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Green-biosynthesis of iron nanoparticles, and its application as an antimicrobial agent

Hassanain Jwad Abidalhussein[1]*, Wejdan Ridha Taj-Aldeen[2]

Abstract

With the progress of science, interest has become in linking modern science with the nano-aspect and the application of nanotechnology in all fields of biological science and medicine, among the most important of which is the biosynthesis of nanoparticles and their application as antimicrobials. In our current research, iron nanoparticles (Fe NPs) were synthesized by using oyster shell as a raw material. oyster shell converted into powder after purification, drying and grinding, and immersing it in de-ionized water in order to be ready for responding through its mixture with hydrochloric acid, as a first step for this synthesis process. The adding of sodium hydroxide is the step with the continuation of the process to extract the iron nanoparticles (Fe NPs) from the oyster shell powder through using a hot plate magnetic stirrer devise (1000 rpm, 50 C). The standard properties of iron nanoparticles were confirmed by using X-ray diffraction (XRD), scanning electron microscopy (SEM), and (FTIR)-based analysis, they were applied as an antibacterial agent that inhibits the action of bacteria. Objective: The objective of our research is to apply the biosynthesize of iron nanoparticles (Fe NPs) as an antimicrobial agent through a serious of steps, starting by the oyster shells grinding within standard specifications that allow them to perform their role as antibiotics. Methods: The working methods includes the collection of oyster shells, then they transformed to powder. The extract of iron nanoparticles (Fe NPs) from oyster shells includes a set of steps interspersed with the break the bonds that bind the basic components of the oyster shell components by using HCL, and NaOH solution. Results and Characterization: The results performed using the (SPSS) statistical program, and the particle sizes of iron nanoparticles (Fe NPs) are calculated by using XRD crystallite (grain) calculator (Scherrer Equation). The characterization of (Fe NPs) are performed by using (X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and FTIR-Based analysis), it gave particles of acceptable size ranging between (30-90) nanometers. Conclusion: We conclude that the biosynthesis of nanoparticles is the successful way to obtain and apply them as an antimicrobial, instead of using antibiotics that are made from chemicals in unsafe and environmentally friendly ways.

Keywords:

Green biosynthesis, Iron nanoparticles, Antimicrobial agent.

[1] Dentistry Collage, Karbala University, Kerbala, IRAQ,
[2]Collage of Science, Babylon University, Babile, IRAQ.

Address for correspondence:
Hassanain Jwad Abidalhussein,
Dentistry Collage, Karbala University, Kerbala, IRAQ.
E-mail: hesinen.J@uokerbala.edu.iq

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Introduction

The sizes of iron nanoparticles ranges between (1-100 nanometers), and they represent iron metal, and they have a high reactive nature, due to their large surface area exposed to the outside in relation to the mass, and they have the ability to quickly oxidize to form free iron ions. It has a wide spectrum in many medical, biological and environmental applications, in which its prominent and clear role is evident in the treatment of pollution resulting from chlorinated organic compounds. There are also other areas of high-tech applications using nano-materials, which has made nanotechnology so important that it has become one of the largest areas of active research [1]. Many studies reported magnetic iron functions represented by its role in functionalized with polymers, amino acids, and biomolecules [2].

The synthesized nanoparticles can determine their biological activity as well as control it to a large extent, and this is done through the biological source used as the green materials used by stabilizing and reducing the ions of certain types of minerals used in this synthesis process.

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Among the most important and optimal properties of iron nanoparticles is their ability to achieve necessary targets, whether applying them as therapeutics or antimicrobial agents, or using them as a means to eliminate pathogens in mammalian cells [3].

The uses of iron nanoparticles are wide in the medical, biological and other fields. There are other applications for iron nanoparticles, for example, iron oxide nanoparticles are used in the treatment of cancer, drug delivery to target tissues or cells, in addition to monitoring and following up on tumor development, and removing all toxins from biological fluids.

There are several methods to synthesis of metal nanoparticles each of these methods has its own characteristics that distinguish it from other methods, in addition to that each method of manufacturing iron nanoparticles has its own specific mechanisms that may differ among them depending on the specifications to be obtained in those particles. The mechanism for the synthesis of iron nanoparticles is not entirely clear, as it has been documented under the same pathway in the synthesis of NPs of zero valent iron and iron oxides as well as mixtures of both [4].

The method of biological manufacture of iron nanoparticles can be carried out with several aspects, as it may be manufactured from plants, fungi, bacteria, and other organisms that can thus give nucleated iron particles after performing a number of necessary procedures for that. Many experiments have been carried out in order to extract or synthesize iron nanoparticles. Some of these experiments were conducted on oyster shells, as they contain a number of different minerals, including iron[5]. The nanoparticles that are produced by green synthesis have a specific size that can be well controlled and can be controlled, in addition to being free of pollutants, and easy to scale, and there are many additional benefits and applications of green synthesis [6].

Experimental aspect

Materials

Heat plate magnetic stirrer device (Heidolph-MR 3001\KMR3001K Magnetic-Stirring Hotplate), SCALTEC-model SBC series, deionized water (di. D.W), oyster shell powder, (HCL, NaOH) solution, filter paper. X-ray diffraction (XRD), Broker company\Germany, Fourier Transform Infrared (FTIR), Perkin-Elmer1725x\Japan, Scanning-electron microscope (SEM)\FEI\Netherland, pH-meter Orient \USA, Refrigerator-Beko\Korea, Fourier Transform Infrared (FTIR)-Perkin-Elmer\1725xJapan.

Methods

Green biosynthesis of iron nanoparticles methods include washing, drying and grinding the oyster shell [7], then (100ml) of deionized water is added to (100gm) of powder, this step followed by the use of heat plate magnetic stirrer device. Then (40 gm) of (NaOH) with (400 ml) of (di D.W) were added to the remaining powder that resulted from the filtration process in previous step.

The mixture was shaken by the magnetic stirrer device for about (4 hours), and (Fe NPs) were extracted by using shaker device. The collection of the iron nanoparticles was examined by XRD, SEM, FTIR analysis.

Iron nanoparticles characterizations

X-ray Diffraction examination of (Fe NPs)

Biosynthesized iron-nanoparticles were subjected to the x-ray determination (XRD-examination) as (Smart-Lab SE., Rigaku-Tokyo\Japan) for determination of its nature as well as average size of the iron NPs, as shown in figure no.1. So, the crystallite sizes were calculated by using Scherrer-Eqn., and the average of size was equal to (60 nm).

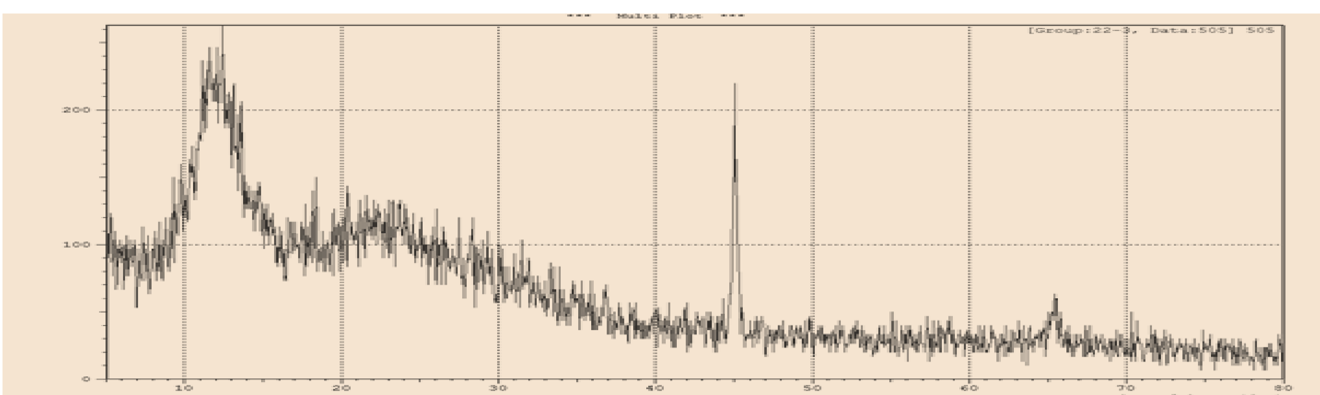

Figure 1: XRD of iron nanoparticles synthesis. there is a normal distribution in the sizes of (Fe-NPs).

Scanning Electron Microscopy (SEM)

(SEM) was according to (JSM-IT\500, Jeol-Boston, MA\USA), which used for the examination of the nanoparticle size of iron.

The dry particles resulted from this green biosynthesis method, diluted with deionized water at a ratio of (10:1 g/ml). These nanoparticles were examined by (SEM-analysis) according to the (National Institute of

Standards and Technology, NIST \2007) [8], as shown in figure (2). It suggests that the study used SEM to characterize the size of iron nanoparticles synthesized through a green method.

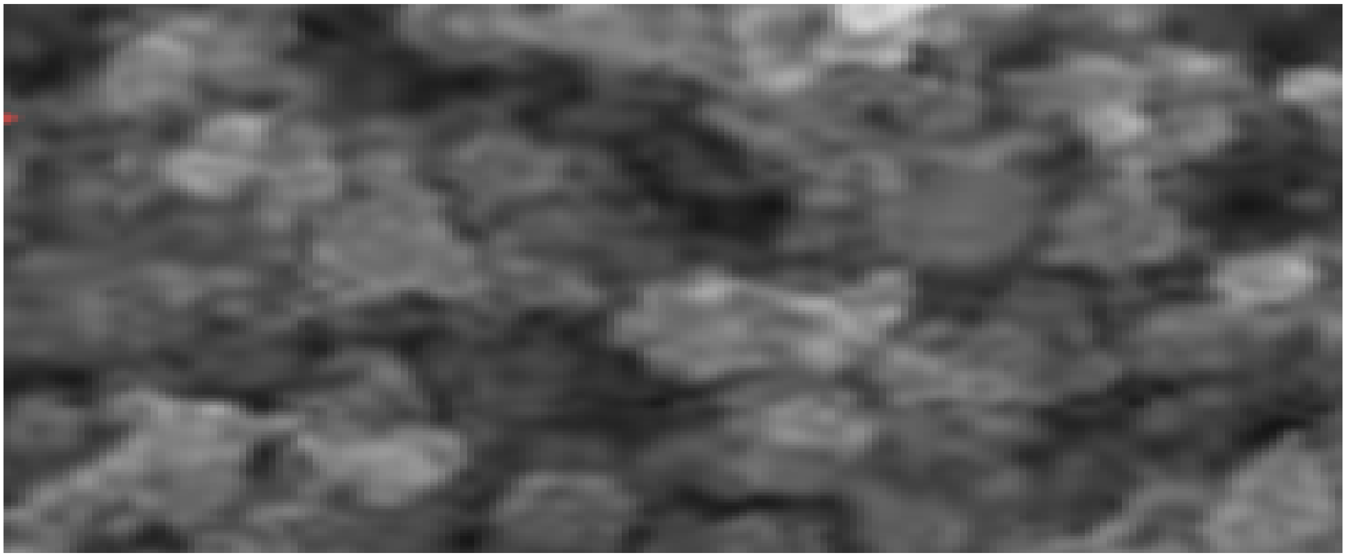

Figure (2): (SEM)-image of (FeNPs)

FTIR-Based examination of (FeNPs)

FTIR- technique used for obtaining an infrared-spectrum of absorption\emission of a solid, liquid, or gas. FTIR-analysis simultaneously collects high resolution spectral-data over high spectral average. FTIR-analysis of (FeNPs) identified by using FTIR-spectroscopy (S\700, Nicolet-MA\USA) [9], as shown in **figure (3)**.

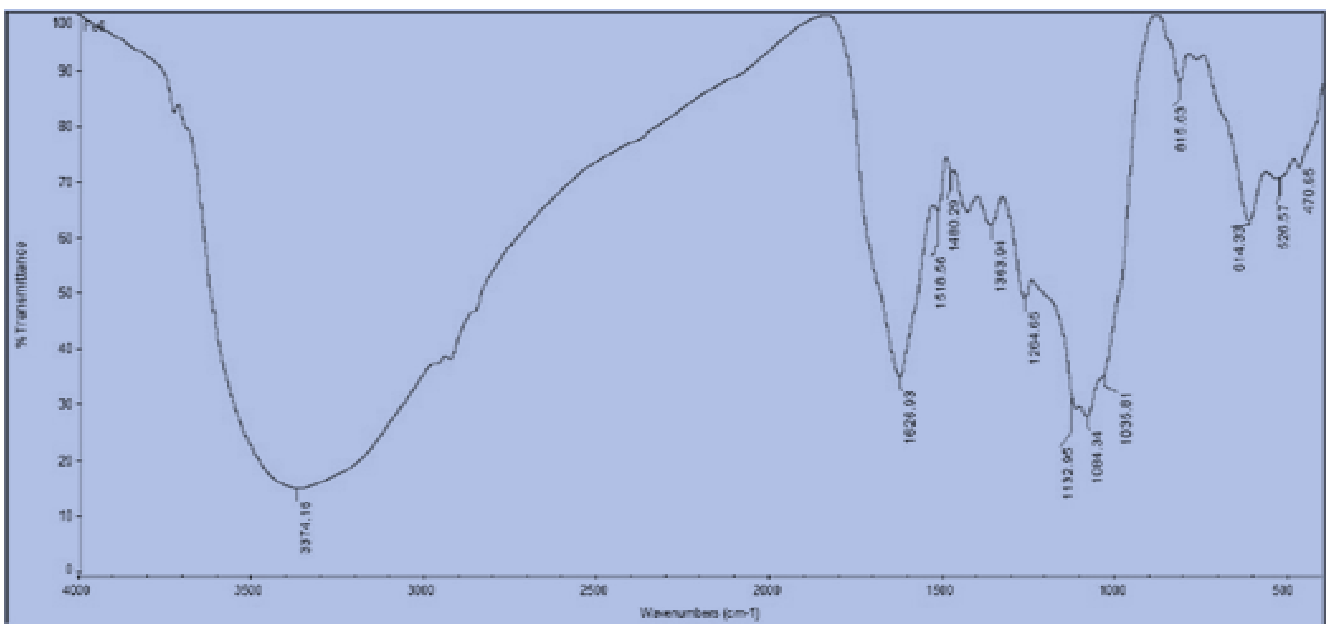

Figure 3: FTIR-analysis for showing the peaks of (FeNPs).

Discussion and Results

Nanoparticles are synthesized in several methods, the biological method is a good way, because its environmentally safe, so the living things have been used in this field. In our study, we used oyster shell powder in the synthesis of iron nanoparticles (as synthesis of green nanomaterial). The particle sizes that we obtained in our study are documented in table (1) ranging between (46.3443 and 70.3432), as a result shown in table (2).

Table (1): Fe NPs nanoparticle sizes.

Particle sizes of iron nanoparticles (Fe NPs) synthesized according to (SEM) measurements (n.m).
79.14
57.62
82.74
69.93
42.69
68.36
78.44
51.83

Table (2): One-Sample Test of Fe NPs nanoparticles

	Test Value = 8					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
N	11.497	7	.000	58.34375	46.3443	70.3432

There are many techniques have been used to clarify the association between iron nanoparticles and oyster shell, including the wet impregnation technique [10]. In view of what iron nanoparticles possess [5], they are characterized by clear interactive properties and distinctive adsorption, and they have received great interest in polluted water treatment and other applications [11]. Oyster shells regarded as the raw material that was used in the biosynthesis process in our current research, because they are belong to the living things. So, they must cleaned and washed and drying [12], then they must be purified and disinfected, when they became ready to use for (FeNPs) synthesis, as shown in figure (no.4)[13].

The optimal properties that recorded through (XRD-analysis), (SEM-examination), and (FTIR Based detection) proved that the (FeNPs) are present. On the biological and medical aspect, standard ascorbic acid showed the greater percentage of its activity in closing the wound, followed by (FeNPs) , they followed by the main plant extracts [14, 15]. Iron nanoparticles have magnetic properties, so they can be used in treatments field. So, the metals nanoparticles like (nickel, cobalt, and their chemical compounds) also have some properties enable them to apply as antimicrobial agents. In our study, the iron nanoparticles possess some properties such as a round shape and small sizes enable them to penetrate the bacterial cell wall, and kill them. As a result of their impressive properties, there are many potential uses in the fields of biopharmaceuticals [16], magnetic particle imaging [17], and environmental remediation [18].

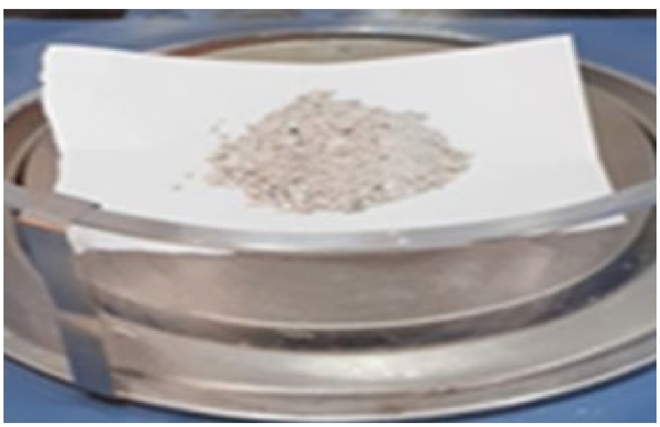

Figure 4: Shown oyster shells as the raw material after grinding.

Antimicrobial Activity of (FeNPs)

Iron nanoparticles have high ability to kill many types of microbes, because they are able to penetrate into cell wall, and they do rupture to the bacterial cell membrane, in which a clear effect on cellular content occurs [19]. The small size of (FeNPs) have a larger surface area, so they have ability to change the molecular components of DNA of the bacterial, and for this reason the bacterial cells will die, so on this basis iron nanoparticles (Fe-NPs) can be considered as an antibiotic that treats bacteria [20], because it is effective and sensitive to bacteria. The explanation for why iron nanoparticles (Fe-NPs) have different antibacterial potential and different types of bacteria is that, since gram positive bacteria have a thick peptidoglycan membrane [21], it is likely that there is a high degree of contact between bacteria, germs and nanoparticles due to their small size. Rather than cytoplasmic membrane, gram negative bacteria possess all of cytoplasmic membrane and an outer cell membrane, in addition of a thin peptidoglycan film between them[22, 23]. It is considerably severe for iron nanoparticles (Fe-NPs) to infiltrate the thin layer in this status. Another putative mechanism for the antibacterial activity by iron nanoparticles (FeNPs) is the ability to generate reactive oxygen species (ROS) [24], such as hydroxyl radicals and lunar oxygen inside the bacterial cell. The phenomenon of reactive oxygen species is formed because of the (Fenton reaction) of iron and metabolic products, for example hydrogen-peroxide in bacterial cells [25]. Reactive oxygen species form oxidative stress in bacteria cells, which leads to bacterial death. Although the exact mechanism of iron nanoparticles (Fe-NPs) antibacterial action is not clear, these nanoparticles can be considered as confirmed antibacterial agents [26].

Conclusion

In our research we conclude that the way of green-biological synthesis of (FeNPs) is better than other chemical and physical methods, since the nanoparticles that we obtained are free from chemical reactions that may not give the desired result and may not perform the desired purpose, and also maintains its properties, and the nanomaterials (iron nanoparticles) that have been synthesized cannot be re-solidified. If (FeNPs) manufactured by a physical method, they will return to its previous position as a solid material, for example. In this situation, the synthesized iron nanoparticles can play their role as a bactericidal antibiotic that maintains its basic properties.

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