National University of Science and Technology
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MAGNETITE-BASED NANOSTRUCTURED MATERIALS OBTAINED BY MICROFLUIDIC TECHNIQUES

- Ph.D. Thesis Summary -

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Part I. Current State of Knowledge

1.1. Introduction

Nanotechnology represents an emergent multidisciplinary field that involves the manipulation and control of matter at the nanoscale level, drawing on knowledge from interconnected scientific domains. It is a continuously evolving field in which phenomena occur at the nanoscale level [1,2]. It is now utilized in numerous remarkable applications across various fields, including communication, energy, electronics, and, most notably, medicine. Thus, nanotechnology deals with the design, manufacture, and characterization of materials, structures, devices, and systems with at least one dimension smaller than 100 nm [3-5].

In the medical field, nanotechnology has been utilized to optimize treatment strategies, enhance therapeutic efficacy, revolutionize diagnostic approaches, provide personalized and targeted treatments, and improve patient outcomes [1,2,6]. In the biomedical field, NPs have started to be utilized for their potential to increase solubility and bioactivity, while providing drug targeting and minimizing systemic toxicity. The advantages of NPs, together with the microfluidic technology approach for synthesis, offer the possibility of achieving precise control and increased efficiency, leading to advanced systems [7].

In this regard, microfluidic technology allows accurate control and manipulation of fluids through micrometer-sized channels. Thus, microfluidics offers advantages such as enhanced mass and heat transfer, laminar flow control, and minimized reagent consumption. Regarding NPs synthesis, microfluidics promotes the tight regulation of nucleation, growth, and aggregation processes, resulting in NPs with uniform size, morphology, and surface characteristics. Moreover, this synthesis method provides reproducibility and scalability for the fabrication of complex nanostructures, overcoming many limitations associated with conventional methods [8].

A crucial field in which nanotechnology holds great promise is overcoming the global burden of antimicrobial resistance (AMR). At this moment, AMR is a critical public health threat, associated with 4.95 million deaths due to drug-resistant infections in 2019 [9]. However, even if global sepsis mortality decreased, AMR-related deaths increased over the past three decades [10]. This crisis was created by the overuse of antibiotics in both humans and animals, with usage exceeding 35% at the beginning of the 21st century [11]. Thus, nanotechnology represents a promising strategy in combating AMR and creates new approaches for the "post-antibiotic" era. NPs have the ability to disrupt bacterial systems and inhibit biofilm formation, while improving drug delivery and pharmacokinetics [12].

This thesis aims to highlight the critical need for novel therapeutic strategies to combat AMR, emphasizing the promising role of iron oxide nanoparticles (IONPs) in the eradication of infections and the development of advanced therapies. The implementation of microfluidic systems for nanoparticle fabrication is presented as a scalable and precise solution for next-generation antimicrobial nanomaterials. Ultimately, this study seeks to stimulate further research and innovation in the field, facilitating the broader application of nanotechnology-based solutions in biomedicine and beyond.

1.2. Iron Oxide Nanoparticles

Iron oxides exist in nature in various forms such as magnetite (Fe_3O_4), hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3), akaganeite (β-FeOOH), goethite FeOH(OH), and wüstite (FeO), and have started to be widely used in their nanoscale form in various applications [13,14]. Among the different possibilities, the most outstanding iron oxides are represented by hematite, magnetite, and maghemite, which contain close-packed planes of oxygen anions with iron cations in octahedral or tetrahedral interstitial sites [15].

One of the most studied iron oxides is represented by magnetite, because of its polymorphism, especially in nanosized forms [15]. Compared to other iron oxides, Fe_3O_4 exhibits superior properties such as ferromagnetism, excellent electrical conductivity at room temperature, and a behavior similar to that of a semi-metal. Another excellent property of Fe_3O_4 is the Verwey transition, representing a phase change at low temperatures, leading to electron reorganization [16,17].

γ-Fe_2O_3 structure, on the other hand, is similar to Fe_3O_4 (Figure 1), being considered an oxidized form of it. Moreover, its charge is neutral, and this is maintained due to the presence of cation vacancies. At the same time, γ-Fe_2O_3 and Fe_3O_4 have close physical and mechanical properties [13,18]. Even if they have a similar cubic spinel structure, compared to Fe_3O_4, γ-Fe_2O_3 exhibits a small magnetic anisotropy because of the lack of orbital angular momentum. Also, unlike Fe_3O_4, which has good conductive properties and can be considered a semiconductor, γ-Fe_2O_3 is viewed as an insulator. About magnetic properties, super-exchange interactions between ions in the tetrahedral and octahedral positions determine the ferrimagnetic ordering in both materials [19].

Furthermore, compared to Fe_3O_4, α-Fe_2O_3 represents a stable form of iron oxide, with an α-Al_2O_3-like crystalline structure (Figure 1). Due to this structure, α-Fe_2O_3 exhibits high thermal and chemical stability, as well as stability in aqueous solutions, under acidic and ambient conditions [13,20,21]. The magnetic properties of α-Fe_2O_3 can also be adjusted by

doping with transition metal ions (such as Sc^{3+}, Ga^{3+}). However, there are disadvantages to using hematite in various applications, such as poor electrical conductivity and the fact that it can be combined with other semiconductors to form type II heterostructures and improve properties.[22].

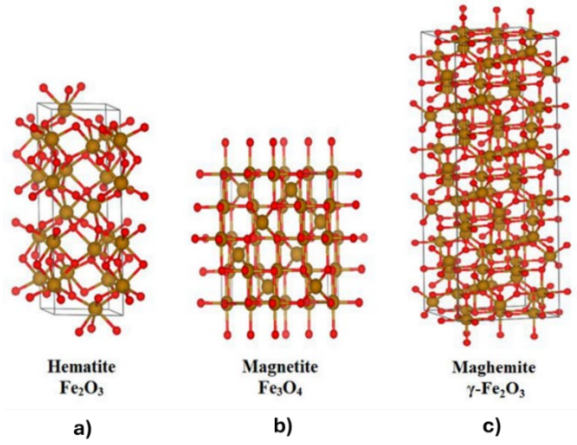


Figure 1. Structural comparison of IONPs: **a**) Hematite - Fe_2O_3, **b**) Magnetite - Fe_3O_4, **c**) Maghemite -γ-Fe_2O_3. Reprinted from an open-access source [23].

The structure and nanosized dimensions of IONPs give them superparamagnetism, a property observed in single-domain nanoparticles with sizes under 10 nm [24]. For example, hematite is antiferromagnetic in bulk form, while magnetite and maghemite are ferrimagnetic [13,25]. Moreover, the synthesis routes influence these properties, and the variation of parameters like temperature, reactant concentration, aging time, and interactions between the surface of the nanoparticles and the surfactants and/or capping agents contributes to the obtaining of IONPs with a variety of shapes [26].

A variety of synthesis method have been explored for iron oxide nanoparticle synthesis, being mainly divided into chemical, physical, and green or biological routes.

Among the chemical synthesis techniques, co-precipitation of ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions in aqueous alkaline media represents the simplest and most employed method. Co-precipitation synthesis route is schematically represented in Figure 2. This method can produce nanoparticles with sizes between 5 and 40 nm, which can be influenced by factors such as precursor type, pH, ionic strength, temperature, and stirring speed [27-31]. However, although this method presents advantages (e.g., scalability, water solubility of the products, and absence of toxic by-products), it also presents challenges, such as maintaining particle size distribution, achieving high crystallinity, and reducing agglomeration [27,32].

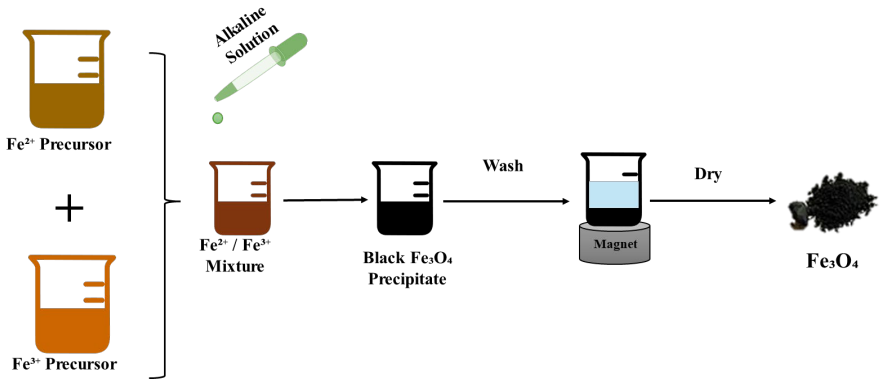


Figure 2. Co-precipitation Fe_3O_4 Synthesis. Based on information from [33,34].

Other chemical synthesis possibilities include hydrothermal and solvothermal methods – which require high temperatures for the chemical reaction to occur [27,35-37], the sol-gel method – depicted in Figure 3 – which consists of the hydroxylation and condensation of a metal precursor in water or alcohol to obtain a colloidal solution, known as a sol. This reaction is followed by gelation, forming iron oxide 3D networks that are further dried by calcination to obtain particles in the 15-50 nm size range [35,38,39]. Microemulsion can also be involved to synthesize nanoparticles between 5 and 40 nm [24,40-42], while the polyol method may lead to a broader size range, with resulting particles having between 4 and 100 nm [27,35].

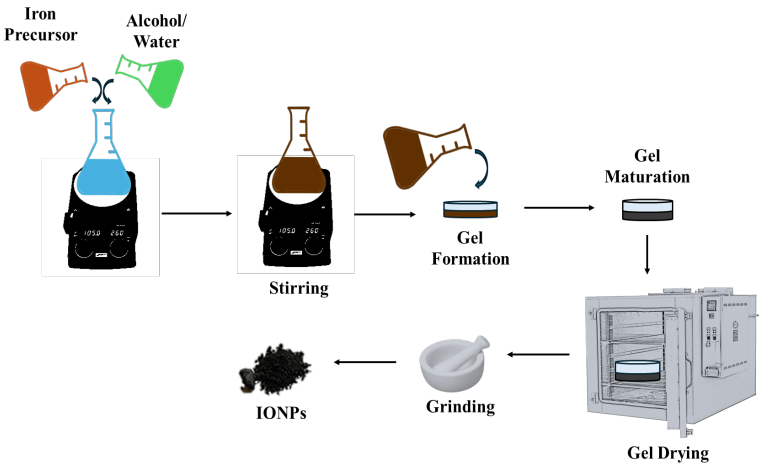


Figure 3. Sol-gel Synthesis of IONPs. Based on information from [27,39].

The second class of conventional synthesis techniques comprises physical methods like pulse laser ablation, laser-induced pyrolysis and sputtering deposition. All have in common the fact that they are top-down techniques, meaning they start from bulk materials and turn into particles of nanometric size. The first two methods involve laser beam irradiation to reduce the

dimensions [36,43,44], while sputtering deposition supposes a solid iron cathode targeted by argon ions [35].

A newer trend is represented by green and biological synthesis routes, which assume the utilization of non-toxic reagents, reducing the risk of obtaining harmful by-products [29]. This type of synthesis, schematically represented in Figure 4, utilizes natural substances (e.g., plant extracts, microbial cultures) as reducing agents or stabilizing agents, being much more cost-efficient and scalable compared to other conventional methods [29,45,46]. However, green synthesis presents challenges such as maintaining particle size uniformity and structure control [47].

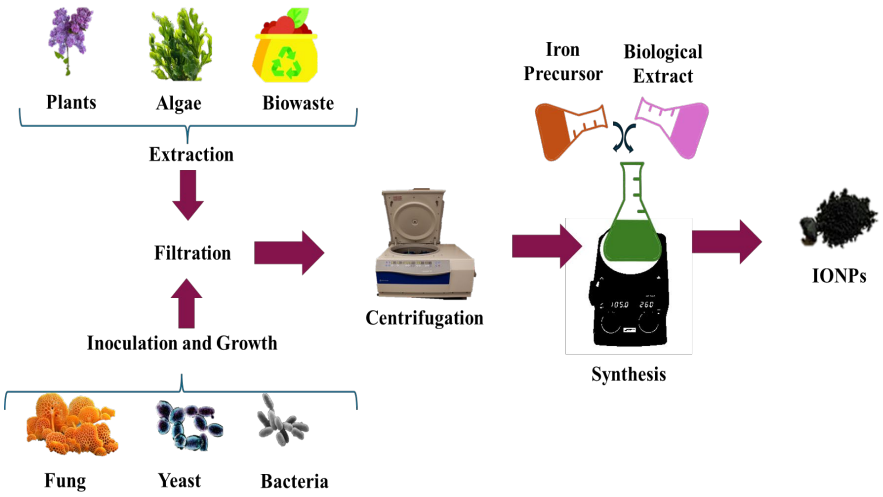


Figure 4. Green synthesis of IONPs. Adapted from an open-access source [48].

An emerging possibility of synthesizing iron oxide nanoparticles with enhanced control over product properties is microfluidic synthesis. This approach employs the manipulation of minimal volumes of fluids (on the scale of µL to pL or less) through micrometer-sized channels, providing precise control, reproducibility, and scalability in nanoparticle synthesis processes [49-51].

Several inter-connected factors impact the results of microfluidic synthesis. One of the most important parameters is the flow regime. From this point of view, microreactors are generally categorized into continuous flow, segmented flow, and droplet-based microreactors, each different mixing patterns within the microfluidic channels [48,52,53], as visually represented in Figure 5.

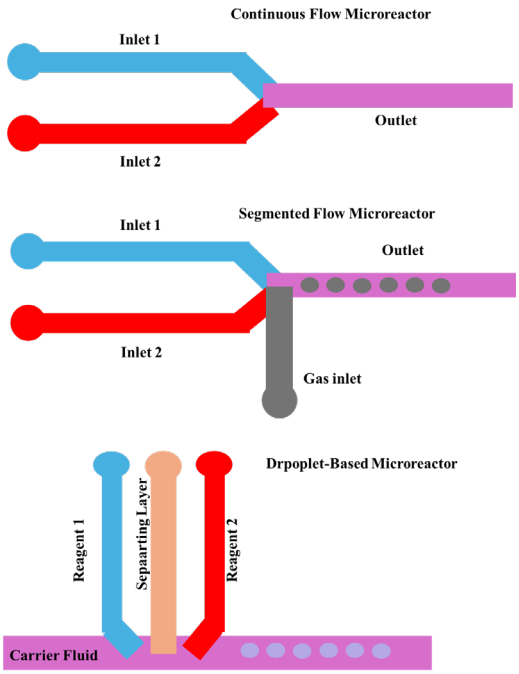


Figure 5. Classification of microreactors according to flow regimes. Created based on information from [52,54].

Mixing is another important factor, helping to avoid particle aggregation or channel blocking [55]. Microfluidic mixers can be passive, with fluid homogenization occurring solely due to how microchannels are design, or they can be active, involving external energy sources for improved efficacy [56]. In terms of design, numerous geometries can be employed for iron oxide on-chip synthesis. The most frequently used are T-type and Y-type geometries [53], but flow-focusing [57], co-flowing [58], s-shaped [59], spiral [59], and serpentine [59] microreactors have also been successfully reported in the literature. More modern platforms have also been developed by combining more geometries into a single chip, creating more complex designs toward achieving improved mixing and ensuring better control over reaction parameters [53,60].

1.3. Functionalization and Bioactivity Enhancement of Iron Oxide Nanoparticles

Besides the synthesis route, iron oxide nanoparticle properties can be engineered and enhanced through various functionalization options. Especially because nanoparticles are prone to be more reactive towards oxidizing agents and moisture, having a high tendency to be unstable and agglomerate, they necessitate surface modification [61].

Regarding the use of NPs in various applications (e.g., biomedical, imaging, sensing, energy), their dispersion in different matrices and their interaction with other molecules must be explored [62]. Thus, the stability of IONPs is essential to explore for their storage and multiple applications. In this case, surface coating and functionalization are key factors

providing dispersion, colloidal stability, and controlled assembly [61-63]. Furthermore, the functionalization of IONPs can provide better control over their magnetic properties, protection, and stabilization on the IONPs surface, thereby enhancing their biocompatibility and reducing their toxic potential [61].

Ligand exchange represents a common strategy used to modify the surface chemistry of IONPs by altering their hydrophobic character to a hydrophilic one, thereby facilitating chemical bonding between the IONPs and functional groups. Moreover, this strategy implies functional groups that enhance nanoparticles' colloidal stability and biocompatibility, replacing stabilizers such as oleic acids with functional group ligands (e.g., amines, carboxylic acids, thiols) [27,64]. Despite the advantages ligand exchange gives to IONPs, this method remains challenging in practice because of the control over surface density, their variable affinity to the substrate, the maintenance of nanoparticle integrity during exchange, and the fact that the strategy is limited to low molecular weight or monodentate ligands because of steric and kinetic constraints [65].

Encapsulation is an alternative strategy used for IONPs functionalization. It implies a consecutive link with an encapsulating agent in solution and creates layers to encompass IONPs in core-shell structure nanocarriers [61,66]. In this type of nanocarrier, IONPs represent the core, and the shell is associated with the coating layer on the surface of nanoparticles [67]. This strategy is often used for IONPs obtained for biomedical applications, creating hydrophilic and biocompatible nanoparticles [27].

Due to the small size of IONPs, the magnetization of each particle is low, which limits the control of particle movement and manipulation using an external magnetic field. Thus, to overcome this limitation, researchers proposed a strategy involving a polymer-assisted self-assembly solution to enhance the incorporation of IONPs in colloidal polymeric particles, thereby controlling their morphology and size. In this regard, polymeric micelles or polymeric vesicles can create layers for IONPs coatings [68]. However, some drawbacks of this technique are that it implies complex environmental parameters for synthesis and can reduce the surface reactivity [69].

Silanization represents a low-cost strategy for coating materials rich in hydroxyl groups (e.g., metal oxides), due to the binding of silicon molecules to the surfaces of particles [70,71]. This strategy provides IONPs with increased colloidal stability, preventing agglomeration formation, and enhances their biocompatibility, representing a suitable method for functionalization [27]. However, despite this approach's advantages, there are limitations, such as being time-consuming and expensive. Moreover, this approach is generally used for

applications involved in decontamination, molecule adsorption, and more, offering the possibility of additional functionalization [72,73].

Stimuli-responsive polymers, are a class of materials with high performance regarding their substantial and reversible changes in their physical and chemical properties in response to external factors (e.g., temperature, pH, redox conditions, glucose, enzymes, humidity, light, magnetic, or electric fields, and ultrasounds) [74,75]. Regarding the surface modification of IONPs with stimuli-responsive polymers, smart coatings are being developed to obtain systems that improve bioavailability in biological systems used for therapy and diagnosis [76].

Moreover, bio-inspired and biomimetic materials are especially engineered and fabricated to resemble biological structures at the chemical, physical, or morphological level. Due to their micro- and sub-micro-molecular level of matter, these materials have the potential to surpass the performance of existing materials [77,78]. Thus, utilizing bioinspired materials, such as cell membranes, proteins, exosomes, and peptides [79], is particularly valuable in biomedical applications, like tissue engineering and regenerative medicine, as well as in other fields [80]. As for IONPs, when combined with these materials, they can be used to create coated nanocarriers for drug delivery, imaging, diagnostic, and sensing systems [81].

1.4. Iron Oxide Nanoparticles Applications

Numerous research studies have demonstrated that IONPs are a promising strategy for various applications across different fields. Due to their small sizes, large surface areas, biodegradability, low toxicity, high magnetic susceptibility, superparamagnetism, and biocompatibility, IONPs can be utilized in various applications, including biomedical, environmental, data storage, glass-ceramic production, and catalysis [82-85].

Regarding biomedical applications, IONPs are utilized in diagnosis, therapy, tissue engineering, and regenerative medicine, with potential applications in anticancer, antibacterial, and cell labeling activities [82,85,86]. IONPs are generally used in MRI as a negative T_2 contrast agent because of their superparamagnetic behavior and high saturation magnetization, effectively shortening the T_2 relaxation time [87]. Magnetic hyperthermia (MH) represents a minimally invasive approach for cancer treatment, which involves IONPs, especially SPIONs, aiming to generate heat during an alternating magnetic field application [88,89]. This method potentiates direct cancer death, but also can increase the tumor's susceptibility to chemotherapy and radiotherapy, promoting a synergistic therapeutic effect. Studies emphasize that the use of SPIONs has increased selectivity and significantly reduces adverse effects compared to traditional hyperthermic or photothermal therapies [90]. IONPs are widely used in targeted drug

delivery systems and gene delivery due to their advantageous properties. They enhance drug efficiency and reduce systemic toxicity, especially as they can be directed to a specific tissue by the aid of an external magnetic field and locally release the therapeutic load through stimulus-responsive mechanisms [91,92].

IONPs can be considered an efficient antimicrobial and antifungal strategy, due to their high surface-to-area ratio, tunable surface charge, and their capacity to generate reactive oxygen species. The antimicrobial activity of IONP is generally achieved through two main mechanisms: membrane disruption, by adhering to electronegative bacterial surfaces, forming clusters that destabilize the cell wall, and oxidative stress, by generating ROS through Fenton or Fenton-like reactions that damage lipids, DNA, and proteins [93,94]. IONPs can represent an alternative to overcome antibiotic resistance, empowering their antimicrobial activity. As mentioned, IONPs have the potential to create cell damage by altering membranes and generating ROS, thus allowing more effective antibiotic penetration, particularly against multidrug-resistant strains. Literature studies have reported the successful use of iron oxide-based formulations against a broad range of Gram-negative and Gram-positive bacteria, as well as a series of fungal strains of clinical importance [95]. These results highlight the promising potential of nanoparticles to be used in therapeutic approaches for different infections.

Besides biomedical applications, IONPs have attracted significant attention due to their properties as an efficient strategy for water purification. Several mechanisms are available for binding contaminants and successfully removing them from polluted water sources [96]. IONPs can be used as sole materials, enabling remediation of fungi and bacteria in water through their intrinsic antimicrobial activity, or they can be included in composites to achieve a performant result in the removal of pesticides and other organic contaminants. In addition, when included in various matrices, IONPs endow them with magnetic separation and recyclability properties [97]. Furthermore, IONPs can also be employed in other applications, such as the manufacturing of semiconductor photocatalysts due to their chemical stability, visible light activity, and cost-effectiveness [98], or for energy storage applications, such as the manufacturing of lithium-ion batteries and supercapacitors [99].

1.5. Challenges and Future Perspectives

Despite the multitude of advantageous properties, iron oxide nanoparticles also exhibit several drawbacks that hinder their at-large utilization.

A major challenge for IONPs is their clinical translation due to the potential cytotoxic effects of these nanoparticles, which are driven by their dimensions, dose, surface coating, and chemical composition [100]. Moreover, the biodistribution of IONPs represents another major challenge, due to their high tendency to accumulate in the liver and spleen, imposing the need for more rigorous studies [101]. Another drawback of IONPs might be represented by the limited explored drug types in creating IONPs drug delivery systems. In this regard, literature can serve as a key starting point in developing such nanocarriers. However, therapeutic agents such as antibiotics, antifungals, and anti-inflammatories require detailed studies for the development of multifunctional systems [102].

Due to the lack of scalability and reproducibility of IONPs synthesis methods, they a need for technological improvement in IONPs synthesis methods. In this respect, microfluidics offers an adequate solution by providing a reproducible and tunable synthesis environment. Unfortunately, current 2D mixing designs do not offer high throughput. Thus, studies began to focus on the synthesis and functionalization of IONPs through three-dimensional microfluidic devices [103,104]. Moreover, artificial intelligence and machine learning can be introduced in adjusting the synthesis parameters to obtain optimized IONPs [101]. Therefore, a multidisciplinary approach, which incorporates technological and biological innovations alongside regulatory frameworks, will facilitate the full potential of IONPs.

1.6. Importance of the Proposed Theme

In the context of AMR, which is marked by a significant increase in deaths from infection with such strains, it is not only an emergency but also a scientific challenge and an urgent medical necessity to avoid persistent infections, prolonged hospitalizations, and increased mortality. Thus, nanotechnology is an emerging field that contributes to the discovery and development of new therapeutic opportunities, significantly reducing risks and improving the quality of medical services, and, implicitly, the quality of life for patients.

Numerous nano-sized materials are being explored for biomedical applications, their variety and versatility having no limits when thoroughly designed, combined, and functionalized to achieve specificity, enhanced biocompatibility, desirable degradability, optimal biodistribution, and non-systemic adverse effects.

Among the plethora of nanotechnological strategies, IONPs have garnered attention due to their biocompatibility, responsiveness to magnetic fields, and ease of surface functionalization, as well as their remarkable antimicrobial potential, particularly when functionalized with bioactive agents. Nonetheless, the applicability of conventionally

synthesized IONPs is often hampered by the drawbacks associated with these techniques, including operational costs, process complexity, and the need for expensive equipment, as well as the poor quality of the produced particles.

In this context, the use of microfluidic platforms to obtain IONPs represents a significant step, as this technology offers precise control over reaction conditions during synthesis (e.g., flow rate, temperature, and precursor concentration). Unlike conventional methods, microfluidic synthesis enables the production of IONPs with uniform sizes and well-defined morphologies, offering the advantages of reproducibility and large-scale synthesis of IONPs. At the same time, microfluidic platforms facilitate a rapid, continuous reaction, reduce reagent consumption, and are cost-effective.

Therefore, this thesis aims to align with international research trends and expand the knowledge in the field of microfluidic production of antimicrobial magnetite nanoparticles. In this respect, the following objectives were set and pursued:

1. Fabrication of IONPs functionalized with salicylic acid and silica on a vortex-mixing microfluidic platform and subsequent antifungal drug loading
2. Fabrication of antimicrobial hybrid iron oxide nanocomposites through a two-step process combining microfluidic-assisted synthesis and post-synthetic surface modification
3. Advanced physicochemical characterization of the synthesized materials
4. Biological investigations for evaluating the biocompatibility and antimicrobial potential of the hybrid coatings

Thus, the experimental work conducted throughout the doctoral stage highlights the promise of microfluidic synthesis methods to obtain tailored therapeutic strategies that have the potential to revolutionize the medical field and improve patient outcomes. The individual study focused on the development of nanocomposites with antibacterial and antifungal properties, utilizing IONP-based nanoparticles and coatings to improve drug delivery systems and achieve better therapeutic results.

Part II. Personal Contributions

This Ph.D. thesis examined the application of microfluidic technologies in the development of multifunctional nanocomposites for controlled drug delivery and antimicrobial coatings. The results were successfully disseminated in prestigious ISI-index journals, highlighting the potential of the on-chip fabricated materials for further biomedical applications.

The published studies have focused on the presentation of two innovative IONP-based antimicrobial strategies, with the produced materials being fabricated using a vortex-mixing 3D microfluidic platform. Specifically, the first material involved the microfluidic synthesis of magnetite nanoparticles coated with salicylic acid, followed by a second on-chip functionalization with silica under a controlled magnetic field, and further encapsulation of the drug (i.e., micafungin). The second study has also started with the simultaneous on-chip synthesis and functionalization of IONPs with salicylic acid. The nanoparticles were then modified with silver and copper oxide nanostructures, being further incorporated into silica-based aerogel coatings. The applicability of the developed materials was demonstrated by desirable drug release profiles, in the first case, and potent antimicrobial activity, in the second.

A series of advanced characterization methods were employed for evaluating the physical, chemical, and biological features of the developed nanocomposites, including X-ray diffraction (XRD), dynamic light scattering (DLS), Fourier Transform Infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM), selected area electron diffraction (SAED), RAMAN spectroscopy and confocal microscopy, Brunauer–Emmett–Teller (BET) analysis, ultraviolet-visible (UV–Vis) spectroscopy, Fourier transform ion cyclotron resonance (FT-ICR) spectrometry, MTT assay, minimum inhibitory concentration (MIC) determination, and histological studies.

2.1. Vortex-Mixing Microfluidic Fabrication of Micafungin-Loaded Magnetite–Salicylic Acid–Silica Nanocomposite with Sustained-Release Capacity





The first study addressed in the doctoral thesis explores the development of a core–shell nanocomposite system, manufactured using a microfluidic platform with vortex mixing, designed for the controlled release of the antifungal agent micafungin. Thus, a vortex microfluidic platform was used, designed with 13 layers of PMMA (Poly(methyl methacrylate)) to facilitate quick and controlled reactions.

The synthesis process involved a magnetic core represented by Fe_3O_4, which was simultaneously functionalized with salicylic acid (SA), resulting in the formation of Fe_3O_4-SA nanocomposite. Fe_3O_4-SA nanoparticles were then used as a core for growing a protective layer of silica (SiO_2). This process involved using a microfluidic platform with two neodymium magnets that generated a magnetic field of 0.3 Tesla, providing stability and orientation to the Fe_3O_4-SA nanoparticles, thereby obtaining Fe_3O_4-SA-SiO_2. The loading of micafungin into the nanocomposite was achieved using a gradual solvent evaporation method, which significantly enhanced the loading efficiency within the porous silica structure.

Compared to traditional methods, this microfluidic approach reduces reaction time, operational complexity, and resource consumption. The efficiency of micafungin as a drug delivery system and its release profile were evaluated using UV-VIS spectroscopy. In this regard, the system presented a biphasic release profile, characterized by an initial rapid release ("burst") followed by a slow, steady release over two hours. Only 50% of the drug was released during this period, which supports the potential for long-lasting antifungal therapy with reduced administration frequency. The results were also confirmed by FT-ICR-MS, which identified the intact molecular structure of micafungin in the release solution. Thus, this nanocomposite represents a promising platform for targeted and controlled release of micafungin, offering significant benefits in the treatment of persistent fungal infections and contributing to increased patient adherence to treatment.

2.2. Antimicrobial Coatings Based on Hybrid Iron Oxide Nanoparticles





The second study involved the development of hybrid nanocomposites based on Fe_3O_4 functionalized with salicylic acid, subsequently modified with silver or copper oxide nanoparticles, and then integrated into silica aerogels to obtain coatings with antimicrobial properties for biomedical applications.

Fe_3O_4 nanoparticles were obtained via microfluidic technology using a 3D vortex chip, and they were functionalized in situ with SA. After that, external layers of Ag or CuO were applied using chemical reduction and precipitation methods, resulting in hybrid nanomaterials—Fe_3O_4/SA-Ag and Fe_3O_4/SA-CuO—designed to enhance antimicrobial efficacy while improving biocompatibility. This configuration minimized the cytotoxicity of Ag and CuO by reducing metal ion leaching and minimizing direct nanoparticle-cell interactions from the Ag and CuO components.

The cytotoxicity tests performed demonstrated that both hybrid systems exhibit improved biocompatibility compared to bulk Ag and CuO nanoparticles, providing insight into the absence of a significant reduction in HaCaT cell viability at concentrations up to 200 µg/mL, as well as variable tolerance in VERO cells, depending on the formulation. Moreover, *in vivo* tests revealed the biodistribution of nanohybrids, indicating the absence of bioaccumulation in vital organs such as the brain, liver, and kidneys, which supports the systemic safety of these nanostructures. Most importantly, the lack of brain accumulation indicates that Fe_3O_4/SA nanoparticles do not cross the blood–brain barrier, confirming their potential use in systemic applications.

Regarding the antimicrobial effect, the Fe_3O_4/SA–based nanomaterials exhibited differential but effective antibacterial properties against both *Staphylococcus aureus* and *Escherichia coli*, with Fe_3O_4/SA-CuO showing the strongest inhibition, followed by Fe_3O_4/SA-Ag. At the same time, Fe_3O_4/SA alone demonstrated only mild activity, which can be attributed to the modest ROS generation by Fe_3O_4 and the intrinsic antimicrobial properties of salicylic acid.

These nanosystems were introduced in a silica-based aerogel, which served as a stabilizing scaffold, preventing nanoparticle agglomeration and ensuring uniform distribution across the coating surface. These results highlight the potential of Fe_3O_4-based hybrids in biomedical applications, with future work focusing on integrating multifunctional therapeutics.

General Conclusions

Nanotechnology has emerged as a continually evolving interdisciplinary field, offering new avenues for numerous and varied applications. Scientists worldwide have explored a wide range of nanomaterial-based strategies for improving biomedical outcomes. Being considered multifunctional agents with great potential in biomedical applications, IONPs have been reported as particularly successful platforms for drug delivery systems, possessing antimicrobial potential that can be enhanced by functionalization with bioactive agents. Thus, IONP-based nanocomposites can be obtained, evaluated, and implemented to reduce clinical risks.

Tackling the benefits of IONP-based materials, researchers have employed various synthesis methods to obtain these materials, each with distinct advantages and disadvantages, depending on the targeted application. Generally, conventional chemical and physical synthesis routes have drawbacks, including the need for bulky, expensive equipment, time-consuming procedures, environmental concerns, and poor reproducibility. On the other hand, green methods offer a more modern approach; however, it has not been fully elucidated how these techniques affect polydispersity, stability, biocompatibility, and biodistribution of nanoparticles.

To overcome existing limitations, microfluidic technology has recently been employed as a promising synthesis technique. Such platforms offer a tighter control over nanoparticle formation, leading to reproducible processes, less time-consuming operations, reduced reagent consumption, and low waste generation. Moreover, the produced nanoparticles exhibit superior quality compared to those obtained through traditional methods, displaying narrow size distribution, tunable morphologies, and controllable surface features. However, the field of microfluidics is still in development, with considerable room for improvement and exploration to expand its potential.

In this respect, the present thesis aligns with the most recent trends in microfluidics, exploring the utilization of a vortex-mixing 3D microfluidic platform for the development of

innovative and effective antimicrobial IONP-based nanocomposites. This goal has been achieved by the subsequent fulfillment of the proposed research objectives:

1. Fabrication of IONPs functionalized with salicylic acid and silica on a vortex-mixing microfluidic platform and subsequent antifungal drug loading
2. Fabrication of antimicrobial hybrid iron oxide nanocomposites through a two-step process combining microfluidic-assisted synthesis and post-synthetic surface modification
3. Advanced physicochemical characterization of the synthesized materials
4. Biological investigations for evaluating the biocompatibility and antimicrobial potential of the hybrid coatings

Thus, the experimental work conducted throughout the doctoral stage has followed the above-mentioned objectives, marking their successful accomplishment through the publication of two research papers, which are briefly summarized below.

Both studies included in this thesis offer valuable insights into the application of microfluidics platforms in the development of innovative nanocomposites for biomedical purposes. Together, these results support the use of these nanosystems in advanced antifungal and antibacterial therapies, with systemic safety and applicability in the medical field.

Thus, the present thesis introduces novelty to the field of nanomaterial synthesis through microfluidic methods, expanding knowledge in designing and fabricating antimicrobial nanoparticles with well-controlled properties and enhanced activity against relevant pathogens.

The importance of the obtained experimental results is also reflected by their publication in Web-of-Science-indexed scientific journals from the first quartile of the impact factor hierarchy. Moreover, during the doctoral stages, results have also been disseminated at international conferences, validating the novelty of proposed nanotechnological solutions and microfluidic synthesis methods.

Dissemination of Results

Articles included in the Ph.D. thesis

- **Mercan, D.-A.;** Niculescu, A.-G.; Bîrcă, A.C.; Cristea, D.-E.; Moroșan, A.; Tudorache, D.-I.; Purcăreanu, B.; Vasile, B.Ș.; Radu, D.; Grigoroscuta, M.A.; *et al*. Vortex-Mixing Microfluidic Fabrication of Micafungin-Loaded Magnetite–Salicylic Acid–Silica Nanocomposite with Sustained-Release Capacity. *Materials* **2024**, *17*, 5816. https://doi.org/10.3390/ma17235816.

- **Mercan, D.-A.;** Tudorache, D.-I.; Niculescu, A.-G.; Mogoantă, L.; Mogoşanu, G.D.; Bîrcă, A.C.; Vasile, B.Ș.; Hudiță, A.; Voinea, I.C.; Stan, M.S.; *et al*. Antimicrobial Coatings Based on Hybrid Iron Oxide Nanoparticles. *Nanomaterials* **2025**, *15*, 637. https://doi.org/10.3390/nano15090637.

Articles related to the topic, but not included in the Ph.D thesis

- **Mercan, D.-A.;** Niculescu, A.-G.; Grumezescu, A.M. Nanoparticles for Antimicrobial Agents Delivery—An Up-to-Date Review. *Int. J. Mol. Sci.* **2022**, *23*, 13862. https://doi.org/10.3390/ijms232213862.
- Grămadă (Pintilie), A.M.; **Mercan, D.-A.;** Niculescu, A.-G.; Grumezescu, A.M.; Moldoveanu, E.T.; Tudorache (Trifa), D.-I.; Balaure, P.C.; Rădulescu, M.; Alberts, A. Shifting From Traditional Antibiotics To Nanoparticle-Based Therapeutics As Promising Alternatives Against Multi-Drug-Resistant Pathogens. *U.P.B. Scientific Bulletin Series B* **2025,** *87*.

Conference participations

- Moldoveanu, E.T.; **Mercan, D.-A.;** Niculescu, A.-G.; Bîrcă, A.C.; Tudorache (Trifa), D.-I.; Vasile, B.Ș.; Hadibarata, T.; Mihaiescu, D.E.; Hudiță, A.; Rădulescu, M.; Grumezescu, A.M. Aerogel Magnetic Nanoparticle Synthesis Using Microfluidic Devices for Healthcare and Environmental Applications. *ICSM 2025 – 10th International Conference on Superconductivity and Magnetism – Session "Magnetism of Nanoparticles, Nano-Wires and Nano-Structures".* Ölüdeniz-Fethiye/Muğla, Turcia, 26 Aprilie – 3 Mai **2025**.

- Tudorache (Trifa), D.-I.; Bîrcă, A.C.; Niculescu, A.-G.; **Mercan, D.-A.;** Trușcă, R.D.; Vasile, B.Ș.; Hadibarata, T.; Mihaiescu, D.E.; Hudiță, A.; Rădulescu, M.; Grumezescu, A.M. Optimization of Magnetic Nanoparticle Synthesis Using Microfluidic Devices. *ICSM 2025 – 10th International Conference on Superconductivity and Magnetism – Session "Magnetism of Nanoparticles, Nano-Wires and Nano-Structures".* Ölüdeniz-Fethiye/Muğla, Turcia, 26 Aprilie – 3 Mai **2025.**

- **Mercan, D.-A.;** Niculescu, A.-G.; Bîrcă, A.C.; Tudorache (Trifa), D.-I.; Burdușel, A.C.; Vasile, B.Ș.; Hadibarata, T.; Mihaiescu, D.E.; Hudiță, A.; Rădulescu, M.; Grumezescu, A.M. Advanced On-Chip Synthesis and Application of Magnetic Core-Shell Nanoparticles and Aerogels for Pollution Mitigation. *ICSM 2025 – 10th International Conference on Superconductivity and Magnetism – Session "Magnetism of Nanoparticles, Nano-Wires and Nano-Structures".* Ölüdeniz-Fethiye/Muğla, Turcia, 26 Aprilie – 3 Mai **2025.**

- **Mercan, D.-A.;** Niculescu, A.-G.; Bîrcă, A.C.; Tudorache (Trifa), D.-I.; Moldoveanu, E.T.; Vasile, B.Ș.; Hadibarata, T.; Mihaiescu, D.E.; Hudiță, A.; Rădulescu, M.; Grumezescu, A.M. Novel 3D-printed microfluidic platform tested for the production of nanocomposites used in water decontamination applications. *ICSM 2025 – 10th International Conference on Superconductivity and Magnetism – Session "Magnetism*

of Nanoparticles, Nano-Wires and Nano-Structures". Ölüdeniz-Fethiye/Muğla, Turcia, 26 Aprilie – 3 Mai **2025.**

References

1. Nasrollahzadeh, M.; Sajadi, S.M.; Sajjadi, M.; Issaabadi, Z. Chapter 1 - An Introduction to Nanotechnology. In *Interface Science and Technology*, Nasrollahzadeh, M., Sajadi, S.M., Sajjadi, M., Issaabadi, Z., Atarod, M., Eds.; Elsevier: 2019; Volume 28, pp. 1-27.
2. Prasad, R.D.; Charmode, N.; Shrivastav, O.P.; Prasad, S.R.; Moghe, A.; AnantSamant, A.; Sarvalkar, P.D.; Prasad, N.R. A Review on Concept of Nanotechnology in Veterinary Medicine. *ES Food & Agroforestry* **2021**, *4*, 28-60, doi:10.30919/esfaf481.
3. Malik, S.; Muhammad, K.; Waheed, Y. Nanotechnology: A Revolution in Modern Industry. *Molecules* **2023**, *28*, doi:10.3390/molecules28020661.
4. Haleem, A.; Javaid, M.; Singh, R.P.; Rab, S.; Suman, R. Applications of nanotechnology in medical field: a brief review. *Global Health Journal* **2023**, *7*, 70-77, doi:https://doi.org/10.1016/j.glohj.2023.02.008.
5. Khan, Y.; Sadia, H.; Ali Shah, S.Z.; Khan, M.N.; Shah, A.A.; Ullah, N.; Ullah, M.F.; Bibi, H.; Bafakeeh, O.T.; Khedher, N.B.; et al. Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in Various Fields of Nanotechnology: A Review. *Catalysts* **2022**, *12*, doi:10.3390/catal12111386.
6. Satalkar, P.; Elger, B.S.; Shaw, D.M. Defining Nano, Nanotechnology and Nanomedicine: Why Should It Matter? *Science and Engineering Ethics* **2016**, *22*, 1255-1276, doi:10.1007/s11948-015-9705-6.
7. Agha, A.; Waheed, W.; Stiharu, I.; Nerguizian, V.; Destgeer, G.; Abu-Nada, E.; Alazzam, A. A review on microfluidic-assisted nanoparticle synthesis, and their applications using multiscale simulation methods. *Discover Nano* **2023**, *18*, 18, doi:10.1186/s11671-023-03792-x.
8. Zhao, X.; Bian, F.; Sun, L.; Cai, L.; Li, L.; Zhao, Y. Microfluidic Generation of Nanomaterials for Biomedical Applications. *Small* **2020**, *16*, 1901943, doi:https://doi.org/10.1002/smll.201901943.
9. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Robles Aguilar, G.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* **2022**, *399*, 629-655, doi:10.1016/S0140-6736(21)02724-0.
10. Naghavi, M.; Vollset, S.E.; Ikuta, K.S.; Swetschinski, L.R.; Gray, A.P.; Wool, E.E.; Robles Aguilar, G.; Mestrovic, T.; Smith, G.; Han, C.; et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *The Lancet* **2024**, *404*, 1199-1226, doi:10.1016/S0140-6736(24)01867-1.
11. Zhu, X.; Tang, Q.; Zhou, X.; Momeni, M.R. Antibiotic resistance and nanotechnology: A narrative review. *Microbial Pathogenesis* **2024**, *193*, 106741, doi:https://doi.org/10.1016/j.micpath.2024.106741.
12. Chakraborty, N.; Jha, D.; Roy, I.; Kumar, P.; Gaurav, S.S.; Marimuthu, K.; Ng, O.-T.; Lakshminarayanan, R.; Verma, N.K.; Gautam, H.K. Nanobiotics against antimicrobial resistance: harnessing the power of nanoscale materials and technologies. *Journal of Nanobiotechnology* **2022**, *20*, 375, doi:10.1186/s12951-022-01573-9.
13. Baabu, P.R.; Kumar, H.K.; Gumpu, M.B.; Babu K, J.; Kulandaisamy, A.J.; Rayappan, J.B. Iron Oxide Nanoparticles: A Review on the Province of Its Compounds, Properties and Biological Applications. *Materials* **2023**, *16*, doi:10.3390/ma16010059.
14. Savari, M.-N.; Jabali, A. Properties of Iron Oxide Nanoparticles (IONPs). In *Theranostic Iron-Oxide Based Nanoplatforms in Oncology: Synthesis, Metabolism, and Toxicity for Simultaneous Imaging and Therapy*, Savari, M.-N., Jabali, A., Eds.; Springer Nature Singapore: Singapore, 2023; pp. 49-65.
15. Campos, E.; Pinto, D.; Oliveira, J.; Mattos, E.; Lazzarini Dutra, R. Synthesis, Characterization and Applications of Iron Oxide Nanoparticles - a Short Review. *Journal of Aerospace Technology and Management* **2015**, *7*, 267-276, doi:10.5028/jatm.v7i3.471.
16. Nguyen, M.D.; Tran, H.-V.; Xu, S.; Lee, T.R. Fe3O4 Nanoparticles: Structures, Synthesis, Magnetic Properties, Surface Functionalization, and Emerging Applications. *Applied Sciences* **2021**, *11*, doi:10.3390/app112311301.
17. Ajinkya, N.; Yu, X.; Kaithal, P.; Luo, H.; Somani, P.; Ramakrishna, S. Magnetic Iron Oxide Nanoparticle (IONP) Synthesis to Applications: Present and Future. *Materials* **2020**, *13*, doi:10.3390/ma13204644.
18. Andronenko, S.I.; Nikolaev, A.M.; Suharzhevsky, S.M.; Sinelnikov, A.A.; Kovalenko, A.S.; Ivanova, A.G.; Shilova, O.A. Phase Composition and Magnetic Properties of Nanoparticles with Magnetite–Maghemite Structure. *Ceramics* **2023**, *6*, 1623-1631, doi:10.3390/ceramics6030099.
19. Shokrollahi, H. A review of the magnetic properties, synthesis methods and applications of maghemite. *Journal of Magnetism and Magnetic Materials* **2017**, *426*, 74-81, doi:https://doi.org/10.1016/j.jmmm.2016.11.033.

20. Wan Ahmad, W.R.; Mamat, M.; Zoolfakar, A.; Khusaimi, Z.; Rusop, M. *A review on hematite α-Fe2O3 focusing on nanostructures, synthesis methods and applications*; 2016; pp. 1-6.
21. Powell, C.D.; Lounsbury, A.W.; Fishman, Z.S.; Coonrod, C.L.; Gallagher, M.J.; Villagran, D.; Zimmerman, J.B.; Pfefferle, L.D.; Wong, M.S. Nano-structural effects on Hematite (α-Fe2O3) nanoparticle radiofrequency heating. *Nano Convergence* **2021**, *8*, 8, doi:10.1186/s40580-021-00258-7.
22. Wan, H.; Hu, L.; Liu, X.; Zhang, Y.; Chen, G.; Zhang, N.; Ma, R. Advanced hematite nanomaterials for newly emerging applications. *Chem Sci* **2023**, *14*, 2776-2798, doi:10.1039/d3sc00180f.
23. Kuchma, E.; Kubrin, S.; Soldatov, A. The Local Atomic Structure of Colloidal Superparamagnetic Iron Oxide Nanoparticles for Theranostics in Oncology. *Biomedicines* **2018**, *6*, doi:10.3390/biomedicines6030078.
24. Savari, M.-N.; Jabali, A. Superparamagnetic Iron Oxide Nanoparticle (SPION) Synthesis. In *Theranostic Iron-Oxide Based Nanoplatforms in Oncology: Synthesis, Metabolism, and Toxicity for Simultaneous Imaging and Therapy*, Savari, M.-N., Jabali, A., Eds.; Springer Nature Singapore: Singapore, 2023; pp. 35-47.
25. Ngoi, K.H.; Wong, J.C.; Chiu, W.S.; Chia, C.H.; Jin, K.S.; Kim, H.-J.; Kim, H.-C.; Ree, M. Morphological structure details, size distributions and magnetic properties of iron oxide nanoparticles. *Journal of Industrial and Engineering Chemistry* **2021**, *95*, 37-50, doi:https://doi.org/10.1016/j.jiec.2020.11.027.
26. Mihai, A.D.; Chircov, C.; Grumezescu, A.M.; Holban, A.M. Magnetite Nanoparticles and Essential Oils Systems for Advanced Antibacterial Therapies. *International Journal of Molecular Sciences* **2020**, *21*, doi:10.3390/ijms21197355.
27. Salehirozveh, M.; Dehghani, P.; Mijakovic, I. Synthesis, Functionalization, and Biomedical Applications of Iron Oxide Nanoparticles (IONPs). *Journal of Functional Biomaterials* **2024**, *15*, doi:10.3390/jfb15110340.
28. Kumar, S.; Manoj, K.; and Singh, A. Synthesis and characterization of iron oxide nanoparticles (Fe2O3, Fe3O4): a brief review. *Contemporary Physics* **2021**, *62*, 144-164, doi:10.1080/00107514.2022.2080910.
29. Jabbar, K.Q.; Barzinjy, A.A.; Hamad, S.M. Iron oxide nanoparticles: Preparation methods, functions, adsorption and coagulation/flocculation in wastewater treatment. *Environmental Nanotechnology, Monitoring & Management* **2022**, *17*, 100661, doi:https://doi.org/10.1016/j.enmm.2022.100661.
30. Muñoz-Leon, M.L.; Zubieta-Otero, L.F.; Coral, D.F.; Villaquiran-Raigoza, C.F.; Rodriguez-García, M.E. Assessing the Effectiveness of the Coprecipitation Method in Synthesizing Magnetic Nanocomposites Based on Iron Oxides Coated with Hydroxyapatite. *ACS omega* **2025**, *10*, 13797-13806, doi:10.1021/acsomega.4c05427.
31. Mao, Y.; Li, Y.; Guo, Z.; Chen, B.; Qin, Z.; Zhang, Z.; Sun, J.; Gu, N. The coprecipitation formation study of iron oxide nanoparticles with the assist of a gas/liquid mixed phase fluidic reactor. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2022**, *647*, 129107, doi:https://doi.org/10.1016/j.colsurfa.2022.129107.
32. Besenhard, M.O.; LaGrow, A.P.; Hodzic, A.; Kriechbaum, M.; Panariello, L.; Bais, G.; Loizou, K.; Damilos, S.; Margarida Cruz, M.; Thanh, N.T.K.; et al. Co-precipitation synthesis of stable iron oxide nanoparticles with NaOH: New insights and continuous production via flow chemistry. *Chemical Engineering Journal* **2020**, *399*, 125740, doi:https://doi.org/10.1016/j.cej.2020.125740.
33. Kritika; Roy, I. Therapeutic applications of magnetic nanoparticles: recent advances. *Materials Advances* **2022**, *3*, 7425-7444, doi:10.1039/D2MA00444E.
34. Antarnusa, G.; Jayanti, P.D.; Denny, Y.R.; Suherman, A. Utilization of co-precipitation method on synthesis of Fe3O4/PEG with different concentrations of PEG for biosensor applications. *Materialia* **2022**, *25*, 101525, doi:https://doi.org/10.1016/j.mtla.2022.101525.
35. Ansari, S.A.; Ficiarà, E.; Ruffinatti, F.A.; Stura, I.; Argenziano, M.; Abollino, O.; Cavalli, R.; Guiot, C.; D'Agata, F. Magnetic Iron Oxide Nanoparticles: Synthesis, Characterization and Functionalization for Biomedical Applications in the Central Nervous System. *Materials* **2019**, *12*, doi:10.3390/ma12030465.
36. Samrot, A.V.; Sahithya, C.S.; Selvarani A, J.; Purayil, S.K.; Ponnaiah, P. A review on synthesis, characterization and potential biological applications of superparamagnetic iron oxide nanoparticles. *Current Research in Green and Sustainable Chemistry* **2021**, *4*, 100042, doi:https://doi.org/10.1016/j.crgsc.2020.100042.
37. Rafienia, M.; Bigham, A.; Hassanzadeh-Tabrizi, S.A. Solvothermal Synthesis of Magnetic Spinel Ferrites. *Journal of Medical Signals & Sensors* **2018**, *8*.
38. Yousif, N.A.; Al-Jawad, S.M.; Taha, A.A.; Stamatis, H. A Review of Structure, Properties, and Chemical Synthesis of Magnetite Nanoparticles. *Journal of Applied Sciences and Nanotechnology* **2023**, *3*, 18-31, doi:10.53293/jasn.2022.5179.1178.
39. Bokov, D.; Turki Jalil, A.; Chupradit, S.; Suksatan, W.; Javed Ansari, M.; Shewael, I.H.; Valiev, G.H.; Kianfar, E. Nanomaterial by Sol-Gel Method: Synthesis and Application. *Advances in Materials Science and Engineering* **2021**, *2021*, 5102014, doi:https://doi.org/10.1155/2021/5102014.
40. Salvador, M.; Gutiérrez, G.; Noriega, S.; Moyano, A.; Blanco-López, M.C.; Matos, M. Microemulsion Synthesis of Superparamagnetic Nanoparticles for Bioapplications. *International Journal of Molecular Sciences* **2021**, *22*, doi:10.3390/ijms22010427.

41. Morán, D.; Gutiérrez, G.; Mendoza, R.; Rayner, M.; Blanco-López, C.; Matos, M. Synthesis of controlled-size starch nanoparticles and superparamagnetic starch nanocomposites by microemulsion method. *Carbohydrate Polymers* **2023**, *299*, 120223, doi:https://doi.org/10.1016/j.carbpol.2022.120223.
42. Chaudhari, D.; Panda, G. A brief overview on iron oxide nanoparticle synthesis, characterization, and applications. *Materials Today: Proceedings* **2023**, doi:https://doi.org/10.1016/j.matpr.2023.10.087.
43. Maneeratanasarn, P.; Khai, T.V.; Kim, S.Y.; Choi, B.G.; Shim, K.B. Synthesis of phase-controlled iron oxide nanoparticles by pulsed laser ablation in different liquid media. *physica status solidi (a)* **2013**, *210*, 563-569, doi:https://doi.org/10.1002/pssa.201228427.
44. Kim, M.; Osone, S.; Kim, T.; Higashi, H.; Seto, T. Synthesis of Nanoparticles by Laser Ablation: A Review. *KONA Powder and Particle Journal* **2017**, *34*, 80-90, doi:10.14356/kona.2017009.
45. Jadoun, S.; Arif, R.; Jangid, N.; Meena, R. Green synthesis of nanoparticles using plant extracts: a review. *Environmental Chemistry Letters* **2021**, *19*, pages355–374, doi:10.1007/s10311-020-01074-x.
46. Aarthye, P.; Sureshkumar, M. Green synthesis of nanomaterials: An overview. *Materials Today: Proceedings* **2021**, *47*, 907-913, doi:https://doi.org/10.1016/j.matpr.2021.04.564.
47. Khan, F.; Shariq, M.; Asif, M.; Siddiqui, M.A.; Malan, P.; Ahmad, F. Green Nanotechnology: Plant-Mediated Nanoparticle Synthesis and Application. *Nanomaterials (Basel, Switzerland)* **2022**, *12*, doi:10.3390/nano12040673.
48. Kulkarni, M.B.; Goel, S. Microfluidic devices for synthesizing nanomaterials—a review. *Nano Express* **2020**, *1*, doi:10.1088/2632-959X/abcca6.
49. Abedini-Nassab, R.; Pouryosef Miandoab, M.; Şaşmaz, M. Microfluidic Synthesis, Control, and Sensing of Magnetic Nanoparticles: A Review. *Micromachines* **2021**, *12*, doi:10.3390/mi12070768.
50. Popa, M.L.; Preda, M.D.; Neacșu, I.A.; Grumezescu, A.M.; Ginghină, O. Traditional vs. Microfluidic Synthesis of ZnO Nanoparticles. *International Journal of Molecular Sciences* **2023**, *24*, doi:10.3390/ijms24031875.
51. Wang, J.; Song, Y. Microfluidic Synthesis of Nanohybrids. *Small* **2017**, *13*, 1604084, doi:https://doi.org/10.1002/smll.201604084.
52. Hao, N.; Nie, Y.; Zhang, J.X.J. Microfluidic synthesis of functional inorganic micro-/nanoparticles and applications in biomedical engineering. *International Materials Reviews* **2018**, *63*, 461-487, doi:10.1080/09506608.2018.1434452.
53. Niculescu, A.-G.; Mihaiescu, D.E.; Grumezescu, A.M. A Review of Microfluidic Experimental Designs for Nanoparticle Synthesis. *International Journal of Molecular Sciences* **2022**, *23*, doi:10.3390/ijms23158293.
54. Amreen, K.; Goel, S. Review—Miniaturized and Microfluidic Devices for Automated Nanoparticle Synthesis. *ECS Journal of Solid State Science and Technology* **2021**, *10*, 017002, doi:10.1149/2162-8777/abdb19.
55. Liu, Y.; Yang, G.; Hui, Y.; Ranaweera, S.; Zhao, C.-X. Microfluidic Nanoparticles for Drug Delivery. *Small* **2022**, *18*, 2106580, doi:https://doi.org/10.1002/smll.202106580.
56. Fan, J.; Li, S.; Wu, Z.; Chen, Z. Chapter 3 - Diffusion and mixing in microfluidic devices. In *Microfluidics for Pharmaceutical Applications*, Santos, H.A., Liu, D., Zhang, H., Eds.; William Andrew Publishing: 2019; pp. 79-100.
57. Wu, J.; Gao, C.; Sun, D.; Yang, L.; Ye, B.; Wang, T.; Zhou, P. Thermally mediated double emulsion droplets formation in a six-way junction microfluidic device. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2023**, *661*, 130961, doi:https://doi.org/10.1016/j.colsurfa.2023.130961.
58. Guerrero, J.; Chang, Y.-W.; Fragkopoulos, A.A.; Fernandez-Nieves, A. Capillary-Based Microfluidics—Coflow, Flow-Focusing, Electro-Coflow, Drops, Jets, and Instabilities. *Small* **2020**, *16*, 1904344, doi:https://doi.org/10.1002/smll.201904344.
59. Wong, W.D.; Majnis, M.F.; Lai, C.W.; Sagadevan, S.; Muhd Julkapli, N. Enhancement of mixing and reaction efficiency of various fluids applications at different microfluidic configuration and design. *Chemical Engineering and Processing - Process Intensification* **2024**, *198*, 109729, doi:https://doi.org/10.1016/j.cep.2024.109729.
60. Yao, F.; Zhu, P.; Chen, J.; Li, S.; Sun, B.; Li, Y.; Zou, M.; Qi, X.; Liang, P.; Chen, Q. Synthesis of nanoparticles via microfluidic devices and integrated applications. *Microchimica Acta* **2023**, *190*, 256, doi:10.1007/s00604-023-05838-4.
61. Nosrati, H.; Salehiabar, M.; Davaran, S.; Ramazani, A.; Manjili, H.K.; Danafar, H. New advances strategies for surface functionalization of iron oxide magnetic nano particles (IONPs). *Research on Chemical Intermediates* **2017**, *43*, 7423-7442, doi:10.1007/s11164-017-3084-3.
62. Rizvi, M.; Gerengi, H.; Gupta, P. Functionalization of Nanomaterials: Synthesis and Characterization. In *Functionalized Nanomaterials for Corrosion Mitigation: Synthesis, Characterization, and Applications*; ACS Symposium Series; American Chemical Society: 2022; Volume 1418, pp. 1-26.
63. Turcheniuk, K.; Tarasevych, A.V.; Kukhar, V.P.; Boukherroub, R.; Szunerits, S. Recent advances in surface chemistry strategies for the fabrication of functional iron oxide based magnetic nanoparticles. *Nanoscale* **2013**, *5*, 10729-10752, doi:10.1039/C3NR04131J.

64. Kumar, Y.; Sinha, A.S.K.; Nigam, K.D.P.; Dwivedi, D.; Sangwai, J.S. Functionalized nanoparticles: Tailoring properties through surface energetics and coordination chemistry for advanced biomedical applications. *Nanoscale* **2023**, *15*, 6075-6104, doi:10.1039/D2NR07163K.
65. Ahmad, F.; Salem-Bekhit, M.M.; Khan, F.; Alshehri, S.; Khan, A.; Ghoneim, M.M.; Wu, H.F.; Taha, E.I.; Elbagory, I. Unique Properties of Surface-Functionalized Nanoparticles for Bio-Application: Functionalization Mechanisms and Importance in Application. *Nanomaterials (Basel, Switzerland)* **2022**, *12*, doi:10.3390/nano12081333.
66. Zhao, S.; Yu, X.; Qian, Y.; Chen, W.; Shen, J. Multifunctional magnetic iron oxide nanoparticles: an advanced platform for cancer theranostics. *Theranostics* **2020**, *10*, 6278-6309, doi:10.7150/thno.42564.
67. Hernández-Hernández, A.A.; Aguirre-Álvarez, G.; Cariño-Cortés, R.; Mendoza-Huizar, L.H.; Jiménez-Alvarado, R. Iron oxide nanoparticles: synthesis, functionalization, and applications in diagnosis and treatment of cancer. *Chemical Papers* **2020**, *74*, 3809-3824, doi:10.1007/s11696-020-01229-8.
68. Yildirim, T.; Pervez, M.; Li, B.; O'Reilly, R.K. Size-controlled clustering of iron oxide nanoparticles within fluorescent nanogels using LCST-driven self-assembly. *Journal of Materials Chemistry B* **2020**, *8*, 5330-5335, doi:10.1039/C9TB02868D.
69. Comanescu, C. Recent Advances in Surface Functionalization of Magnetic Nanoparticles. *Coatings* **2023**, *13*, doi:10.3390/coatings13101772.
70. Boccafoschi, F.; Fusaro, L.; Cannas, M. 15 - Immobilization of peptides on cardiovascular stent. In *Functionalised Cardiovascular Stents*, Wall, J.G., Podbielska, H., Wawrzyńska, M., Eds.; Woodhead Publishing: 2018; pp. 305-318.
71. Elkholy, A.E.A.; Poon, K.; Singh, G.; Giansiracusa, M.; Callaghan, K.L.; Boskovic, C.; Ellis, A.V.; Kingshott, P. Electrosynthesis of Silane-Modified Magnetic Nanoparticles for Efficient Lead Ion Removal. *ChemSusChem* **2025**, *18*, e202402098, doi:https://doi.org/10.1002/cssc.202402098.
72. Villa, S.; Riani, P.; Locardi, F.; Canepa, F. Functionalization of Fe3O4 NPs by Silanization: Use of Amine (APTES) and Thiol (MPTMS) Silanes and Their Physical Characterization. *Materials* **2016**, *9*, doi:10.3390/ma9100826.
73. Roto, R. Surface Modification of Fe3O4 as Magnetic Adsorbents for Recovery of Precious Metals. In *Advanced Surface Engineering Research*, Chowdhury, M.A., Ed.; IntechOpen: Rijeka, 2018.
74. García, M.C. 14 - Ionic-strength-responsive polymers for drug delivery applications. In *Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications*, Makhlouf, A.S.H., Abu-Thabit, N.Y., Eds.; Woodhead Publishing: 2019; pp. 393-409.
75. Jose, J.; Athira, V.P.; Michel, H.; Hafeela, A.R.; Bhat, S.G.; Thomas, S.; Pereira Maria, L. Chapter 1 - Hydrogels: An overview of the history, classification, principles, applications, and kinetics. In *Sustainable Hydrogels*, Thomas, S., Sharma, B., Jain, P., Shekhar, S., Eds.; Elsevier: 2023; pp. 1-22.
76. Javanbakht, S.; Shadi, M.; Mohammadian, R.; Shaabani, A.; Ghorbani, M.; Rabiee, G.; Amini, M.M. Preparation of Fe3O4@SiO2@Tannic acid double core-shell magnetic nanoparticles via the Ugi multicomponent reaction strategy as a pH-responsive co-delivery of doxorubicin and methotrexate. *Materials Chemistry and Physics* **2020**, *247*, 122857, doi:https://doi.org/10.1016/j.matchemphys.2020.122857.
77. Harun-Ur-Rashid, M.; Jahan, I.; Foyez, T.; Imran, A.B. Bio-Inspired Nanomaterials for Micro/Nanodevices: A New Era in Biomedical Applications. *Micromachines* **2023**, *14*, doi:10.3390/mi14091786.
78. Gong, Z.; Zhang, Y.; Yu, X. Review on Bio-inspired Materials with Nanotechnology Applications in Medical Devices. *Journal of Physics: Conference Series* **2021**, *1948*, 012227, doi:10.1088/1742-6596/1948/1/012227.
79. Cui, K.; Ren, F.; Yu, J.; Pan, H. Bioinspired nanomedicines for the management of osteosarcoma: Recent progress and perspectives. *Materials Today Bio* **2025**, *32*, 101607, doi:https://doi.org/10.1016/j.mtbio.2025.101607.
80. Siddiqui, M.A.S.; Rabbi, M.S.; Ahmed, R.U.; Alam, F.; Hossain, M.A.M.; Ahsan, S.; Miah, N.M. Bioinspired composite structures: A comprehensive review of natural materials, fabrication methods, and engineering applications. *Composites Part C: Open Access* **2025**, *17*, 100578, doi:https://doi.org/10.1016/j.jcomc.2025.100578.
81. Tietze, R.; Alexiou, C. SPION based nanoformulations: bio-inspired design and functionalization strategies for applications in medicine. *Precision Nanomedicine* **2022**, *5*, doi:10.33218/001c.35650.
82. de Jesus Andrade Fidelis, R.; Pires, M.; de Resende, D.S.; Costa Lima, G.F.; de Paiva, P.R.P.; Bezerra, A.C.d.S. Magnetite: Properties and applications – A review. *Journal of Magnetism and Magnetic Materials* **2025**, *614*, 172770, doi:https://doi.org/10.1016/j.jmmm.2025.172770.
83. Lakshmipriya, T.; Gopinath, S.C.B. 1 - Introduction to nanoparticles and analytical devices. In *Nanoparticles in Analytical and Medical Devices*, Gopinath, S.C.B., Gang, F., Eds.; Elsevier: 2021; pp. 1-29.
84. Ali, A.F.; Atwa, S.M.; El-Giar, E.M. 6 - Development of magnetic nanoparticles for fluoride and organic matter removal from drinking water. In *Water Purification*, Grumezescu, A.M., Ed.; Academic Press: 2017; pp. 209-262.

85. Meng, Y.Q.; Shi, Y.N.; Zhu, Y.P.; Liu, Y.Q.; Gu, L.W.; Liu, D.D.; Ma, A.; Xia, F.; Guo, Q.Y.; Xu, C.C.; et al. Recent trends in preparation and biomedical applications of iron oxide nanoparticles. *Journal of Nanobiotechnology* **2024**, *22*, 24, doi:10.1186/s12951-023-02235-0.
86. Pillarisetti, S.; Uthaman, S.; Huh, K.M.; Koh, Y.S.; Lee, S.; Park, I.-K. Multimodal Composite Iron Oxide Nanoparticles for Biomedical Applications. *Tissue Engineering and Regenerative Medicine* **2019**, *16*, 451-465, doi:10.1007/s13770-019-00218-7.
87. Lu, H.; Chen, A.; Zhang, X.; Wei, Z.; Cao, R.; Zhu, Y.; Lu, J.; Wang, Z.; Tian, L. A pH-responsive T1-T2 dual-modal MRI contrast agent for cancer imaging. *Nature Communications* **2022**, *13*, 7948, doi:10.1038/s41467-022-35655-x.
88. Dadfar, S.M.; Roemhild, K.; Drude, N.I.; von Stillfried, S.; Knüchel, R.; Kiessling, F.; Lammers, T. Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. *Advanced drug delivery reviews* **2019**, *138*, 302-325, doi:10.1016/j.addr.2019.01.005.
89. Farzanegan, Z.; Tahmasbi, M. Evaluating the applications and effectiveness of magnetic nanoparticle-based hyperthermia for cancer treatment: A systematic review. *Applied Radiation and Isotopes* **2023**, *198*, 110873, doi:https://doi.org/10.1016/j.apradiso.2023.110873.
90. Pucci, C.; Degl'Innocenti, A.; Belenli Gümüş, M.; Ciofani, G. Superparamagnetic iron oxide nanoparticles for magnetic hyperthermia: recent advancements, molecular effects, and future directions in the omics era. *Biomaterials Science* **2022**, *10*, 2103-2121, doi:10.1039/D1BM01963E.
91. Alphandéry, E. Iron oxide nanoparticles for therapeutic applications. *Drug Discovery Today* **2020**, *25*, 141-149, doi:https://doi.org/10.1016/j.drudis.2019.09.020.
92. Ahmadi, M. 16 - Iron oxide nanoparticles for delivery purposes. In *Nanoengineered Biomaterials for Advanced Drug Delivery*, Mozafari, M., Ed.; Elsevier: 2020; pp. 373-393.
93. Shoudho, K.N.; Uddin, S.; Rumon, M.M.H.; Shakil, M.S. Influence of Physicochemical Properties of Iron Oxide Nanoparticles on Their Antibacterial Activity. *ACS omega* **2024**, *9*, 33303-33334, doi:10.1021/acsomega.4c02822.
94. Gudkov, S.V.; Burmistrov, D.E.; Serov, D.A.; Rebezov, M.B.; Semenova, A.A.; Lisitsyn, A.B. Do Iron Oxide Nanoparticles Have Significant Antibacterial Properties? *Antibiotics (Basel, Switzerland)* **2021**, *10*, doi:10.3390/antibiotics10070884.
95. Rumyantceva, V.; Rumyantceva, V.; Andreeva, Y.; Tsvetikova, S.; Radaev, A.; Vishnevskaya, M.; Vinogradov, V.; Drozdov, A.S.; Koshel, E. Magnetically Controlled Carbonate Nanocomposite with Ciprofloxacin for Biofilm Eradication. *International Journal of Molecular Sciences* **2021**, *22*, doi:10.3390/ijms22126187.
96. Aragaw, T.A.; Bogale, F.M.; Aragaw, B.A. Iron-based nanoparticles in wastewater treatment: A review on synthesis methods, applications, and removal mechanisms. *Journal of Saudi Chemical Society* **2021**, *25*, 101280, doi:https://doi.org/10.1016/j.jscs.2021.101280.
97. Furlan, P.Y.; Fisher, A.J.; Furlan, A.Y.; Melcer, M.E.; Shinn, D.W.; Warren, J.B. Magnetically Recoverable and Reusable Antimicrobial Nanocomposite Based on Activated Carbon, Magnetite Nanoparticles, and Silver Nanoparticles for Water Disinfection. *Inventions* **2017**, *2*, doi:10.3390/inventions2020010.
98. Tabasum, N.; Saeed, A.; Shafiq, R.; Khan, B.S.; Bashir, M.; Yousaf, M.; Rafiq, S.; Shaik, M.R.; Khan, M.; Alwarthan, A.; et al. Synthesis of Iron Oxide Nanoparticles via Atmospheric Pressure Microplasma for High-Performance Energy Storage and Environmental Applications. *Catalysts* **2025**, *15*, doi:10.3390/catal15050444.
99. Tanaka, S.; Kaneti, Y.V.; Septiani, N.L.W.; Dou, S.X.; Bando, Y.; Hossain, M.S.A.; Kim, J.; Yamauchi, Y. A Review on Iron Oxide-Based Nanoarchitectures for Biomedical, Energy Storage, and Environmental Applications. *Small Methods* **2019**, *3*, 1800512, doi:https://doi.org/10.1002/smtd.201800512.
100. Wu, L.; Wen, W.; Wang, X.; Huang, D.; Cao, J.; Qi, X.; Shen, S. Ultrasmall iron oxide nanoparticles cause significant toxicity by specifically inducing acute oxidative stress to multiple organs. *Particle and Fibre Toxicology* **2022**, *19*, 24, doi:10.1186/s12989-022-00465-y.
101. Zúñiga-Miranda, J.; Guerra, J.; Mueller, A.; Mayorga-Ramos, A.; Carrera-Pacheco, S.E.; Barba-Ostria, C.; Heredia-Moya, J.; Guamán, L.P. Iron Oxide Nanoparticles: Green Synthesis and Their Antimicrobial Activity. *Nanomaterials (Basel, Switzerland)* **2023**, *13*, doi:10.3390/nano13222919.
102. Arias, L.S.; Pessan, J.P.; Vieira, A.P.; Lima, T.M.; Delbem, A.C.; Monteiro, D.R. Iron Oxide Nanoparticles for Biomedical Applications: A Perspective on Synthesis, Drugs, Antimicrobial Activity, and Toxicity. *Antibiotics* **2018**, *7*, doi:10.3390/antibiotics7020046.
103. Niculescu, A.; Alexandra Catalina, B.; Moroşan, A.; Oprea, O.; Vasile, B.; Purcareanu, B.; Mihaiescu, D.; Grumezescu, A. SIMULTANEOUS MICROFLUIDIC SYNTHESIS AND SILANIZATION OF IRON OXIDE NANOPARTICLES. *UPB Scientific Bulletin, Series B: Chemistry and Materials Science* **2025**, *87*, 2025.
104. James, M.; Revia, R.A.; Stephen, Z.; Zhang, M. Microfluidic Synthesis of Iron Oxide Nanoparticles. *Nanomaterials (Basel, Switzerland)* **2020**, *10*, doi:10.3390/nano10112113.