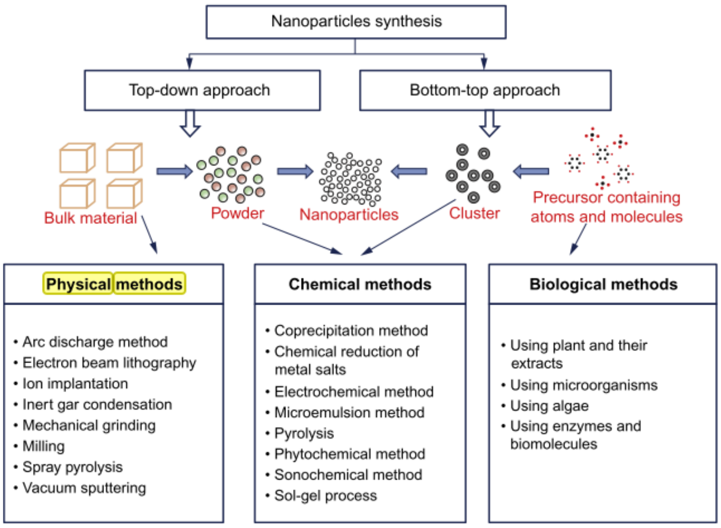

Figure 4. Methods for the synthesis of nanoparticles [35]

reduction-oxidation procedures. Although electron beam lithography produces tiny particles, it has high production costs, time-consuming processes, potential difficulties with electron scanning, resolution limits, etc. Also, the laser-induced pyrolysis method produces <10 nm nanoparticles by heating gaseous precursors in the air with a laser beam. Even though this method produces tiny particles, getting a consistent nanoparticle size for the starting droplets or gas mixture is challenging [36].

Chemical Method

Chemical methods, such as conventional iron chloride co-precipitation, thermal decomposition, sol-gel method, microemulsion, hydrothermal synthesis, and sonochemical methods, provide greater size and composition uniformity [37]. These methods use a bottom-up approach, accumulating iron oxide from monomers or atoms. Hasany et al. [38] explored sol-gel processing by adding a common surfactant, sodium benzene sulfonate (NaDDBS), which reduced the free energy mechanism and permitted the formation of stable nano-sized iron oxide particles without producing a 3D gel network. Kumar et al. [39] synthesized irregular shaped nickel oxide (NiO) nanoparticles with a size of 92 nm from $NiSO_4$ as a precursor. Nanoparticles demonstrated a strong inhibition zone against *Bacillus subtilis, S. aureus, P. vulgaris,* and *E. Coli.* Nevertheless, chemical processes often use hazardous chemicals such as lead oxide and sodium citrate. As a result, the byproducts generated during synthesis create cytotoxic effects, which may exacerbate unfavorable outcomes in biomedical applications [40].

Biological Method

The biological method is classified as a bottom-up approach. Iron oxide nanoparticles can be synthesized via biological entities such as bacteria, fungi, plant extracts, and protein-mediated synthesis. Although these are less harmful to the environment, the particles obtained may be unstable, non-uniform, with much less homogeneity, and thus more agglomeration [18]. Anandan et al. [41] studied cobalt-doped zinc oxide nanoparticles with a single-phase crystalline size of 31-41 nm. These nanoparticles were evaluated against gram-positive *Bacillus subtilis*, as well as gram-negative *Klebsiella pneumoniae*. The antibacterial behavior of zinc oxide was proven to increase when cobalt was doped. Plant extracts, in addition to microorganisms, may be used in synthesizing iron oxide nanoparticles or metal nanoparticles, in general, using a bottom-up method. Using extracts of *Psidium guavaja* and *Moringa oleifera*, Madubuonu et al. [42] produced iron oxide nanoparticles with sizes of less than 50 nm. According to their findings, these iron oxide nanoparticles have antibacterial activities and photocatalytic methylene blue degradation capability. *Moringa oleifera* leaf extract was used to produce iron oxide nanoparticles with a rod-like shape. Rod-shaped iron oxide nanoparticles, like spherical iron oxide nanoparticles, were discovered to have strong antibacterial activities.

Drawbacks of the Conventional Approach Methods

Over the years, researchers have realized how harmful these conventional methods are due to the hazardous solvents and chemicals. These techniques commonly require a larger amount of toxic solvents and are very expensive in many cases [43]. In addition, these methods consume too much energy, involve chemicals of high concerns where many difficulties occur, and include wasteful purifications. For example, hydrothermal techniques and thermal decomposition are common synthesis methods for higher temperatures, huge amounts of harmful organic solvents, and high pressure [44]. Therefore, their use in the medicinal and biomedical fields is banned due to toxic chemicals, non-polar solvents, and synthetic additives or capsizing agents. By this virtue, the need for a safe, efficient, eco-friendly, benign, and biocompatible nanoparticle synthesizing method is crucial [34]. Consequently, many researchers have been working tirelessly to discover and initiate new approaches that are more effective, facile, and reliable in producing nanoparticles. Therefore, “green synthesis” is the alternative to producing NPs [43].

Azadirachta indica (Neem)

Azadirachta indica, commonly known as neem, Indian lilac, or nim, is a popular medicinal plant in India. This plant belongs to the family *Meliaceae* and the genus *Azadirachta.* In parts of the subcontinent of Asia, neem leaves, as shown in Figure 5, are taken as a vegetable and mostly as traditional medicine for treating human illnesses and diseases. Aqueous neem leaf extract possesses good medicinal properties like antifungal, anthelmintic, and antibacterial [45]. Moreover, neem leaf extract has been shown to control high blood sugar levels and clean up the blood [46]. Further studies on all parts of the neem tree, such as leaves, fruits, seeds, and flowers, resulted in a few advantages in pre-clinical research, like therapeutic and promising chemo-preventive effects. Additionally, *Azadirachta indica* is less toxic and always available as it can be found naturally.

A previous work had shown that neem leaf extract in an alkaline medium causes nanoparticle synthesis with a one-pot reaction occurring along with the reduction of Fe^{2+} and Fe^{3+} ions [1]. Besides, the presence of phytochemicals could be observed in neem leaf extractions, namely as flavonoids, terpenoids, alkaloids, etc., which act as reducing agents of metal ions and capping agents, and also help to stabilize nanoparticles [3]. There are, however, many difficulties and concerns involved with green extraction that need to be further discussed.

Green Synthesis Method of Nanoparticles from Neem Leaves

Realizing there are enormous environmental effects from the conventional routes, researchers have found an alternative way to create green-environmental methods. In the past few years, nanoparticle synthesis through the green route method has received significant recognition and become a favorable method. The purpose is to provide uncontaminated nanoparticles via uncomplicated, repetitive, and profitable methods. The green synthesis approach is environmentally safe, budget-friendly, safe, and biocompatible. Besides, it is applicable in various applications, such as opto-electronics, catalysis, and even nanomedicine [44, 47].

Moreover, production via green techniques will employ natural adaptations without the impediment of harmful chemicals [14]. At the same time, this is an excellent opportunity for nanomaterial evolution, and tremendous progress has been made in synthesizing nanoparticles using biological resources like plants and microorganisms. However, plants are favored over microorganisms. The reason is that metal ions can be reduced within a short period throughout the extraction of plants. A longer time is required for microorganism methods.

Despite challenges and factors that could affect green synthesis, works on green extraction of iron oxide nanoparticles continues, as to achieve better and more successful results. Pushkar and Sevak (2019) [48] studied green nanoparticle synthesis and degradation of organochlorine pesticides, such as DDT, by Fe-oxide nanoparticles. The extraction of neem leaves was used in the synthesis of Fe-oxide nanoparticles, which act as reduction and stabilizing agents. In the presence of neem leaf extract, the color of the aqueous solution of the precursor for iron, ferric chloride, rapidly changed from yellowish to vivid black, indicating the production of Fe_3O_4-NPs. SEM analysis revealed that the nanoparticles were spherical, with an average size of 80 nm. The effects of pH, DDT concentration, and contact time were crucial to obtain the adsorption at maximum; as a result, maximum DDT adsorption at pH 3 was achieved after an incubation time of two hours, and 88-92% of DDT was extracted by as-synthesized Fe_3O_4 nanoparticles with a maximum concentration of 500 ppm of DDT. Therefore, for optimal decontamination, various conditions should be modified according to the type of pollutant to be eliminated.

Figure 5. *Azadirachta indica* leaves

On the other hand, Taib et al. [1] reported that rapid production of stable nanoparticles of iron oxide (Fe_3O_4-NPs) or magnetite nanoparticles was observed to treat ferrous and ferric chloride aqueous solutions in an alkaline medium with the extract of neem leaves. Hence, numerous biomolecules, such as flavonoids and aqueous leaf extract terpenoids, have been shown to play a significant role in the synthesis of Fe_3O_4-NPs, through the analysis of infrared spectra. Moreover, this study confirmed the production of Fe_3O_4-NPs by an immediate black color. As a result, strong peak characteristics were also observed by UV-Vis spectroscopy at 249 nm for Fe_3O_4-NPs. Furthermore, spherical and oval shapes and the diameter from 9 to 14 nm were observed with transmission electron microscopy (TEM). They confirmed that the technique's results were simple, quick, non-toxic, one-step, and eco-friendly, which can be used in various biomedical applications, including the magnetic targeting drug delivery system.

Based on Kanase et al. (2020) [49], it is highly desirable to establish a safe approach in synthesizing biocompatible MNPs using the redox characteristics of natural compounds from plant extracts. Therefore, the iron precursor used in this study was ferric nitrate. Next, the biological and physical properties of MNPs synthesized with the adjusted green hydrothermal process at various reaction temperatures and times were investigated. Upon that, most iron oxalate hydrate ($Fe(C_2O_4).2(H_2O)$) was produced at a lower reaction time and temperature of 200°C for 2 hours. Moreover, increasing reaction temperature showed the phase transition from iron oxalate hydrate to pure magnetite (Fe_3O_4) stage. The MNPs prepared for 4 hours with an optimal temperature of 220°C showed superparamagnetic character with an enhanced high saturation magnetization (M_s) value of 58 emu g^{-1}. Apart from that, at optimal reaction conditions, which were 220ºC for 4 hours, spherical MNPs with a size range of 13–15 nm and excellent crystallinity were produced. Thus, MNPs acquired through this modified biogenic approach will widen the scope and applicability in future biomedical applications.

Pattanayak and co-workers (2013) successfully synthesized nano-scaled zero valent irons (nZVI) from the extractions of plants under ambient circumstances [5]. The iron nanoparticles produced were mostly in the zero-valent oxidation state during the iron nanoparticle synthesis. Ferric chloride ($FeCl_3$) is widely recognized for its vivid yellowish color in distilled water. When the plant extract was mixed with the aqueous $FeCl_3$ solution, it quickly changed the color of the solution and reduced the pH, which might be a sign of the production of iron nanoparticles. The UV-Vis analysis showed that nanoparticles were synthesized in the range of 216-265 nm; meanwhile, the SEM analysis showed that nanoparticles with a diameter of about 100 nm were spherical.

The concentration of polyphenols plays a pivotal role in capping and decreases efficiency, which might also be among the probable causes of a wide range of sizes. Besides, the use of nanoparticles for different applications might also influence the size of nanoparticles obtained. In addition, shape and size are crucial properties of iron oxide NPs because nanoparticles will exhibit superparamagnetic behavior when the size is smaller than 20 nm. As could be seen, nanoparticle sizes in Taib et al. [1] and Kanase et al. (2020) [49] studies were between 9-15 nm. Thus, it is proven that acquired nanoparticles are superparamagnetic NPs. On that account, many findings also showed that green synthesis of iron oxide nanoparticles is clean and devoid of harmful chemicals, making them acceptable for biological, pharmacological, and medicinal applications, suggesting that green synthesis may have an advantage over conventional synthesis.

Factors That Impact Synthesis

As reported by many research before, green synthesis advancement may be affected by shortcomings, issues, and challenges. In addition to that, as seen in Figure 6, there are also significant problems that come from engineering, technical, and economic limits related to plant extract concentration, reagents ratio, optimal conditions for experimental, characterization of product, and yield of product [50].

Optimal Analysis Conditions

The experimental conditions would differ for every procedure because different plant extracts have different properties [50]. Optimal experimental parameters are crucial to synthesize nanoparticles (NPs) from plant extracts. For instance, particle size, pH, temperature, and extract concentration are the parameters that may affect the synthesis [35]. The concentration of polyphenols plays a pivotal role in capping and reducing performance, which could be one of the potential reasons for a wide variety of sizes [51].

Besides, pH alterations like alkaline and acidic conditions might force agglomeration to occur. For example, nucleation will eventuate due to low pH or acidic conditions; meanwhile, nanoparticles lack stability when pH increases. In essence, a stable and fast process of nanoparticles (NPs) will be formed only in alkaline conditions. In terms of temperature, dynamics grow faster and lead defects to occur simultaneously at a faster rate, thus affecting crystal quality at high temperatures rather than room temperature [50]. Moreover, even though the nucleation rate moves faster than the growth rate, the nuclei activity surface will increase once temperature increases. The nuclei will be forwarded to agglomerated and colloid [45]. Thus, the optimal conditions for analysis are crucial because these conditions may influence the synthesis.

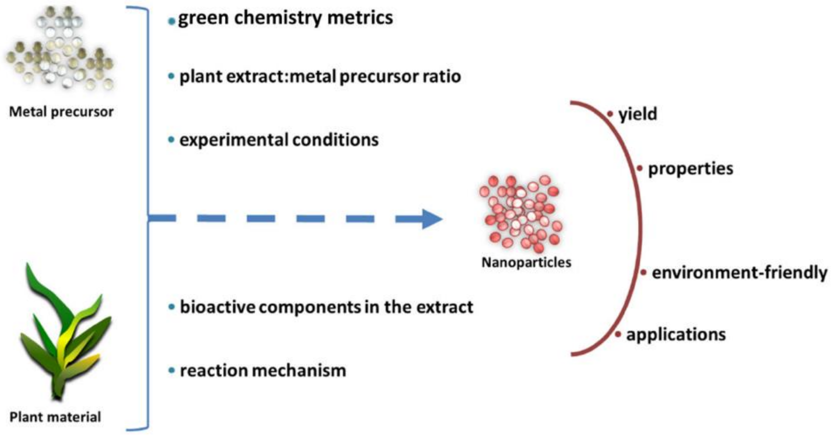

Figure 6. Key concerns influencing the development of green nanoparticle production technologies [50]

Technical Analysis Factor

There are limitations like source variability, for example, bioactive compounds of plant extracts in standardization for green synthesis of metal-based nanoparticles (NPs). The formation of NPs is sometimes triggered by many active ingredients, but not so many details were given about these active ingredients. For instance, Nagarajan and co-workers [52] compared seaweed extracts' ability in synthesizing zinc oxide nanoparticles (ZnO-NPs). The seaweed used were *Caulerpa peltata*, *Hypnea valencia*, and *Sargassum myriocystum*. From the results, the only extract that could disturb ZnO-NPs formation was *S. myriocystum* extract.

Furthermore, in the case of CeO_2 NPs, Thovhogi et al. (2015) [53] reported various bioactive compounds in *Hibiscus sabdariffa* flower extract (hibiscetin, quercetin, gossypetin, cyanidin 3-sambubioside, delphinidin, and pectin). Still, they could not determine the substance responsible in producing CeO_2 NPs. The antioxidant capacity of the leaf extractions of 26 different plant species has been shown to have a direct impact on their potential to reduce Fe(III) to form NPs of ZVIs; however, scientists only categorized the extracts by evaluating the "ferric decreasing antioxidant power" [51]. Therefore, to obtain effectiveness in green synthesis, the sterile solutions of the active components found in plant extractions should be used in many studies. However, a similar problem had also been reported in many articles on the synthesis of biogenic of other NPs, causing a significant gap in knowledge as to which metabolites in plant extracts are the reduction or capping agents to convert metallic substrates into NPs [50].

Engineering Factor

Products should be produced with comprehensive and careful standards for the performance and quality of commercial nanoparticles. For instance, the composition of surface and size uniformity guarantees similar behavior of products. The morphology for small nanoparticles is spherical. Gold nanoparticles (Au-NPs) and gold platelets diverse from 7 to 3000 nm from aqueous extractions of *Madhuca longifolia* leaves [54]. Meanwhile, larger particles have mixed geometries containing spherical and triangular platelets [50]. The stoichiometric ratio of extract, precursor of metal, and concentration of plant extract is the main factor that influences size, yield, crystallinity, shape, and other attributes of green synthesis NPs [50]. For instance, Ag NPs synthesized with 1 to 5 mL of maize husk extract had a smaller particle size than those generated at a higher volume of extract, which was 8 mL [55].

Plant extract concentration is also a key element affecting metal nanoparticle shapes and crystallinity [50]. The formation of zinc oxide nanoparticles (ZnO NPs) was only detected when using 5 mL of *S. myriocystum* extract [52]. Meanwhile, Davar et al. [56] reported that size distribution was found in 30 mL lemon juice content, which increased to submicrometer size for 50 and 70 mL volume extraction. This indicates that when the volume of lemon extract used in the synthesis increased, the size distribution of ZnO NPs also increased. In addition, few publications reported that synthesized plant-based NPs were surface capped, and none mentioned capping characterization [50]. Plant-based synthesis of metal nanoparticles can be coated using several organic molecules [57]. Therefore, much more data is required to analyze and assess the viability of the entire process and obtain a thorough overview of the path in which the green synthesis is going.

Economical Limit and Environmental Factor

In the current studies, some factors might be overlooked. There is a limiting factor for long-term demands to be achieved. For example, the required volume from plant sources and extracts will tremendously increase when green synthesis production

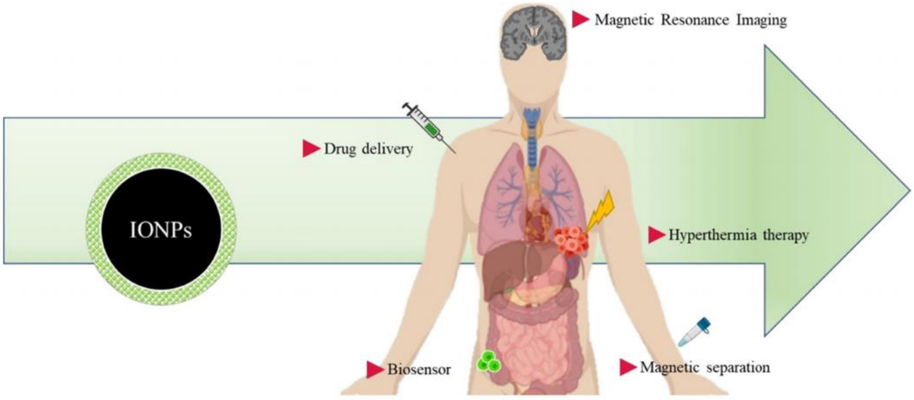

Figure 7. Fe_3O_4-NPs in biomedical applications [59]

rises daily. Besides, the yield and stability of green synthesis nanoparticles (NPs) are other factors that can be evaluated. This is because yield can be crucial in measuring green synthesis efficiency, profitability, and practicality. However, the efficiency of the yield from the process and economic analysis of green production is usually not stated in the reports. In essence, mass yield, stoichiometric factor, and mass intensity are important parameters for an effective analysis of the plant-based synthesis of metal nanoparticles [50].

Biomedical Applications of Iron Oxide Nanoparticles

Magnetic nanoparticles, such as iron oxide nanoparticles, have been widely explored for various biological uses during the last 20 years. These nanoparticles are commonly used in targeted drug administration, magnetic resonance imaging (MRI), treatment of magnetic hyperthermia, and cell separation due to their unique magnetic characteristics, nontoxicity, and biodegradability [58]. Besides that, as can be seen in Figure 7, biosensor is also one of the most used applications [59].

Targeted Drug Administration

Typically, medications are given orally or intravenously. Furthermore, the delivered medications will first enter the systemic circulation before reaching the site of damage. However, while this process continues, some drugs are lost before they hit the targeted location, resulting in poor drug bioavailability [18]. The notion of enhanced drug delivery can play an essential complementary role in developing modern medicine. Superparamagnetic iron oxide nanoparticles (SPIONs) have shown considerable promise in nanomedicine, among other nanoscale drug transporters.

Recent studies have confirmed that SPIONs may be employed as capable drug carriers after appropriate fabrication, which may prevent oxidation, preserve the drug molecules, and restrict the incursion of the reticuloendothelial system (RES), enhancing the in-vivo retention period inside the circulatory system [37]. Moreover, SPIONs have the best drug-targeting effectiveness of any carrier because an external magnetic field applied locally to the target organ promotes the aggregation of magnetic nanoparticles at the drug's site of action. Because these nanoparticles are so tiny, they can readily get through the biological barrier. SPIONs are encased in biocompatible and biodegradable biopolymers to maximize their bioavailability and dispersibility [60]. Rather than the traditional direct delivery of bare medicine, these SPIONs can be utilized to provide medication directed to a more particular spot where therapy is required. However, numerous toxicological issues, such as oxidative stress, unanticipated physiological responses, signaling pathway stimulation, changes in gene expression patterns, and potential disruption in iron homeostasis, must be carefully evaluated despite these advantages. Also, the protein corona on the surface of SPIONs may cause a few faults, such as decreased SPIONs targeting effectiveness.

Imaging

Superparamagnetic iron oxide nanoparticles (SPIONS) complements Magnetic Resonance Imaging (MRI), ushering in a new era in diagnosis. Because SPIONs are less poisonous and superparamagnetic, they have frequently been employed as MRI contrast agents to monitor delivered medicines. Yang et al. [61] claimed MRI employs nanoparticles no larger than 5 nm. SPIONs have recently been used to monitor stem cells following recent advancements.

Besides, SPIONs can also be used to determine the effectiveness or failure of an administered therapy and better understand organ structure [18]. Magnetic particle imaging (MPI) is a relatively new imaging technology for inflammatory imaging, cancer cells, neuroimaging, etc. However, magnetic particle imaging (MPI) has recently received much attention due to its higher sensitivity than MRI. MPI is also more preferable to MRI in cell localization accuracy. Furthermore, MRI and MPI have recently been coupled as dual or multi-modal imaging technology to increase the signal in the brain for the early diagnosis and treatment of brain disorders. Magnetic and iron oxide nanoparticles appeal to brain delivery since they are diverse and may be applied in various applications such as imaging and therapy. The prime limits for using MRI/MPI for imaging and therapy are in brain delivery, one of which is the brain-blood barrier, or BBB [60].

Treatment of Magnetic Hyperthermia

Iron oxide nanoparticles are also used for magnetic hyperthermia (MH) treatment as well. The favorable influence of MH in treatment will enable us to anticipate significant advances in biological research. The reaction of iron oxide nanoparticles towards an external alternating magnetic field (AMF) in the presence of heat energy dissipation when the temperature of iron oxide NPs elevated above 45ºC is called magnetic hyperthermia [18]. When subjected to adequate amplitude and frequency alternating magnetic fields, iron oxide nanoparticles produce heat locally where they would be concentrated, reducing the survival of cancer cells. Furthermore, SPIONs exhibit magnetization reversal dynamics due to AMF, driven by two rotating processes: Neels rotation and Brownian alignment. According to Shah et al. [62], Brownian alignment is described as the force imposed on a particle in solution to rotating on itself with the fixed magnetic moment, while Neels rotation happens when the magnetic moment rebuilds the particle's electronic spins, causing the particle to reorient towards the applied field.

Furthermore, SPIONs in the alternating magnetic field (AMF) will generate heat energy. To improve biocompatibility, stable SPIONs are covered with a biopolymer. SPIONs can be injected intravenously into a targeted area, like a tumor, and then subjected to an external AMF. Therefore, quick temperature rise destroys the malignant cells in the area. Unfortunately, one of the drawbacks of MH is the poor power heating of typical magnetic nanoparticles, which necessitates a substantial local injection of NPs. Considering the therapeutic significance of these findings, there is an urgent need to develop optimal NPs for MH effectiveness. Nevertheless, optimization is still poorly understood, and experimental data obtained from NP systems differ greatly. Thus, only certain physical qualities necessary for MH to effectively convert magnetic energy to heat are provided [58].

Separation of Cells

Specific cell type separation in a mixed cell population and some other biomolecules might be accomplished through immunomagnetic separation based on specific antibodies immobilization against target cells on MNPs. The target cells have excessive expression of receptors on the cell membrane against antibodies with higher sensitivity. Aside from antibodies, MNPs showing particular biomolecules against cell membrane receptors like lectins, peptides, and carbohydrates might have significant potential in this field [63]. For instance, Xu et al. [64] studied a separation of cancer cell circulation by using antibody-linked iron oxide nanoparticles (IO-Ab) in a low magnetic field gradient. Iron oxide nanoparticles were produced in an organic solvent via a pyrolysis-based process that carefully controlled crystallinity and particle size. Before being treated with an antibody to trap the cancer cells, iron oxide nanoparticles exclusively soluble in an organic solvent were altered with polymers to make them water-soluble. Gel electrophoresis was used to examine the efficacy of the attachment to the iron oxide nanoparticles, while Prussian blue staining and TEM were used to examine the effectiveness of cell capture. After testing for stability, this antibody, attached with iron oxide nanoparticles, was utilized to separate cancer cells in a buffer. However, the presence of antibodies applied to the target organism's surface and the requirement for a large concentration of free antigens in the target cell are drawbacks of this approach [65].

LIMITATIONS AND RECOMMENDATIONS

More studies and improvements are needed in investigating the uniqueness of green-synthesized nanoparticles, and other accessible resources should have been used for this synthesis. Certain areas, however, require a more in-depth grasp, including:

i. A dependable and sustainable technique for nanomaterial synthesis should pave the way for an economically viable technology.

ii. More emphasis should be placed on addressing and managing challenges such as technical analysis factors, engineering factors, economic constraints, environmental constraints, and optimal analysis conditions of green-produced iron nanoparticles to produce nanoparticles with specified sizes, shapes, and structures that can be used in biomedical applications.

iii. Agglomeration was formerly a significant concern for iron oxide nanoparticles. Hence, multiple strategies or techniques should be utilized to keep nanoparticles from agglomerating and remain in a stable monodispersed form.

iv. As nanoparticles tend to lack stability at higher pH, future research studies should be conducted in alkaline conditions to produce a stable and rapid process of NPs.

v. Plants are chosen over microbes because metal ions may be reduced quickly during plant extraction, while a longer time is required through microbial methods. Thus, future studies should introduce new augmentations to address this issue while also spotlighting this microorganism method.

vi. A thorough analysis of green nanoparticles' distribution and mechanism of action is required to improve biomedical applications.

CONCLUSION

Iron oxide nanoparticles (Fe_3O_4-NPs) have received a lot of interest due to their characteristics in biomedical applications such as targeted drug administration, magnetic resonance imaging (MRI), treatment of magnetic hyperthermia, cell separation, and others. Previously, iron oxide nanoparticles were synthesized using various methods, including physical, biological, and chemical methods. These conventional methods are costly and employ poisonous substances that may have unintended consequences. Green approaches, on the other hand, are eco-friendly, cost-effective, safe, and biocompatible. The following conclusions may be derived from the present study:

i. Pure solutions of the active components discovered in plant extractions should be utilized in numerous studies to achieve effectiveness in green synthesis.

ii. For commercial nanoparticle performance and quality, goods should be made in accordance with extensive and meticulous requirements, like surface and size uniformity, which ensure that products behave similarly.

iii. The main factors influencing the size, yield, crystallinity, morphology, and other characteristics of green-synthesized NPs are extract stoichiometric ratio, metal precursor, and plant extract concentration.

iv. Dynamics develop quicker, causing defects to form faster, compromising crystal quality at high temperatures than at room temperature.

Therefore, since iron oxide nanoparticles are less toxic yet biocompatible than other nanoparticles, they are excellent metal nanoparticles for biomedical applications.

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