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Biosynthesis of Iron Nanoparticles (Fe NPs), and their Antibacterial Activity

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Abstract:

Our ongoing research involves the synthesis of iron nanoparticles through a multi-step procedure that includes intricate processes resulting in the disruption of the bonds between the fundamental components of the oyster shell. As a result, we successfully extract iron nanoparticles from oyster shell powder. The obtained iron nanoparticles were characterized using X-ray Diffraction Analysis (XRD), Scanning Electron Microscopy (SEM), and FTIR-Based Analysis. These techniques confirmed that the nanoparticles possess the standard properties and meet the optimal specifications necessary for their function as an antibiotic to suppress bacterial action.

Objective: Our research aims to biologically generate iron nanoparticles from oyster shells, meeting conventional standards that enable them to function as antibiotics.

Methods: Our research employs a method for producing iron nanoparticles that involves a series of processes combined with the addition of chemicals that disrupt the bonds between the fundamental constituents of the oyster shell. This shell is first purified and then ground into a powder.

Results And Characterization: The results were obtained using the SPSS statistical program, and the size of the iron particles was determined using the XRD crystallite (grain) calculator, specifically the Scherrer Equation. The characteristics acquired from the steps involving Fe NPs in our ongoing study are confirmed based on the measurements conducted using X-ray Diffraction Analysis (XRD), Scanning Electron Microscopy (SEM), and FTIR-Based Analysis. These measurements indicate that the particles have a satisfactory size range of 30-100 nanometers.

Conclusion: According to this study, we produced iron nanoparticles through a series of steps from a biological source, involving many chemical reactions to retain their desired properties. Our research demonstrates that biologically produced iron nanoparticles cannot be re-solidified and can act as bactericidal antibiotics.

Keywords: (Biosynthesis, Iron nanoparticles, Antibacterial activity).

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1. Introduction.

Iron nanoparticles have a size less than one micrometer and are made up of iron metal. By virtue of their extensive surface area in relation to their mass, they possess a natural inclination to react strongly and rapidly oxidize, resulting in the formation of free iron ions. It has extensive and diverse uses in numerous medical and biological applications, as well as in the treatment of pollution caused by chlorinated organic chemicals. The wide range of technical applications utilizing nanomaterials has significantly elevated the importance of nanotechnology, establishing it as one of the most prominent and actively researched topics [1,2].

Iron nanoparticles, similar to other metal nanoparticles, can be generated through several methods, such as physical, chemical, and biological processes. 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 precise process for the production of iron nanoparticles remains unclear, as it has been observed to occur through the same pathway as the synthesis of zero valent iron nanoparticles, iron oxide nanoparticles, and combinations of both [3, 4]. The process of biogenic production of iron nanoparticles can be accomplished through several means, such as utilizing plants, fungus, bacteria, and other species. These organisms can generate iron particles with nuclei by undergoing a series of essential steps. Several studies have been conducted to extract or manufacture iron nanoparticles. Several investigations were carried out on oyster shells due to its composition, which includes various minerals, such as iron.

The iron nanoparticles obtained during extraction are utilized in a wide range of industries, medical practices, and biological applications. These applications encompass many disciplines and include the eradication of bacteria and the treatment of microbial contamination. The iron particles are classified as non-valent nanoparticles, which endows them with distinctive interaction and absorption characteristics that contribute to their efficacy in biological treatments. Consequently, they have garnered significant interest in the remediation of water pollution [5, 6].

The nanoparticles generated using green synthesis exhibit precise size control, absence of contaminants, and ease of scalability. Furthermore, green synthesis offers numerous other advantages and applications [7]. The biological activity of the produced nanoparticles is predominantly influenced and regulated by the green materials employed to stabilize and diminish the ions of different types of metals. An essential trait of NPs is their capacity to effectively accomplish desired objectives, such as acting as therapeutic agents against bacteria, eliminating pathogens in certain disorders, or targeting mammalian cells (host) [8].

Iron nanoparticles have a wide range of applications in the fields of medicine, biology, and other areas. Similarly, iron oxide nanoparticles are also used in various therapeutic applications, including cancer treatment, drug delivery, tissue repair, tumor monitoring, toxin removal from biological fluids, and magnetic resonance imaging.

2. Materials and Methods.

2.1. Materials:

The user mentions the use of a specific magnetic stirrer device, the Heidolph MR 3001 K MR3001K Magnetic Stirring Hotplate, along with the SCALTEC model SBC series. The user also lists the materials being used, which include deionized water, oyster shell powder, HCL (hydrochloric acid), sodium hydroxide, and filter paper.

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2.2. Methods:

The biosynthesis of iron nanoparticles (Fe NPs) method includes four stages represented by mixing, separating, washing, and drying [9-11], then diluted to many concentrations for application as antimicrobial, or antibacterial.

1. (50ml) deionized water is added to (10gm) of oyster shell powder, and then kept for one day at room temperature.
2. Prepare (10% HCL): (10ml HCL) complete to (100ml) with deionized water.
3. (100 ml) of (HCL 10%) is added to the preserved mixture that contains (10 gm) oyster shell powder + (50 ml) deionized water, and the mixture is kept for 4 hours.
4. We take a filter paper weighing (0.65 g), and the resulting mixture from the previous step is filtered after four hours of mixing in the magnetic stirrer (rotor) device.
5. Then weigh (10 gm) of sodium hydroxide, add to (100 ml) of deionized water and cover for one day, then added to the remaining powder that resulted from the previous step, including the filtration process.
6. The magnetic stirrer device shook the mixture for four hours, and the iron particles were drawn to the metal sphere that spins inside the beaker that was set on top of the magnetic stirrer device.
7. Collect the iron nanoparticles from the capsule for preparing and checking (examine) them by XRD, SEM, FTIR examination.

3. Results:

Table no.1: Different sizes of iron nanoparticles:

Particle sizes of iron nanoparticles (FeNPs) synthesized according to (SEM) measurements (n.m).
70.22
67.53
84.36
73.65
34.72
52.58
82.35
46.73

Table no.2: The normal distribution of iron nanoparticles size.

Descriptives				
			Statistic	**Std. Error**
Iron (Fe-NPs):	Mean		64.0175	**6.24144**
	95% Confidence Interval for Mean	Lower Bound	49.2588	
		Upper Bound	78.7762	
	5% Trimmed Mean		64.5150	
	Median		**68.8750**	
	Variance		311.644	
	Std. Deviation		17.65345	
	Minimum		34.72	
	Maximum		84.36	
	Range		**49.64**	
	Interquartile Range		31.98	
	Skewness		-.556-	.752
	Kurtosis		-.884-	1.481

4. Characterization of Iron Nanoparticles (Fe-NPs):

4.1. X-ray Diffraction Analysis (XRD) Analysis:

The iron nanoparticles, manufactured using green methods, were analyzed using X-ray determination (XRD-analysis) by Smart. Lab SE., Rigaku, Tokyo, Japan. This analysis was conducted to determine the type of the nanoparticles and their average size, as shown in **figure no.1.**

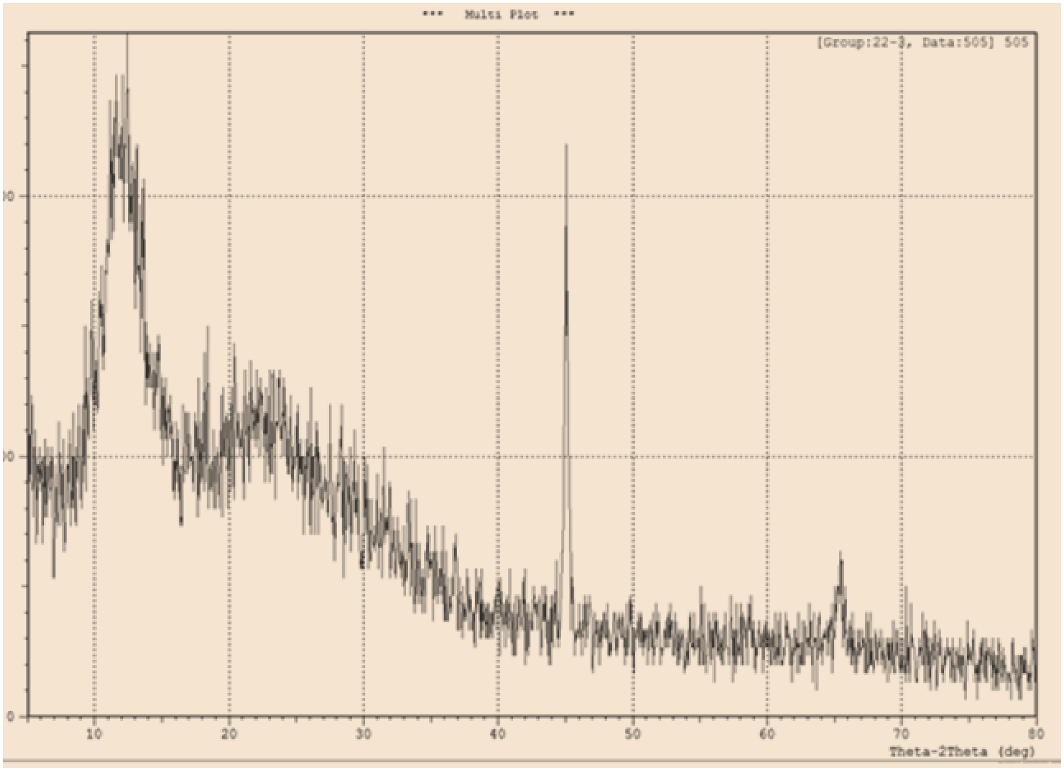

Figure no. 1: XRD analysis of iron nanoparticles synthesis (Fe-NPs) [12].

4.2. Scanning Electron Microscopy (SEM):

The iron nanoparticles were examined, and their surface shape was confirmed using scanning electron microscopy (JSM-IT\500, Jeol, Boston, MA, USA). The dry granules produced using the

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green biosynthesis process described earlier can be diluted with deionized water at a ratio of 10 grams per milliliter. The dried granules underwent structural characterization using SEM analysis, following the guidelines provided by the National Institute of Standards and Technology (NIST\2007) [11], as shown in **figure no.2 (A, and B.**)

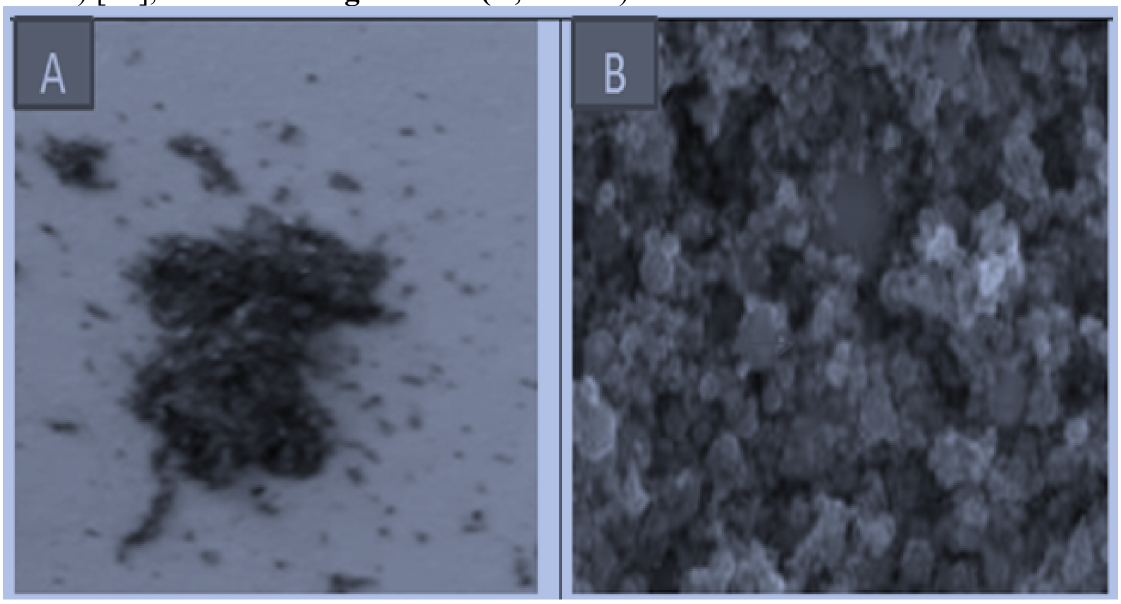

Figure no. 2: A) Showing the iron nanoparticles that have been synthesized by the biological **methods. B) Scanning Electron Microscope (SEM) image of iron nanoparticles synthesized (Fe-NPs) [12].**

4.3. FTIR-Based Analysis:

The identification of the functional groups on the iron surface that were involved in the green biosynthesis of Fe-NPs was carried out using FTIR-spectroscopy (S\700, Nicolet, MA, USA) [13]. The mixture was agitated by the magnetic stirrer for a duration of 4 hours. Subsequently, the iron particles that clustered around the rotating metal capsule inside the beaker, which was positioned on the magnetic stirrer, were gathered and subjected to analysis using FTIR-spectroscopy, as shown in **figure no.3**.

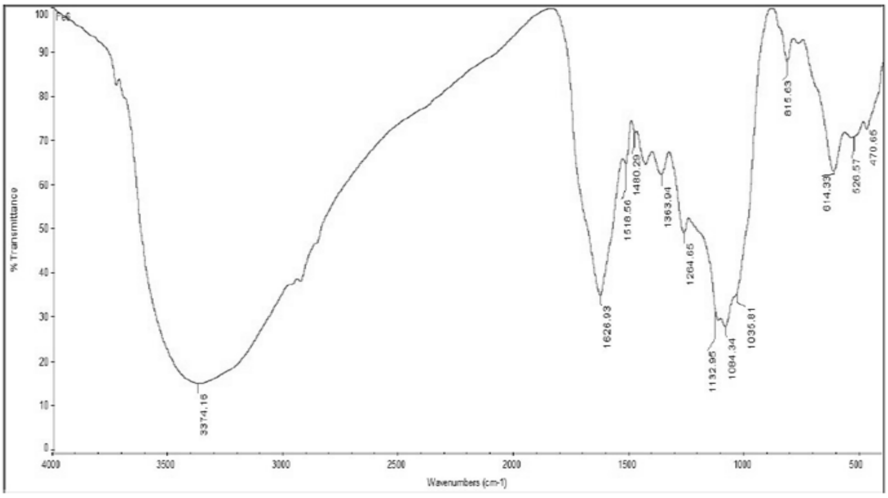

Figure no. 3: FTIR-spectra showing the peaks of iron nanoparticle synthesis (Fe-NPs) [12].

5. Antibacterial Activity of Iron Nanoparticles (Fe-NPs):

As the size of nanoparticles decreases, their capacity to infiltrate the bacterial cell and gather in the bacterial cell wall increases. In order to achieve effectiveness, it is necessary for the iron nanoparticles to cause the bacterial cell membrane to break, resulting in a noticeable impact on the cellular content [14, 15]. Furthermore, small iron nanoparticles possess a greater surface area, leading to molecular-level alterations in the DNA of bacterial cells. Consequently, the bacterial cells perish. Based on this mechanism, iron nanoparticles (Fe-NPs) can be regarded as an antibiotic for combating bacteria [16], owing to their efficacy and sensitivity towards bacterial pathogens. The variation in antibacterial efficacy of iron nanoparticles (Fe-NPs) against different types of bacteria can be attributed to the fact that gram positive bacteria possess a substantial peptidoglycan membrane, which increases the likelihood of extensive interaction between bacteria, pathogens, and nanoparticles owing to their diminutive dimensions. The outer cell membrane and the inner membrane of Gram-negative bacteria both have a thin peptidoglycan film in between them. In this case, it is very bad that iron nanoparticles (Fe-NPs) are getting into the thin layer [17]. One idea for how iron nanoparticles (Fe-NPs) might kill bacteria is that they can make reactive oxygen species (ROS), such as hydroxyl radicals and moon oxygen, inside the bacterial cell. The Fenton reaction takes place in bacterial cells and involves iron and metabolic products like hydrogen peroxide [18,19]. This reaction makes reactive oxygen species. When reactive oxygen species form, they put bacterial cells under oxidative stress, which kills the bacteria. Iron nanoparticles (Fe-NPs) are definitely good at killing bacteria, even though no one fully understands how they do it. Iron nanoparticles (Fe-NPs) are used in health, catalysis (including sensing), environmental science, magnetism, and other fields because they are very good at killing bacteria [20-22].

6. Discussion.

Nanotechnology is a contemporary technology that has permeated various domains, such as biological, medicinal, and others. Nanoparticles are produced by many synthesis methods. The biological approach is a simple, rapid, and cost-effective solution. Furthermore, live creatures and plant extracts have been utilized due of their environmentally friendly nature. For our recent study, we employed oyster shell powder to synthesize iron nanoparticles (Fe NPs), which are considered a type of green nanomaterial. The interaction between iron nanoparticles and oyster shell has been investigated using many methodologies and procedures, such as the wet impregnation approach [23, 24]. Iron nanoparticles have notable interaction features and specific adsorption capabilities, making them highly sought after for various applications, particularly in the treatment of dirty water. [25, 26]. Although there are multiple and varied methods for the synthesis and production of nanoparticles, biological methods remain the best and most efficient because they give good efficacy in application, as the manufactured nanoparticles cannot lose their properties, and do not return to their first formula that they were on it before synthesis.

Oyster shells are the raw material that was used in the biosynthesis process in our current research. After cleaning and washing them well and drying them, they were purified and disinfected, and after they were ground, they became ready for use as a raw material from which iron nanoparticles could be obtained, as shown in **figure (no.4)**. The thing that gives reassurance in the process of creating these iron nanoparticles in this biological way is that oysters are considered living organisms that can be eaten (suitable for human use as food), and in this case they are safe and non-toxic. The standard properties reached through X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and (FTIR-Based detection) allow iron nanoparticles to be applicable as an antibiotic against bacteria, since their size enables them to penetrate the wall of bacteria and get rid of them completely. One of the most important characteristics of NPs in general is that they exhibit superior activity [27]. In order to synthesize various different types of NPs, a range of methods are now available at the biological, physical, chemical, hybrid and other methods [28]. In our research, green synthesis processes were used to make iron nanoparticles, and it is a known fact that biologically extracted chemicals are safe and end up with desirable optical-metallic properties with acceptable structural specifications. On the medical level, standard ascorbic acid showed the highest percentage of its effectiveness in closing the wound, followed by iron nanoparticles (Fe NPs), and then followed by the rest of the plant extracts and others [29, 30]. There are materials that make cancerous cells more visible during tomography, among these materials are gadolinium metal particles (Gd) and metallic iron particles, as they have distinctive magnetic properties under the influence of the external magnetic field. In addition, exotic drugs taken by humans can carry potential risks. This problem can be solved by an injection system based on magnetic ferritin, which is represented by a magnetic core (iron oxide nanoparticles) that is located inside the human ferritin protein membrane, which is directly responsible for iron metabolism. In the body. This compound is biocompatible as it interacts inside the body without causing side reactions. In this context, scientists suggested the use of magnetic ferritin injections intravenously, so that target cells can pick it up and interact positively with it, while the injected ferritin spreads in the body with the bloodstream. Iron nanoparticles possess magnetic characteristics, rendering them suitable for utilization in therapies involving the application of a magnetic field. Furthermore, these particles exhibit magnetic characteristics, and there are also nanoparticles composed of different metals like nickel, cobalt, and their chemical compounds possess same features. Recent research has extensively studied magnetic nanoparticles due to their remarkable properties, which hold potential applications in various fields such as stimulation [31], biopharmaceuticals [32], magnetic resonance imaging [33], magnetic particle imaging [34], data storage, and environmental remediation [35].

Figure no.4: Oyster shells as the raw material before grinding, and preparation for synthesis.

8. Conclusion:

Based on our ongoing research, we have determined that the biological synthesis method for producing iron nanoparticles is superior to other chemical and physical methods. This is because the nanoparticles obtained through this method are devoid of any undesired chemical reactions and retain their desired properties. Additionally, synthesized nanomaterials, specifically the iron nanoparticles, cannot be re-solidified. If they were produced through physical means, we cannot ensure that their configuration would not revert back to its original state as a solid material, for instance. Under these circumstances, the produced iron nanoparticles can function as a bactericidal antibiotic while preserving their fundamental characteristics.

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