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Biosynthesis of iron nanoparticles (Fe NPs), and its antibacterial activity

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Abstract
Our current research includes biosynthesis of iron nanoparticles by a series of steps as a procedure that included complex processes that led to the breaking of the bonds that bind the basic components of the oyster shell, and thus ended up obtaining iron nanoparticles that were extracted from oyster shell powder. The standard properties of the gold nanoparticles that we obtained were confirmed by the use of (X-ray Diffraction Analysis (XRD) Analysis, Scanning Electron Microscopy (SEM), FTIR-Based Analysis) in comparison with the optimal specifications through which the required function can be performed as an antibiotic that suppresses the action of bacteria.

Objective: The objective of our research is to synthesize iron nanoparticles in a biological way, from oyster shells within standard specifications that allow them to perform their role as antibiotics.

Methods: The working method used in our research to prepare iron nanoparticles includes a set of steps interspersed with the introduction of chemicals that break the bonds that bind the basic components of the oyster shell that we have converted into powder by grinding it after purification.

Results and characterization: The results performed according to (SPSS) statistical program, and iron particles size calculated as XRD crystallite (grain) calculator (Scherrer Equation). The characteristics obtained through the (Fe NPs) steps in our current research are verified according to the measurements shown in (X-ray Diffraction Analysis (XRD) Analysis, Scanning Electron Microscopy (SEM), FTIR-Based Analysis), it gave particles of acceptable size ranging between (30-100) nanometers.

Conclusion: We conclude from our current study that there are no significant differences between the number of patients and the three stages type that recorded through this periodic time, even if there is a difference in age and gender.

Keywords: Biosynthesis, iron nanoparticles, antibacterial activity

Introduction

Iron nanoparticles are smaller than one micrometer, and they are composed of iron metal. They are by nature highly reactive, due to their large surface area exposed to the outside relative to the mass, and they have the ability to oxidize quickly to form free iron ions. It is widely and varied in many medical and biological applications, in addition to its role in treating pollution resulting from chlorinated organic compounds. There wide area of technological applications by using nanomaterial's, it has made nanotechnology very important and made it one of the largest active domains of research [1, 2]. The iron nanoparticles are like the rest of the metal nanoparticles are synthesized in several ways, including physical, chemical, and biological. 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 [3, 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. The extracted iron nanoparticles are used for many industrial, medical and biological applications in various fields, including killing bacteria and treating microbial contamination. The iron particles are considered non-valent nanoparticles, so they have unique interaction and absorption properties that make them successful in biological treatments, and they have received great attention in the treatment of water contaminated [5, 6]. 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 [7]. The biological activity of the synthesized nanoparticles is largely determined and controlled by the green materials used in order to stabilize and reduce the ions of various kinds of metals. One of the most ideal characteristics of NPs is that they should have the distinct ability to achieve the necessary goals, whether they are therapeutic means such as antibacterial agents, or a way to get rid of pathogens of some diseases, or in mammalian cells (host) [8]. The uses of iron nanoparticles are broad-spectrum in terms of medical, biological, and other uses, as well as the uses of iron oxide

nanoparticles, which are also used in the therapeutic aspect, such as cancer treatment, drug delivery, treatment of damaged tissues, monitoring and follow-up of tumor development, and removal of various toxins from biological fluids and magnetic resonance imaging.

Materials and methods

1. Materials

Magnetic stirrer (rotor) device (Heidolph MR 3001 K MR3001K Magnetic Stirring Hotplate), SCALTEC model SBC series, deionized water, oyster shell powder, HCL, sodium hydroxide, filter paper.

2. Methods

The biosynthesis of iron nanoparticles (Fe NPs) method include four stages represented by mixing, separating, washing, and drying [9, 10], then diluted to many concentration 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 covered for one day, then added to the remaining powder that resulted from the previous step, including the filtration process.
6. The mixture was shaken by the magnetic stirrer device for about (4 hours), and the iron particles were attracted to the metal capsule that rotates inside the beaker that was placed on the magnetic stirrer device.
7. Collect the iron nanoparticles from the capsule for preparing and checking (examine) them by XRD, SEM, FTIR examination.

Results

Table 1: To show different sizes of three types of synthesized 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 2: To show that there is a normal distribution in the sizes of the synthesized iron nanoparticles (Fe-NPs).

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

Table 3: To show that there is a normal distribution in the sizes of the synthesized Iron nanoparticles (Fe-NPs).

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
I	.204	8	.200*	.934	8	.555

*. This is a lower bound of the true significance.
a. Lillie or s significance correction

Table 4: XRD crystallite (grain) ag-particle size calculator (Scherrer Equation).

Peak Position (2θ)	46
FWHM (2θ)	0.13
X-Ray Wavelength	0.15418
Results	
69.37	nm

Characterization of iron nanoparticles (Fe-NPs)

1. X-ray diffraction analysis (XRD) analysis

The green-synthesized iron-nanoparticles that extract by biological processes were subjected to the x-ray determination (XRD-analysis) 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.

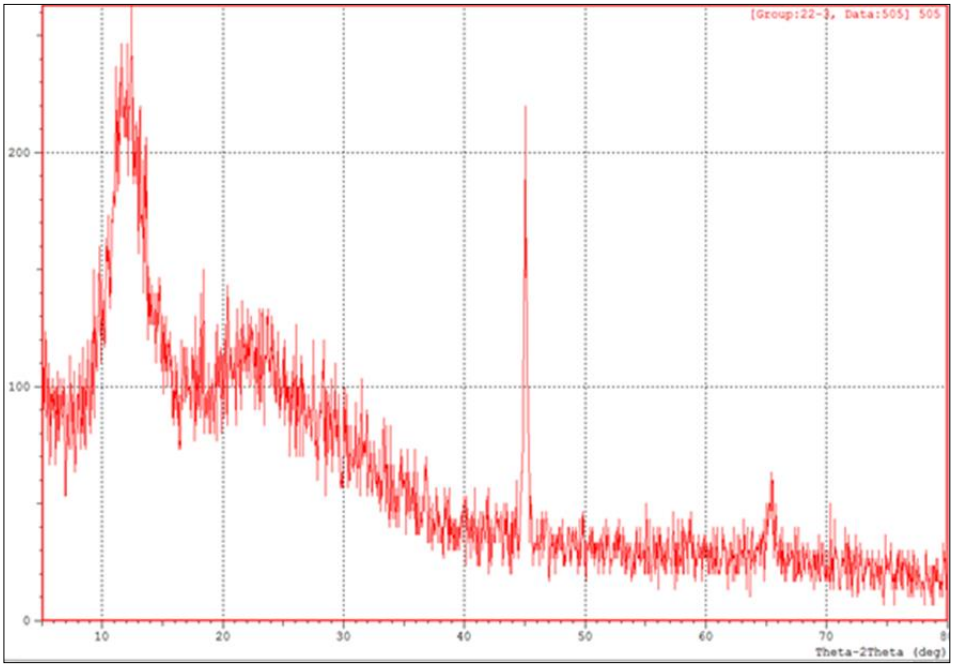

Fig 1: XRD analysis of iron nanoparticles synthesis (Fe-NPs).

2. Scanning electron microscopy (SEM)

Scanning electron microscopy as (JSM- IT\500, Jeol, Boston., MA. USA), used for the examination of the iron nanoparticles, and confirm their surface shape. The dry granules obtained from this green biosynthesis method by the steps in the aforementioned working methods, diluted with deionized water at a ratio of 10:1 g/ml. These dry granules were subjected to structural characterization by SEM analysis according to the (National Institute of Standards and Technology, NIST\2007) [11], as shown in figure no.2 (A, and B.

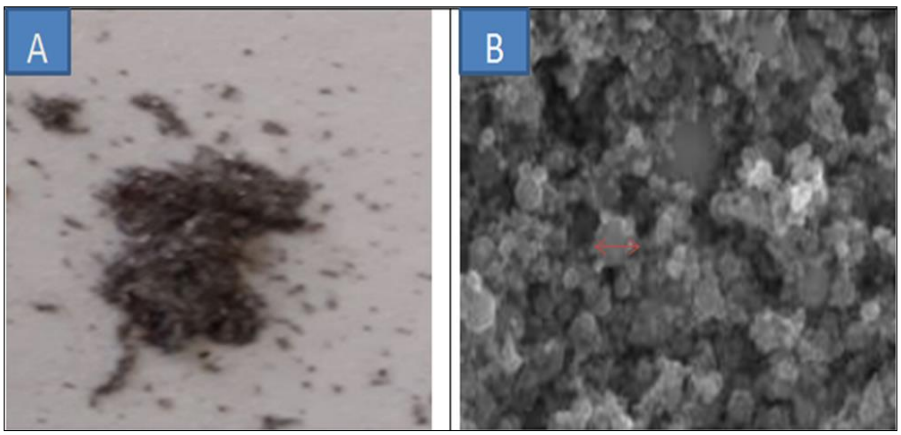

Fig 2: A) Showing the iron nanoparticles that have been synthesized by the biological methods. **B)** Scanning Electron Microscope (SEM) image of iron nanoparticles synthesized (Fe-NPs).

3. FTIR-based analysis

The functional groups of iron surface and were involved in the green biosynthesis of Fe-NPs were identified by using FTIR-spectroscopy (S\700, Nicolet, MA. USA) [12]. The mixture that was shaken by the magnetic stirrer for a period of (4 hours), and after the attraction of the iron particles around the metal capsule that rotates inside the beaker that was placed on the magnetic stirrer, was collected and analyzed by FTIR-spectroscopy, as shown in figure no.3.

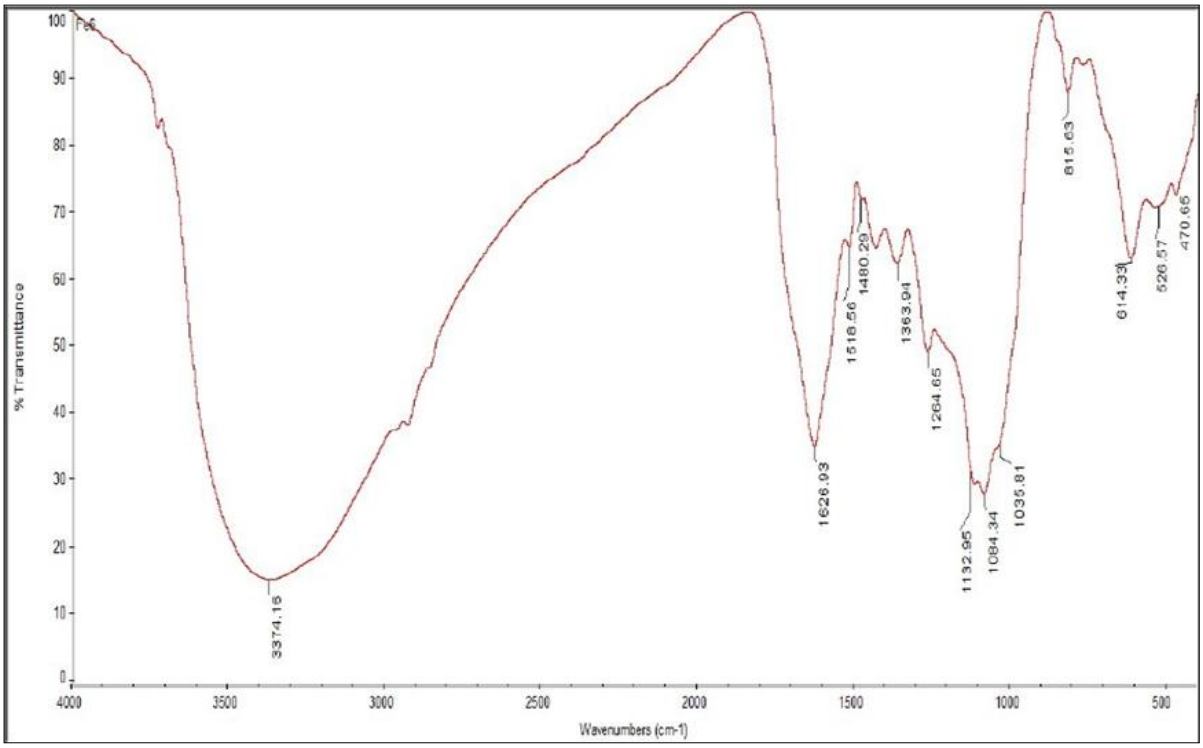

Fig 3: FTIR-spectra showing the peaks of iron nanoparticles synthesis (Fe-NPs).

Antibacterial activity of **iron nanoparticles (Fe-NPs)**

The smaller the nanoparticles are, the more they have the ability to penetrate the bacterial cell and accumulate within the bacterial cell wall. To be effective, the iron nanoparticles must rupture the bacterial cell membrane, in which a clear effect on cellular content occurs [13, 14]. In addition, small iron nanoparticles have a larger surface area, and this causes changes at the molecular level in the DNA of the bacterial cell 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 [15], 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, 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. It is considerably severe for iron nanoparticles (Fe-NPs) to infiltrate the thin layer in this status [16]. Another putative mechanism for the antibacterial activity by iron nanoparticles (Fe-NPs) is the ability to generate reactive oxygen species (ROS), 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 [17, 18]. 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. With all this outstanding antimicrobial activity, iron nanoparticles (Fe-NPs) also have many other applications in a variety of fields including medical, catalytic (including sensing), environmental, and magnetic fields [19, 20, 21].

Discussion

Nanotechnology is a modern technology that has entered many fields, including biological, medical and others. Nanoparticles are synthesized in many different ways. The biological method is one of the easy, fast, and cheap methods. In addition to being environmentally safe, living organisms and plant extracts have also been used. In our current research, we used oyster shell powder in the synthesis of iron nanoparticles (Fe NPs) (as synthesis of green nanomaterial). Various methods and techniques have been used to clarify the association between iron nanoparticles and oyster shell, including the wet impregnation technique [22, 23]. In view of what iron nanoparticles possess, they are characterized by clear interactive properties and distinctive adsorption, and they have received great interest in polluted water treatment and other applications [24, 25]. Although there are multiple and varied methods for the synthesis and production of nanoparticles, but 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 [26]. 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 [19]. 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 [28, 29]. 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 have magnetic properties, so they can be used in treatments using a magnetic field. And as these particles have magnetic properties, there are nanoparticles of other metals such as nickel, cobalt, and their chemical compounds have the same properties. Many areas of recent research have focused on magnetic nanoparticles as a result of their impressive properties, which may see potential uses in the fields of stimulation [30], biopharmaceuticals [31], magnetic resonance imaging [32], magnetic particle imaging [33], data storage as well as environmental remediation [34].

Fig 4: To show oyster shells as the raw material before grinding, and preparation for synthesis.

Conclusion

We conclude from our current research that the method of biological synthesis of iron nanoparticles 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 they were manufactured in a physical way, we cannot guarantee that their formation will not 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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