RESEARCH ARTICLE

A Study of Iron Oxide Nanoparticles Synthesis by Using Bacteria

Abdulsahib S. Jubran[1*], Oda M. Al-Zamely[2], Mahdi H. Al-Ammar[3]

[1]*Dentistry Faculty, University of Alkafeel, Najaf, Iraq*
[2]*College of Science, University of Babylon, Babylon, Iraq*
[3]*College of Science, University of Kufa, Kufa, Iraq*

Received: 22nd Dec, 2019; Revised: 24th Jan, 2020; Accepted: 17th Feb, 2020; Available Online: 25th Mar, 2020

ABSTRACT

The biosynthesis of nanoparticles by using microorganisms is developing as an ecofriendly method for nanoparticle synthesis because of its cheap, simple and non-toxic. *Bacillus sp.* can be used for producing iron oxide nanoparticles. In addition, it has the ability for the biosynthesis of Fe_3O_4 nanoparticles. The nanoparticles producing was evaluated by using ultra violate-visible (UV-visible) and Fourier-transform infrared spectroscopy (FT-IR) methods also the production and size of the nanoparticle was confirmed by X-ray Diffraction and Field Emission Scanning Electron Microscope (FESEM) to confirm the accuracy of iron oxide nanoparticles. pH, Temperature, and Incubation time of production of iron oxide nano-particle also studied.

Keywords: Bacteria, Drug delivery, Iron oxide, Nanoparticles.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.1.13

How to cite this article: Jubran AS, Al-Zamely OM, Al-Ammar MH. A Study of Iron Oxide Nanoparticles Synthesis by Using Bacteria. International Journal of Pharmaceutical Quality Assurance. 2020;11(1):88-92.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Nanotechnology is a multi-disciplinary kind of science that covers many areas of scientific techniques, like biomedical, pharmaceutical, agricultural, environmental, materials, general chemistry, general physics, electronics, data sciences and technology, etc.[1-4] Nanotechnology is become applied now in the pharmaceutical industry, medicine, electronics, robotics, and tissue engineering. The usage of nanomaterials in the enhancement of delivery systems for various molecules, like DNA, RNA, plasmids, and proteins it is very important today and has been considered widely throughout the last years.[2] Nanoparticles have been used to deliver drugs to target tissues and to increase stability against degradation by enzymes.[3] Their exclusive size-dependent properties make these materials indispensable and superior in many areas of human activities.[3,4] Green synthesis methods are eco-friendly approaches and compatible with pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods.[5] Iron oxide nanoparticles are most suitable for biomedical applications due to their proven biocompatibility. These particles have an ability to interact with various biological molecules in different ways due to their superparamagnetic properties, high specific area and wide choice of surface functionalization.[6] The potential of drug delivery systems based on the use of nano- and microparticles stems from significant advantages such as;[7,8] The ability to target specific locations in the body. The reduction of the drug quantity needed to attain a particular concentration approximately the target. The reduction of the concentration of the drug at non-target sites minimizing severe side effects. Living microorganisms, especially *Bacillus sp.* have a remarkable ability to form exquisite inorganic structures often in nano-dimensions. The development of these eco-friendly methods for the fabrication of nanoparticles is developing into an important division of nanotechnology, especially iron oxide nanoparticles.[7,9] Microbes play direct or indirect roles in several biological activities. So use them in the biosynthesis of nanoparticles is a more demanding approach for the bio-production of nanoparticles via a highly stable, eco-friendly process with no toxic chemical and large scale production.[10] Our study aims to investigate and detect iron oxide nanoparticles produced by *Bacillusims to Sp.* Bacteria.

METHODOLOGY

Bacillus Identification

Large gram-positive rods, often in pairs or chains with rounded or square ends (which may have a single endospore). Some species may be Gram variable. The identification was done by using spore stain method, which used to stain the spores of *Bacillus* species. Spores were in light green, and vegetative cell walls were pick up the counterstain safranin. The media used with conditions ware blood agar incubated in air/CO_2 at 35°C-37°C for 24 – 48-hour.[11]

Author for Correspondence: abdulsahebs@gmail.com

Preparation of Supernatant Solution of *B. subtitles*[12]

A nutrient broth medium was prepared by dissolving 30 gm of nutrient broth in one liter of distilled water (D.W.). After that, it was put in the autoclave at 110°C with one atm for 20 min. then cooled to room temperature. Next, Freshly *B. subtilis* were grownup on nutrient broth medium and left for one day at 37 ^{0}C. Finally, the fresh grownup cells were centrifuged at 6000 rpm for 12 min. to be removed, then supernatant solution collected for preparation of nanoparticles.

Biosynthesis of Iron Oxide Nanoparticles

The supernatant of *B. subtilis* was used as an aqueous solution of 2 mM $FeCl_3$ was added of *B. subtilis* supernatant solution by (1:1) addition in a 250 mL Erlenmeyer flask (pH adjusted to 9). The solution was incubated at 35^{o}C (200 rpm) for 48 hours (in dark condition). UV-visible spectroscopy (UV-Vis) in the range of 200~1,100 nm was made in a biotech spectrophotometer to assess the Fe_3O_4 formation.[13]

Temperature Effect on Iron Oxide Nanoparticle Production

To study the influence of temperature on the nanoparticle production, five different temperatures (25, 30, 35, 40 and 45°C) was approved. For these conditions, the pH was constant at nine, the incubation time dated kept as 48 hours with rotation system 200 rpm. At last, the absorbance was read at 258 nm.[14]

pH Effect on Iron Oxide Nanoparticle Production

To study the influence of pH on the nanoparticle production, seven different pH value (1, 3, 5, 7, 9, 11, and 13) was approved. For these conditions, the temperature was constant at 35°C, the incubation time dated kept as 48 hours with rotation system 200 rpm. At last, the absorbance was read at 258 nm.

Incubation Time Effect on Iron Oxide Nanoparticle Production

To study the influence of incubation time of the reaction on the nanoparticle production, five different periods (24, 48, 72, 96, and 120 hours) was approved. For these conditions, the temperature was constant at 35°C, and the pH value kept as nine with a rotation system 200 rpm. At last, the absorbance was read at 258 nm.

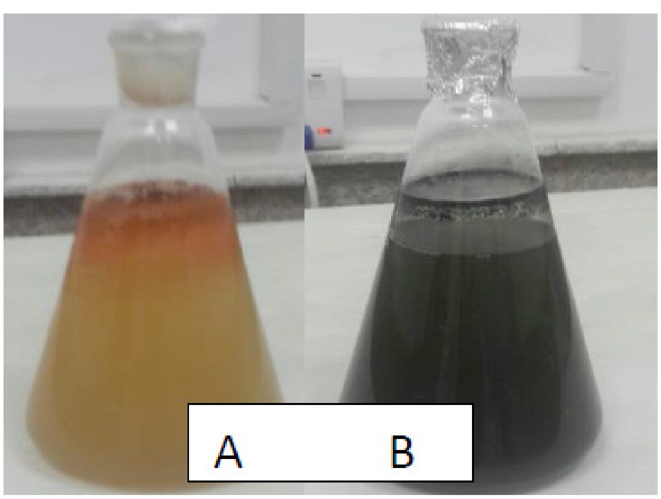

Figure 1: color change before (A) and after (B) the production of nanoparticles

Characterization of the synthesized nanoparticles

Scanning electron microscopy was used to study the size, morphology, and composition of the nanoparticles. In addition, X-ray Diffraction was used to perform the chemical formula and the particle size of the nanoparticles by using Sherrer's equation, ($D = k\lambda/\beta \cos \theta$) where D is the average crystallite size, k is an arithmetical factor, λ is the X-ray wavelength, θ is the Bragg angle, β is the line broadening in radiation. The FT-IR spectra was used before and after the formation of Fe_3O_4 for the description of nanoparticles, and they were ordered at a range between 400 – 4,000 cm^{-1}.

RESULTS AND DISCUSSION

Bacteria Identification

The *B. subtilis* group are closely related and are not easily distinguishable. Cells of these organisms are less than 1μm wide, sporangia are not swollen, and spores are ellipsoidal. They are in general mesophilic with regard to temperature and neutrophilic with respect to pH for growth, while often being tolerant to higher pH levels. All species were differentiated on the genetic level and it is to be expected that when genotypic analyses are applied to a wider range of strains of the classical species mentioned above, additional genospecies will be detected. The colonial appearance in blood agar media was large (2–7mm) with a frosted-glass appearance. Variable colonial morphology foe some species was produced mucoid, smooth, and some raised wrinkly colonies.

Biosynthesis of Iron Oxide Nanoparticles

The ferric chloride solution was added to the *B. subtilis* supernatant. The color-changing (Figure 1) was confirmed the production of Fe_3O_4 nanoparticles. Where $FeCl_3$ was dark green in color while Fe_3O_4 was black-brown in color.

The biosynthesized iron oxide nanoparticles band was indicated by using the UV–Visible scanning (Figure 2) at 258 nm. That refers to the dispersion of particles in the aqueous solution.[15]

Temperature Effect on Iron Oxide Nanoparticle Production

Figure 3 shows that the temperature influence on the rate of nanoparticles production were temperature less than 35°C reduced the formation of nanoparticles and this effect is increased with increasing the temperature than 35°C within a period time of 48 hours. this may be because of the deactivation or squalor of molecules accountable for the reduction of Fe^{3+}. This is contentious to the synthesis of nanoparticles by chemical methods where rising the temperature of the reaction improved the rate of iron oxide nanoparticles formation.[16]

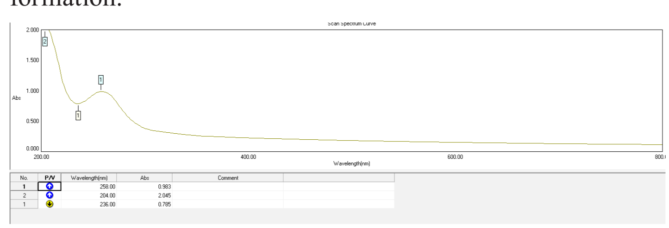

Fig. 2: Absorption spectra of iron oxide nanoparticles

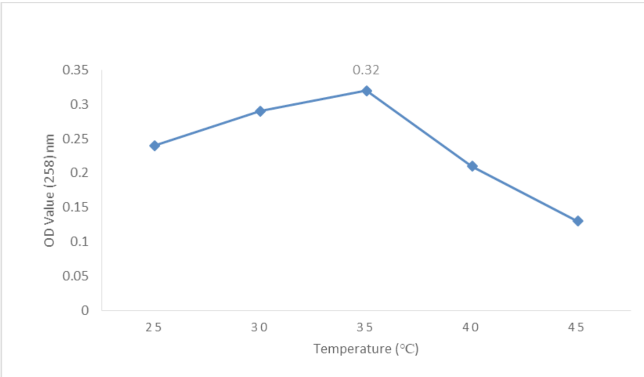

Figure 3: Temperature effect on iron oxide nanoparticle production

pH Effect on Iron Oxide Nanoparticle Production

The result of (Figure 4) shows the optimum pH for the production of iron oxide NPs is approximately nine, more than nine, an abrupt decrease in amount formation observed. Reach to 12 and more the formation was very low. This decline might be because of the absence or defect of the molecules or enzymes responsible for the formation of Fe^{2+}, ferric oxide might be

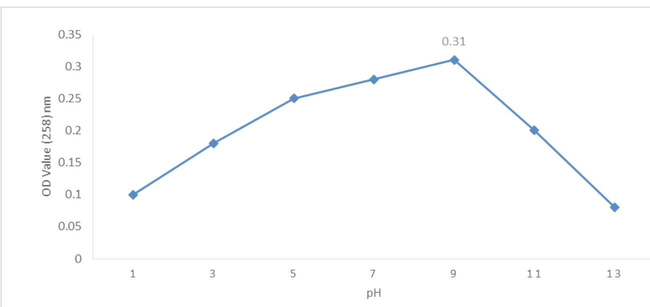

Figure 4: pH effect on iron oxide nanoparticle production

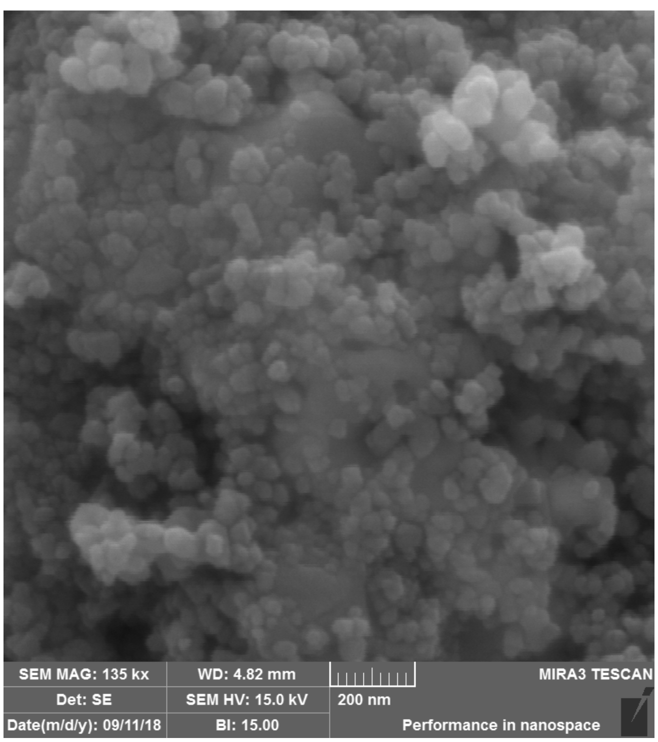

Figure 6: FESEM image of iron oxide NPs

formed, and this formation increased with pH increasing. The generation of the Fe_3O_4 nuclei occur simply at the medium pH is inferior to eleven, though the growth of the Fe_3O_4 NPs happens more definitely at the medium pH is upper than 11.[17]

Incubation Time Effect on Iron Oxide Nanoparticle Production

The finding of the study referred (Figure 5) that the optimum incubation time for iron oxide NPs formation was approximately 48 hours. Subsequently, a decrease for the formation of NPs was noticed. Where less than that time, there was no enough amount of Fe^{3+} available for NPs formation. While incubation time more than 48 hours there were enough yield of iron oxide NPs.[18]

Characterization of the Synthesized Nanoparticles

The result of the FESEM image of iron oxide NPs. Where this image appears the delivery of iron oxide NPs with groups. In addition, the FESEM image has approved the formation of iron oxide NPs by *Bacillus sp.* The morphology of NPs was spherical with size ranged between (3.6 ~ 7) nm. Also, from image noticed that the NPs with high stability by a coating means. This coating might be by proteins other molecules generated by *Bacillus sp.*[19]

In Figure (7,8) illustrated the FT-IR spectrum of Fe_3O_4 NPs synthesized by bacteria that determined by the band between 800–400 cm^{-1} for iron oxide, in comparison with Figure 7 of $FeCl_3$ FT-IR spectrum, the strong band between 800 – 400 cm^{-1} are referred to Fe-O bond stretching vibration in iron oxide. In addition, the specific vibrations for the Fe-O

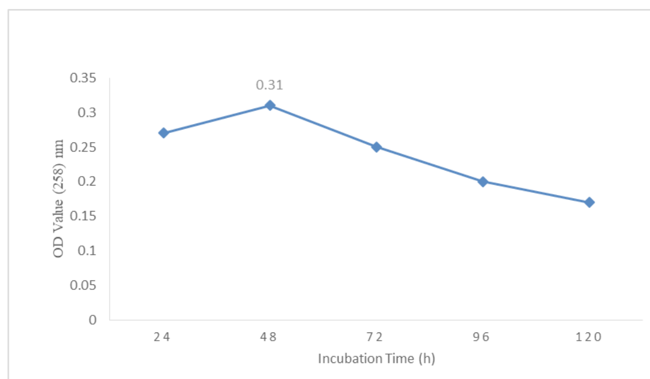

Figure 5: incubation time effect on iron oxide nanoparticle production

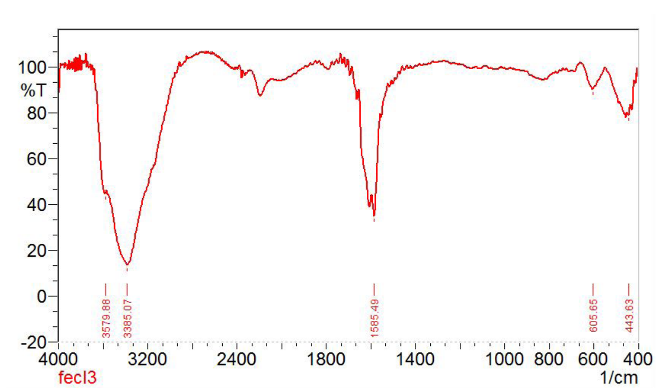

Figure 7: FTIR image of $FeCl_3$

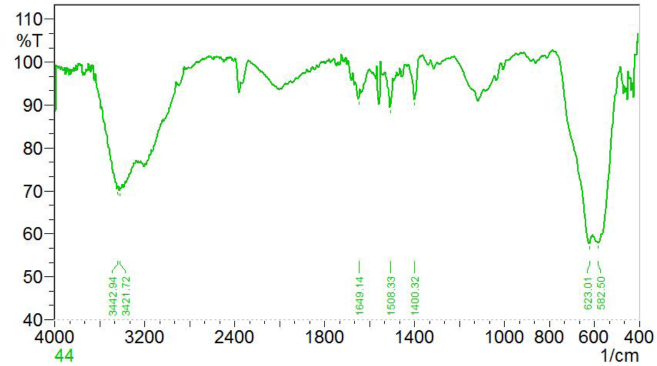

Figure 8: FTIR image of Fe_3O_4

Table 1: Summary of the average particles size projected from XRD outlines by means of the Scherer formula of biosynthesized Fe_3O_4 NPs.

Sample	*2θ*	*FWHM (deg°)*	*Particle size (nm)*	*Average particle size*
Fe_3O_4	30.43	0.74	11.63	11.94
	35.763	0.94	9.28	
	43.42	1.1	8.13	
	53.7	0.4	23.27	
	57.7	1	9.48	
	63	0.99	9.84	

bonds are allocated at 623, and 582 $cm^{-1,}$ where these bands are disappearing in the spectrum of $FeCl_3$ also the band at 1585 for Fe-Cl stretching vibration is missing in iron oxide spectrum.[20]

X-ray diffraction image in Figure 9 appears the biosynthesized of iron oxide NPs where the series of distinguishing peaks at 2θ and Bragg reflection, as mentioned in Table 1, which comparable with pattern references magnitude of XRD infers that the biosynthesized of iron oxide NPs are cubic back in creation. In addition, the average particle size of iron oxide NPs was calculated by using Sherrer's equation ($D = k\lambda / B \cos\theta$) and is about 18.17 nm, as shown in Table 1.[19]

IN CONCLUSION

The freshly *Bacillus sp.* was used to generation iron oxide NPs where this bacterium has the capability to biosynthesized of Fe_3O_4 NPs. The extracellular excretion of enzymes or minor metabolites arbitrates the biosynthesis of iron oxide NPs with a faster rate and slight toxicity. The presence of O-H was depicted by FTIR spectrum, which may work as stabilizers by link the molecules onto the functional forms. The formation of iron oxide NPs was confirmed by SEM analysis, and particle size ranged between (12–32) nm that supported by XRD pattern with average particle size 11.94 nm, which also authorizes the morphology of NPs to be cubic back in creation.

REFERENCES

1. Chatterjee K, Sarkar S, Jagajjanani Rao K, Paria S. Core/shell nanoparticles in biomedical applications. Adv. Colloid Interface Sci. 2014; 209: 8–39.
2. Dena D, Mohd ZBH, Aminu UK, Sharida F, Abdul HS, Zalinah A. Preparation of Fe_3O_4 magnetic nanoparticles coated with gallic acid for drug delivery. International Journal of Nanomedicine. 2012; 7: 5745–5756.

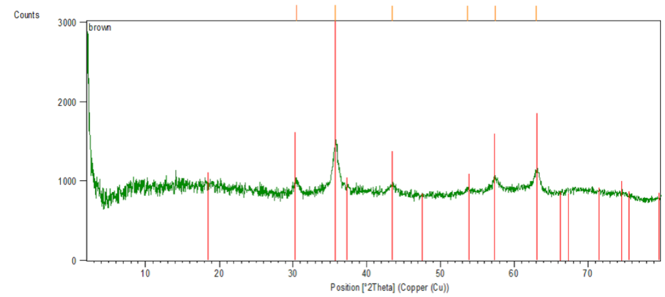

Figure 9: XRD image of Fe_3O_4

3. Prijic S, Sersa G. Magnetic nanoparticles as targeted delivery systems in oncology. Radiol. Oncol. 2011; 45: 1–16.
4. Mohammed L, Gomaa, HG, Ragab D, Zhu J. Magnetic nanoparticles for environmental and biomedical applications: A review. Particuology. 2017; 30: 1–4.
5. Willner B, Basnar B. Nanoparticle-enzyme hybrid system for nanobiotechnology. FEBS Journal. 2007; 274: 302-309.
6. Wei Wu, Quanguo H, and Changzhong J. Magnetic Iron Oxide Nanoparticles: Synthesis and Surface Functionalization Strategies. Nanoscale Research Letters. 2008; 3(11): 397-415.
7. Arruebo M, Fernandez-Pacheco R, Ibarra MR, Santamaria J. Magnetic nanoparticles for drug delivery. Nano Today. 2007; 2:22–32.
8. Mornet S, Vasseur S, Grasset F, Veverka P, Goglio G, Demourgues A, et al. Magnetic nanoparticle design for medical applications. In: Meeting of the European-Materials-Research-Society. Strasbourg, France: Pergamon-Elseviern Science Ltd.; 2005. p. 237–247.
9. Jositta Sherine, Annie Sujatha, Maheshwaran Rathinam. Biological synthesis of iron oxide nanoparticles using Streptomyces sp. and its antibacterial activity. JCHPS, 2016: 8; 58–60.
10. Ankit Chokriwal, Madan Mohan Sharma and Abhijeet Singh. Biological Synthesis of Nanoparticles Using Bacteria and Their Applications. Am. J. PharmTech Res. 2014; 4(6): 38–61.
11. Halket G, Dinsdale AE, Logan NA. Evaluation of the VITEK2 BCL card for identification of Bacillus species and other aerobic endosporeformers. Lett Appl Microbiol 2010;50:120-126.
12. P. Arul Jose and Solomon RD. Jebakumar. Appraisal of solar saltern based diverse actinomycetes as a source of antimicrobial natural compounds. International Conference on Biotechnology for Innovative Applications (Amrita BioQuest 2013) organised by Amrita University, Kerala, India, 10.07.2013 to 14.07.2013.
13. Jagathesan and Rajiv. Biosynthesis and characterization of iron oxide nanoparticles using Eichhornia crassipes leaf extract and assessing their antibacterial activity. Biocatalysis and Agricultural Biotechnology. 2018; 13: 90-94.
14. Irwan Nurdin, Ridwan, Satriananda. The Effect of Temperature on Synthesis and Stability of Superparamagnetic Maghemite Nanoparticles Suspension. Journal of Materials Science and Chemical Engineering, 2016, 4, 35-41.
15. Harald U, László D, Jasmin M, Christina J, Marina P, Jutta J, Tobias B, János S, Tobias F, Aldo RB, Christoph A, Iwona C. Dextran-coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging: evaluation of size-dependent imaging properties, storage stability and safety. International Journal of Nanomedicine: 2018; Volume 2018 (13): 1899-1915.
16. XC Jiang, WM Chen, CY Chen, SX Xiong, and AB Yu. Role of Temperature in the Growth of Silver Nanoparticles Through

a Synergetic Reduction Approach. Nanoscale Res Lett. 2011; 6(1): 32.

17. Reyman D, Serrano R, and Garcia-Leis A. Sonochemical synthesis of iron oxide nanoparticles loaded with folate and cisplatin: effect of ultrasonic frequency. Ultrasonics Sonochemistry. 2015;23:391-398.
18. Naseem T and Farrukh MA. Antibacterial activity of green synthesis of iron nanoparticles using Lawsonia inermis and Gardenia jasminoides leaves extract. J Chem. 2015; 2015:7.
19. Tang, S.C. and Lo, I.M. (2013) Magnetic Nanoparticles: Essential Factors for Sustainable Environmental Applications. Water Research, 2013;47:2613-2632.
20. Paiva DL, Andrade AL, Pereira MC, Fabris JD, Domingues RZ, Alvarenga ME. Novel protocol for the solid-state synthesis of magnetite for medical practices. Hyperfine Interact. 2015; 232:19–27.