



Synthesis and Characterization of Biogenic Iron Sulfide Nanoparticles in Cancer and Other Biomedical Applications: A Review

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Abstract

This review focuses on recent breakthroughs in the physicochemical approaches and biological approaches of metallic iron, iron sulfides, and iron oxide nanoparticles for a variety of applications. The range of kinds, topologies, and physicochemical features of nano-sized iron sulfides has piqued researchers' curiosity. Furthermore, this study examines the medicinal, environmental, and technical uses of biogenically synthesized NPs, as well as the hurdles that must be overcome in order to optimize the environmentally friendly production of these critical nanoparticles. FeS NPs have a good effect on biological activity due to their ease of production, magnetic properties, biocompatibility, and biodegradability. Also canvassed are the FeS NPs nanoparticle-specific biomedical applications in cancer treatments. The goal of this review is to discuss the synthesis, characteristics, and uses of nano sized FeS NPs in biomedical domains, revealing that they have significant promise for enhancing human health and excellence of life.

Keywords: Iron Sulfides; Surface Modification; Biocompatibility; Cancer Therapy; Photothermal Therapy; Biosensors; Antifungal Agents; Enzyme-Like Catalysis

Introduction

Nanoscience can be explained as the study of the property of matter and structure on a nanoscale between 1 -100 nm. The several properties of the matter like interfacial and surface chemistry useful in nanotechnology's applications. The high surface-to-volume ratio useful in catalysis, it enhances the chemical properties of nanomaterials. Mechanical properties boost the strength hardness in lightweight nanomaterial and Nanocomposites [1]. It purely depends on the unique and size-dependent property of solid matter.

The biomedical nanotechnology is an interdisciplinary field of study that spans health, science and engineering. It also has a

wide variety of molecular diagnostic, molecular imaging, and targeted treatment applications. The amalgamation of nanotechnology with molecular biology has begotten a new research area or sector that transforms biomedical research by providing specific imaging techniques, nano-robotics, and nano-devices, etc [2-3]. Nanomaterials with a spherical shape are very useful in the field of nanomedicine as they are similar in dimension to biological macromolecules, and smaller than cellular organelles. The nano-sized particles such as iron oxide and quantum dots have magnetically, optically, or structural properties that differ from the bulk materials. These biocompatible nanoparticles have a high affinity and selectivity for sick cells and organ-like malignant tumors. Nanopar-

ticles can also be linked with bio-targeting ligands like peptides and monoclonal antibodies. They have a variety of advantages for medical applications, including high surface areas and functional groups, cross-linking to various diagnostic and therapeutic agents such as magnetic, radio-isotopic, and optical, and encapsulation of bioactive agents such as fluorophores and drugs, among others. And quantum properties, which are used for conjugating multiple diagnostics like magnetic, radio isotropic and optical, and therapeutic agents. Because of the enhanced permeability effect (EPR), the nanomaterial (< 100 nm) has the capacity to acquire and gather tumor locations, which aids in early tumor identification and treatment. After the interaction, they should maintain their physical properties after modification and remain non-toxic. Nanomaterials have unique properties that show their ultra-small dimension. And it is designed to assist the transport of diagnostic or therapeutic agents through biological barriers to acquire access within cells. And arbitrate molecular interactions and trace molecular changes in an intuitive and out-turn manner. The main applications of nanomaterials in biology are drug delivery [4-5], bio-detection of pathogens [6], fluorescent and biological labelling, probing of DNA structure [7], tissue engineering [8,9], tumor ruination by hyperthermia [10], MRI contrast enhancement [11], detection of protein [12], etc.

metals have sparked a lot of attention recently, and their characteristics [13] have led to their usage in superconductors [14], magnetic semiconductors [15], photovoltaics [16], sensors [17], thermoelectrics, optoelectronic behavior, their indirect bandgaps, and the catalysts might absorb visible light, which is plentiful in solar radiation. Iron chalcogenides have been identified as an important and promising nanomaterial among the transition metal chalcogenides [17-23]. The structural features, semiconducting characteristics, magnetic characteristics, and biocompatibility are all reasons for this interest [18-24]. They have also been employed in biological applications including antibacterial activity [24], magnetic resonance imaging (MRI), photothermal therapy, magnetically guided drug delivery, and biocatalysis [21] based on these features. Other benefits of iron chalcogenides are their ease of production and ultra-small size [25]. There are several types of iron sulfide, including mackinawite (FeS), troilite (FeS), greigite (Fe₃S₄), pyrrhotite (Fe_{1-x}S, frequently Fe₇S₈ and Fe₈S₉), and pyrite is a mineral that is found in cubic form (FeS₂). While some exclusively have Fe (II), others, like greigite, have both Fe (II) and Fe (III). Based on its characteristics, the iron sulfide (FeS) nanostructure has a greater potential for biological applications [23,25]. Because of their biological action, xanthene derivatives are extremely essential molecules in medicine. They are also used as dye components in lasers, pigments, and fluorescent biomolecule detection systems. Because of the pyran ring, 9-aryl-1, 8-dioxooctahydroxanthenes, and 14-substituted-14H dibenzoxanthene derivatives are found in a variety of natural compounds and are employed as multimodal synthons [25].

FeS nanoparticles (NPs) are being used in biological and catalysts for organic synthesis and many more applications such as the interactions of FeS NPs with proteins and enzyme is critical. In biological processes, proteins and enzymes play a crucial role [26]. FeS is an innocuous, widely distributed mineral that acts as a precursor to the more stable iron FeS minerals greigite and pyrite. As a consequence, chlorinated organic compounds (COCs), heavy metals, arsenic, selenium, and other inorganic and organic contaminants in groundwater and soil have been remedied using FeS. Because they combine with Hg to produce an insoluble product, HgS, many naturally occurring sulfide minerals have been identified as the primary sink for Hg in the atmosphere. Previous research suggested that adsorption, inclusion, precipitation, and surface complexation might be involved in the interaction between Hg (II) and FeS. Furthermore, FeS nanoparticles were produced and proven

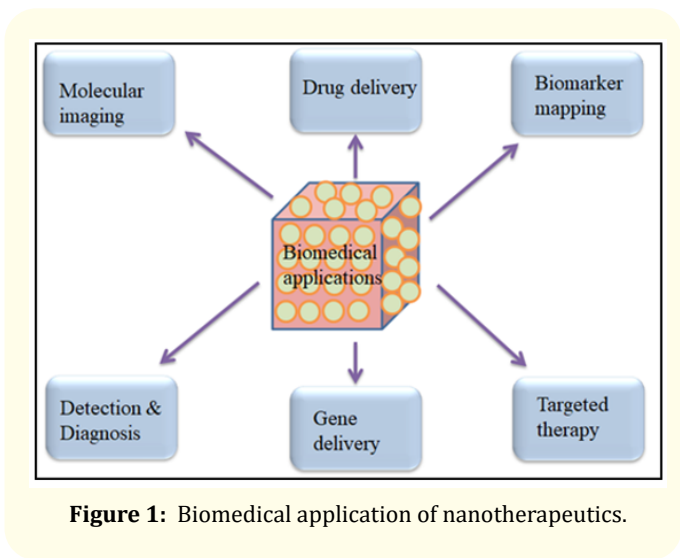


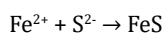
Figure 1: Biomedical application of nanotherapeutics.

Metallic nanoparticles have attracted a lot of interest in the past few years because of their unusual physical, chemical, and biological features, which are linked to their large surface-to-volume ratio and quantum confinement effects. Chalcogenides of transition

to be efficient in collecting Hg, demonstrating a much higher sorption capacity than that of the other sorbents FeS NPs are smaller, have a larger specific surface area, have more active sorption sites, and stronger surface reactivity. FeS particles are nanoscale materials are projected to provide increased soil deliverability and adsorption capacity. They have a tendency to clump together and develop fast to micrometer or millimeter scale particles, reducing their mobility and chemical responsiveness. Previous investigation has shown that carboxymethyl cellulose (CMC), an improved natural polysaccharide, may efficiently stabilize FeS NPs via steric hindrance and electrostatic repulsion, resulting in very stable CMC-FeS nanoparticles [27].

Iron sulfide nanoparticle synthesis

There are two types of synthetic techniques for FeS nanostructured materials and conventional production techniques for bare nano-sized FeS particles; physicochemical approaches and biological approaches. Chemical precipitation is the standard approach for making bare nanoscale FeS. It proposes that FeS nanoparticles might be produced by co-precipitating Fe^{2+} and S^{2-} ions in an aqueous phase under anaerobic circumstances while stirring constantly [28]. Iron (Fe^{2+}) source precursors include iron sulfate heptahydrate, ferrous chloride (FeCl_2), and ammonium ferrous sulfate while thiourea ($\text{CH}_4\text{N}_2\text{S}$), Sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) and sodium sulfide ($\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$) were used to provide S^{2-} ions. The co-precipitation of FeS in the aqueous phase under anoxic conditions is the most frequent traditional physicochemical technique.



FeS NPs synthesize by reacting the ammonium ferrous sulfate (Mohr's salt) with sodium sulfide at room temperature under N_2 atmosphere, and a black precipitate formed.

FeS NPs can be synthesized by the biosynthesis method (biological process), recent studies have lots of interest due to its "green synthesis" and environmentally friendly nature. Sulfate-reducing bacteria (SRB) may create sulfide by using sulfur species (Sulphate, thiosulfate, etc.) as a terminal electron acceptor. Ferric reducing bacteria (FRB) reduce Fe^{3+} ions; and S^{2-} ions and Fe^{2+} ions respectively to produce FeS NPs [29-31]. FeS NPs are produced by the *Acidiphilium cryptum* JF-5 and SRB (*Desulfovibrio vulgaris mizakii*), and it is black in color and its XRD pattern resembles the mackinawite ($\text{FeS}_{0.9}$) [32]. Moreover, FeS nanoparticles synthesized

by *Shewanella* species; can instantaneously reduce Fe^{3+} ions, and sulfur works as a terminal electron acceptor when lactate worked as an electron acceptor [33].

Microwave-assisted procedures have several gains over traditional heating methods, including decreased time of reaction, narrower nanoparticle size dispersion, and greater pureness. Ethylene glycol (CH_2OH)₂ is an excellent solvent for microwave-assisted technique because of its large dipole moment. iron sulfate heptahydrate, PVP-K30, and sulfur (S) in ethylene glycol can be microwaved in a nitrogen (N_2) gas environment to make FeS/ FeS_2 microspherulites [34]. Although this new methodology appears to be more desired, the clump phenomena do not seem to have improved.

Bala and co-workers designated the conventional sonochemical approaches to synthesize the FeS nanoparticles. To begin, $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ was dissolved in distilled water (DW). And then, ferrous sulfate ($\text{FeSO}_4\cdot 7\text{H}_2\text{O}$) was dissolved separately in DW; and a 1:1 mole ratio of polyethylene glycol while the sodium sulfide solution was being continuously sonicated for 30 minutes, Triton-X surfactant was added dropwise to the solution. After adding PVP, the solution was stirred for another 30 min using an ultrasound [35,36].

Synthesis of the modified FeS nanoparticles

To generate FeS nanoparticles with normalized particle shape and size distribution, several particle stabilizing approaches have been investigated. SRB-assisted manufacturing, Reverse micelle, polymer-stabilized wet-chemical synthesis, high-energy mechanical milling, and poly(amidoamine) dendrimer stabilization are among these methods. The latest research has demonstrated that adding polymeric stabilizers to the FeS particle manufacturing process, such as starch and CMC, can help with size control in aqueous solutions. Using CMC as a stabilizer, a unique class of stabilized FeS nanoparticles has been developed. This may efficiently regulate the nucleation and progress of nanoparticles by combining electrostatic repulsion and steric hindrance. A stabilizer called CMC or chitosan was utilized to prevent the FeS NPs from aggregating, and these fabricated nanoparticles performed well in dispersion. FeS NPs have a tendency to clump together and develop fast to micrometer or millimeter scale particles, reducing their mobility and chemical responsiveness. In addition to CMC, several macromolecular biomaterials with similar physicochemical characteristics have sparked attention, including cyclodextrin (CD), starch, and polysaccharide sodium alginate (SA) [34,37,38].

Applications of FeS nanoparticles

Photothermal therapy

Photothermal therapy (PTT) is a non-invasive therapeutic method that has recently received a lot of interest [39,40]. Photo-absorbing nanoparticles generate heat through uninterrupted laser irradiation, killing cancer cells while leaving healthy tissue untouched [41]. Imaging-guided PTT, for example, might not only show sizes, tumor locations, and forms to guarantee that tumors are effectively exposed to the laser during therapy, but it could also indicate the ideal moment for laser irradiation when the photothermal agent reaches a top-level in the tumor. As a consequence, the photothermal characteristics of iron sulfide (FeS) must be taken into consideration. The near-infrared absorption of FeS nanoparticles that have been PEGylated (FeS-PEG) is quite high. The multi-enzyme activities, severe environmental resistance, storage strength, and vital advantages of nanomaterials are shown by infrared thermal imaging, suggesting that excellent replacements for natural enzymes are possible. Iron sulfide's enzymatic activity has gotten a lot of attention. The peroxidase-like activity was discovered in FeS nanosheets in 2010. In the existence of H_2O_2 , FeS suspensions were demonstrated to catalyze the oxidation of peroxidase substrates TMB (3, 3', 5, 5'-Tetramethylbenzidine) to yield a blue color substance [42]. FeS-PEG outperforms other known iron oxides in terms of photothermal transformation efficiency [43].

Enzyme-like catalysis

FeS clusters are required as cofactors by several proteins and enzymes that execute redox reactions and control oxidative stress. As a result, FeS nanoparticles are projected to serve as enzymes and conduct comparable catalysis. Iron sulfide has been demonstrated to successfully activate peroxymonosulfate (PMS, HSO_5^-) or persulfate (PS, $S_2O_8^{2-}$) to create sulfate radicals in previous research [44]. Since the discovery of intrinsic peroxidase activity in iron oxide nanoparticles in 2007 [45], it has been assumed that iron sulfide possesses comparable capabilities. Iron sulfide catalyzes a variety of reactions such as high catalytic activity, extreme environmental resistance; multi-enzyme activities, storage stability, and the inherent benefits of nanomaterials all contribute to the advancement of biomedicine, implying that good substitutes for natural enzymes exist. Iron sulfide's enzymatic activity has been extensively studied. FeS nanosheets were discovered to have peroxidase-like activity in 2010.

In 2020, it was observed that light irradiation of FeS NPs might increase intrabacterial ROS levels in bacteria. This was discovered to be FeS NPs' principal antibacterial mechanism [24].

Cancer therapy

Iron is a vital nutrient that affects a variety of bodily activities, including energy metabolism, hematopoiesis, oxygen metabolism, muscular function, and more. On the other side, iron is important in the development of cancer and cancer-related disorders. Iron is a potent driver of cancer development, invasion, and metastasis in previous research [46]. Furthermore, iron homeostasis is frequently disrupted in cancer patients, making iron deficiency anemia a common consequence. However, in terms of iron speciation, cancer-related iron distortion is not entirely understood [47,48]. The ferrous-sulfide-like iron and ferritin chemical forms of iron identified in the tumors suggest that iron sulfide might be utilized to treat cancer [49]. FeS NPs are synthesized by the co-precipitation method and are used in hyperthermia, opening up new possibilities for multimodal anticancer treatments [50]. High dosages of FeS are harmless and efficacious in mice using PEGylated FeS (FeS-PEG) NPs as nanoagents for in-vivo MRI-targeted photothermal cancer therapy. FeS might be used in the clinical for MRI as well as PTT, according to the study [51]. The high effectiveness of iron sulfide nanosheets for MRI-targeted photothermal and chemodynamic synergistic therapy has paved the way for future clinical trials of inorganic iron sulfide. Due to the localized heat generated by PTT (synergistic photothermal therapy) from the defect-rich structure, which could cause overproduction of H_2O_2 by the Fenton process, and the formed $\cdot OH$ could hinder tumor growth and reappearance after PPT, Fe_3S_4 TNSs have high proficiency for MRI guidance and can achieve CDT (chemodynamic therapy) and PTT. As a consequence, we were able to create a high-efficiency inorganic theranostic platform that was effectively evacuated from the body. This will open the path for the future development of inorganic substances like iron sulfide for medicinal purposes [52]. FeS-Dox@bLf NZs were shown to be not only effective in treating and managing breast cancer with minimum adverse effects *in vitro* and *in vivo*, but also to give a clear method to synchronize diverse treatments, including chemotherapy, PDT, and so on [53].

Biosensors

The FeS nanostructure holds the scope for increased biosensing and biocatalysis applications. The sheet-like FeS nanostructures'

capacity to activate the oxidation of organic compounds to create a color shift is backed up by their appealing functionality of an in-built peroxidase-like activity. This H_2O_2 sensor was also more sensitive than others that used spherical FeS nanoparticles. Compared to the HRP, the mimic peroxidase was more stable when unprotected to high temperatures and pH extremes. The sheet-like FeS has an extensive variety of potential accomplishments in biotechnology, biosensors, and environmental chemistry due to its high stability, simplicity of manufacture, and distinctive characteristics. According to the researchers, the unique sheet-like FeS nanosheets worked as an “artificial peroxidase” in the growth of amperometric transducers and biocatalysts. Because of their natural peroxidase-like activity, FeS has been employed as glucose sensors. Colorimetric techniques, in which cascade reactions constitute the primary mechanism of glucose detection [54], can be used to create glucose sensors.

Antifungal agents

In in-vitro conditions, iron sulfide NPs show antifungal properties against *F. verticillioides*; at a considerably lower dosage than the conventional fungicide, FeS-NPs considerably suppressed the development of the test fungus. When the concentration was increased from 10 to 30 $\mu\text{g/ml}$, radial growth inhibition increased gradually. FeS-NPs have the potential to be employed as a future substitute to high-dose organic fungicides as ecologically acceptable seed priming antifungal agents, exclusively in iron-deficient soils [55].

Conclusion

This study focuses on current advancements in environmentally friendly inorganic NPs used for biomedical applications. Although significant progress has been made in the regulated biogenic production of metallic NPs, there are still certain obstacles to overcome. In the foreseeable future, precise control of particle size, morphological form, and monodispersity is still seen as a critical challenge to address. Due to its high photothermal and magnetic performance, nano-sized iron sulfide has also gained interest due to its large potential in-vivo use, notably in cancer treatment. In summary, we have presented the most current techniques of nano-iron sulfide production, including nano-iron sulfide alterations and characterizations. Remarkably, nano-sized iron sulfides display varied physiochemical characteristics, enzyme-like catalysis, excel-

lent stability, and cytocompatibility, which assist their biological applications. A spectrum of nano-iron sulfides has been tested in catalysis, tumor treatment, antibiotic ointments and antifungals, biosensors, and in plants. Furthermore, studying multimodal FeS NPs characteristics in a live organism might lead to improved disease detection and therapy in the future.

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Conflict of Interest

The authors state that they have no financial or other conflicts of interest.

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