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Biosynthesis of Iron Oxid Nanoparticles Coated with Folic Acid by Bacillus Megaterium

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ABSTRACT:
Background: Magnetic nanoparticles have stability in different physiological conditions, suitable biocompatibility, high chemical stability, low toxicity, etc. and can be considered as novel nanocarriers in diagnostic and drug delivery systems.
Purpose: This study was biosynthesis of iron oxide nanoparticles coated with folic acid by Bacillus megaterium.
Methods: In experimental part, nanoparticles were biosynthesis with Bacillus megaterium PTCC1656 suspension in the presence of folic acid and then characterized.
Results: In the experimental part, with the results obtained from DLS, we found that the particle size distribution is relatively narrow around 110 nm, and most of the particles are between 30 and 200 nm. According to the obtained zeta potential, the biosynthesized magnetite nanoparticles are stable. According to the XRD results, the nanoparticles have a cubic crystal structure and the space group was Fd-3m form. The examination of FTIR showed us the specific bands of folic acid indicated that this molecule was placed on the surface of nanoparticles and the coating was done well.

Keywords: Folic asid, Iron oxide, Biosynthesis, Bacillus megaterium, Nanoparticle

INTRODUCTION:

In order to increase the stability of drugs in the body and to increase the shelf life and medicinal activity, it is tried to use nanomaterials.(1) Among these nanostructures are magnetic nanoparticles due to their characteristics such as stability in various physiological conditions, suitable biocompatibility, high chemical stability, low toxicity, and high image contrast in MRI and strong super magnetic properties and easy synthesis, they are always considered as suitable nanocarriers in modern diagnostic and drug delivery systems.(2) In this research, iron oxide magnetic nanoparticles coated with folic acid from Bacillus megatrium were biosynthesized and characterized. It is expected that the designed magnetic nanoparticles will be in better conditions in terms of stability, structure and biological activity in order to improve drug efficiency.

Nanoparticles can have technological applications in various fields, from material science to medicine. The performance of a nanoparticle is determined by its chemical structure, which can have complex states of sizes and shapes. In addition, nanoparticles can have a coating layer of different molecules, which strongly affects their properties and performance. In this way, coating molecules can be used to design drugs and produce new tools or systems that are able to identify, signal and interact with different molecules or cells. In this way, coating molecules can be used to design drugs and produce new tools or systems that are able to identify, signal and interact with different molecules or cells. However, there is still a poor understanding of the behavior of nanoparticles, especially when coating molecules are placed on their surface. Modeling, molecular simulation and computational software integrated with experimental experiments is a powerful approach to increase our understanding of fundamental nanoparticle structure and behavior. Molecular simulations such as molecular dynamics or Monte Carlo simulations can clarify the interaction of coating molecules with their environment. Molecular dynamics is a widely used computational analysis method in chemistry, physics, biology and other experimental sciences and can briefly define Newton's equation of motion for a particle system as a step-by-step numerical solution. Molecular dynamics is a type of computer simulation in which atoms and molecules are allowed to interact for a period of time under the known laws of physics, giving a view of the motion of the atoms (1, 2).

Some methods for designing the synthesis of materials are taken from nature to create or improve an ability by imitating the structure and function of biological systems, and in this way they create or improve the structure, function or structures. The formation of magnetic particles in biological membranes is an attractive topic for the production of small nanoparticles under highly controlled conditions. Among the methods of nanoparticle production, the biomimetic synthesis method of MNP2s in the laboratory by means of biomineralization systems of organisms exerts a high level of control over the nucleation and growth of inorganic materials such as carbonate, silicate, and iron oxides. In addition, the production of nanoparticles using bacteria or fungi has received much attention due to advantages such as the absence of biological and environmental hazards, low toxicity and high biocompatibility, cheap production and available resources (3, 4).

1-Magnetic particles:

Magnetic particles are solid phase materials that respond to magnetism, which can be in the form of individual nanoparticles or aggregates of micro and nano particles. Each type of nanoparticles is used in a specific field. The composition, size and synthesis route of magnetic nanoparticles are different according to their usage, but super-paramagnetic, ferro and ferromagnetic particles can be used for various drug delivery applications. Such materials are strongly affected by the external magnetic field due to the magnetic moment of the network unit and the structure of the fields, so that in the absence of the external magnetic field, they act as an inactive particle. Single domain and superparamagnetism are the characteristics of magnetic nanoparticles, which are the origin of many of their unique properties (5).

2- Nanoparticles:

Nanoparticles are particles whose dimensions are between 1 and 100 nanometers. These particles with sizes less than 100 nanometers have different properties compared to particles larger than 100 nanometers. . Nanoparticles show completely new or developed characteristics based on specific characteristics (size, dispersion, morphology, phase, etc.) in comparison with the bulk sizes of the same particles. Nanoparticles can be produced from a wide range of materials, the most common of which are metal oxide ceramics, silicates and non-oxide ceramics. Although nanoparticles made of other materials (such as those based on polymer materials or compound semiconductors) also exist; But the first category is available in many applications today. Nanoparticles show different shapes (sheet, spherical, tree-like, etc.). Metal nanoparticles and metal oxide used are usually spherical and silicate nanoparticles have sheet shapes with dimensions of 100-1000 nm. They are usually produced with appropriate physical characteristics for specific application needs (6).

3-Magnetic nanoparticles (MNP):

Magnetic nanoparticles, which account for a large part of nanomaterials, due to their unique properties such as magnetic resonance and super-paramagnetic momentum and the power of biological interactions at the cellular and molecular levels, potential They have a high level of clinical diagnosis and treatment (7). Based on nanotechnology, MNPs have facilitated a wide range of diagnostic and therapeutic applications in diseases including cancer, heart and neurological diseases (8). Magnetic nanoparticles are widely used in the targeted delivery of therapeutic agents, and they act based on magnetic drug targeting, which includes a strong affinity between ligand and receptor, or through magnetic absorption of specific tissue. MNPs are very significant due to the possibility of remote control of therapeutic agents in the transfer of particles to the desired tissue, and for this reason they are called magnetic targeted carriers. (5)

4-Nano carrier:

Nanoparticles are used as carriers for drug delivery. These carriers hold the drug in the form of a reservoir and release it in a certain amount and in a specific place. As a result, they are effective on the pharmacokinetics, distribution of the drug in the body and reduction of its side effects. Also, nano-carriers due to the control and slowing down of the drug release, protection from the drug molecule, smaller particle size than the cell, the ability to pass through biological barriers, in order to deliver the drug to the target site, increase the durability of the drug in the blood stream, Targeted and biocompatible drug delivery can be considered as a very effective drug delivery system and increase the therapeutic efficiency of the drug (9).

5- Biosynthesis:

Biosynthesis of nanoparticles by microorganisms is a green and environmentally friendly technology. This review focuses on the use of different microorganisms belonging to prokaryotes and eukaryotes for the synthesis of metal nanoparticles. Silver, gold, platinum, zirconium, palladium, iron, cadmium and metal oxides such as titanium oxide, zinc oxide, etc. These microorganisms include bacteria, actinomists , fungi and algae.

Multifunctional iron oxide superparamagnetic nanoparticles

Beneficial Tools in Cancer Diagnostics-Therapeutics Small-sized iron oxide nanoparticles that have superparamagnetic properties show great potential to revolutionize the future of cancer theranostics, combined diagnosis and therapeutic approaches to cancer. Iron oxide superparamagnetic nanoparticles have unique magnetic properties, which is why they show great efficiency in tumor targeting, and this paves the way for specific cancer treatment. The purpose of this review is to focus on the ability of SPIONs to perform multiple roles in cancer biology, such as diagnosis, monitoring, targeting, and therapy. Cancer includes a wide range of diseases that result from the unregulated growth and proliferation of malignant cells. To control this growing burden and improve the quality of life of cancer patients, new technologies with high tumor targeting and drug delivery efficiency are being conceptualized. Among different types of nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) are considered as promising options in cancer theranostics (targeted specific cancer therapy) due to their superparamagnetic behavior and surface modification properties. When the size of iron oxide nanoparticles is reduced to a few nanometers (1-20 nm), they are forced to have a unit domain and become paramagnetic. Unlike multi-domain ferromagnetic materials that retain their magnetism even after the magnetic field is removed, SPIONs lose their magnetism and become highly scattered when the magnetic field is turned off. This feature is very important in clinical applications; Because if nanoparticles tend to aggregate, they can easily be recognized and surrounded by macrophages, thus making them inaccessible for disease treatment (10).

Synthesis of SPIONs:

Depending on the specific requirements, SPIONs can be synthesized by different techniques. Co-deposition is the simplest and most common method to prepare SPIONs. The synthesis of magnetic nanomaterials occurs when iron and iron salts are precipitated in aqueous solutions. The advantages of co-deposition method are: fast synthesis, versatility and high yield of nanoparticles with favorable morphology and characteristics. On the other hand, its disadvantages include large particles with different diameters, polydispersity, and poor crystallization, which can lead to low concentration magnetic surfaces. Microemulsions are formed when dispersions of colloidal materials (such as water in oil or oil in water) Stabilize with the help of surfactants. Then these emulsions can be easily used as nanodevices to perform chemical reactions. This method has the advantage of producing very small nanoparticles with uniform morphology. The weak yield of nanoparticles and the need for large amounts of solvent are the main disadvantages of this method. Thermal decomposition of iron precursors in the presence of high-temperature organic surfactants is another widely used method to produce SPIONs. SPIONs produced by this method have high single scattering speed, good crystallinity and narrow size range. The hydrothermal method, which is considered as one of the oldest methods, produces SPIONs by heating iron precursors in aqueous solutions under controlled temperature and pressure conditions. A new method uses microwaves to synthesize SPIONs of uniform size, and this can be used for large-scale processes. Another new approach to produce monodisperse nanoparticles is the use of sonochemical routes (11).

Metal nanoparticles have attracted attention due to their unique optical properties, catalytic properties, and electrical and magnetic applications (12) and are produced by various chemical and physical methods, which production methods vary in size, catalytic properties, and properties. Its level is impressive (13). Among the various methods and sources of metal nanoparticles biosynthesis, the use of bacteria as a source of metal nanoparticles production is of particular importance. Biomimetic is a new science that takes inspiration from nature to implement plans and processes to solve human problems (14). In other words, in biomimetic methods, inspiration is drawn from nature to design materials and systems in order to create or improve an ability by imitating the structure and function of biological systems and in this way create or improve the structure, function or structures. Following the discovery of magnetotactic bacteria, various scientific fields, including microbiology, geology, mineralogy, crystallography, etc., were transformed (15). Biomimetic synthesis of MNPs in the laboratory by the biomineralization systems of organisms exert a high level of control over the nucleation and growth of inorganic materials such as carbonate, silicate, and iron oxides (16, 17).

Magnetotactic bacteria in some microorganisms have this internal compass and have an organelle called magnetosome (18). Magnetotaxic behavior is also the result of the presence of magnetosome, which surrounds magnetite (Fe3O4) or greigite (Fe3S4) with its membrane (10). The formation of magnetic particles in biological membranes is an attractive topic for the production of synthetic small particles under highly controlled conditions. Small magnetic particles are produced by different physical and chemical methods. However, the particles that are made in this way are not of the same shape and are not completely crystallized, they are not homogeneous in terms of composition and make the production process problematic. Biosynthesis has advantages such as growth control and morphological properties. Magnetosomes are the key components of MTBs, which are intracellular organelles composed of magnetic iron mineral crystals that are individually surrounded by a phospholipid layer and are often located next to or near the cytoplasmic membrane, or in some cases are attached to the membrane. The number of chains and The number of magnetosomes in a chain varies from one bacterial species to another depending on environmental conditions (19). Considering the advantages mentioned, therefore, the synthesis of nanoparticles from biological sources such as bacteria, fungi, algae, yeasts and plants is considered a valuable method because it does not have the possible health and environmental risks of other methods and also uses low-cost production sources (3). Bacillus megaterium bacterium has a deep root in the Bacillus phylogeny, which has made it an important evolutionary species of bacilli. This organism was introduced as a gram-positive model organism long before Bacillus subtilis. Unlike gram-negative organisms such as Escherichia coli (Escherichia coli), Bacillus megatrium does not produce endotoxin at the same time as the growth of the outer membrane, therefore it is used as a non-pathogenic commercial host for the production of biotechnological products as well as biochemical studies. It becomes (20). Iron-oxidizing bacteria are found among both gram-positive and gram-negative groups. Iron-oxidizing bacteria convert ferrous ions to ferric, and different species do this at different rates. Microbial oxidation of iron can be done in both aerobic and anaerobic environments. We can also mention the applications of iron nanostructures in important fields such as biochemical processes, MRI, hyperthermia, DNA and cell labeling, tissue engineering and targeted drug delivery, etc. Due to their source and synthesis method, these nanostructures have high biocompatibility, as well as their high physicochemical stability, allergenicity and low toxicity, in addition to the advantages of their size, they are important (21). Among the advantages of using iron oxide nanoparticles in pharmaceutical research are suitable magnetic properties, low toxicity, high compatibility, relative ease of synthesis compared to other opinions (22, 23). Also, its diagnostic capabilities are due to its magnetic property, which can simultaneously perform targeted drug delivery in addition to disease diagnosis. On the other hand, due to the targeted drug delivery, the toxic side effects of the drug in non-diseased (healthy) tissues are reduced, thus keeping the systemic effect at the lowest level in order to prevent the accumulation of iron nanoparticles and to create superior properties in the drug-targeted systems of the surface of these nanoparticles with different coatings are covered (24). Magnetic nanoparticles have been modified with various organic and biological molecules such as proteins, enzymes, antigens, antibodies, RNA, DNA, as well as biological cells and cellular components, etc. (25).

The introduction of iron oxide nanoparticles into biological and medical sciences and the research of researchers in this field led to the US Food and Drug Administration (FDA) emphasizing only dextran-coated iron oxide nanoparticles for pharmaceutical and medical use (26). Biomimetic methods will be more promising when our understanding of biomineralization pathways improves to the molecular level. This issue will enable the accurate production of quasi-magnetosomal nanoparticles in the test tube by biomimetic means.

In the study of Li et al., polyethyleneimine (PEI) was used to synthesize magnetic iron oxide nanoparticles (FA3O4 NP) coated with folic acid for tumor imaging. This study showed that these coated nanoparticles were able to target a cancer cell model. Also, this nanostructure can be used for MRI imaging (27).

In the study of Landmark et al., iron oxide nanoparticles were conjugated with poly(amidoamine), Tamra fluorescent dye, and folic acid molecules. This study showed that these coated nanoparticles are able to detect and image cells and provide the possibility of being used in targeted drug delivery (28).

In the study of Huang et al., iron oxide nanoparticles coated with PEG and PEI polymers and conjugated with folic acid were made. In this study, this advanced nanostructure was able to deliver targeted drugs to cancer cells in in vitro and in vivo models and was capable of imaging with MRI (29).

Krais et al showed that the targeted absorption of iron oxide nanoparticles coated with folic acid by ovarian cancer cells can take place. Decreasing the expression of folate receptor using siRNA led to a significant decrease in cellular uptake of coated nanoparticles. This phenomenon takes place in the presence of serum (30).

Fatemi et al succeeded in synthesizing MIONPs using a bacterial supernatant extracted with Lactobacillus megantrum bacteria. The produced nanoparticles were characterized using SEM, DLS, VSM, UV-VIS, FT IR and EDS. The analyzes showed that the average particle size of highly stable spherical MIIPS was about 29.3 nm. Bacterial protein profiles obtained from SDS plate analysis indicated the presence of different proteins. Cytotoxicity of nanoparticles was investigated in laboratory environment using MTT test. The results show that the toxicity of nanoparticles was low and dependent on the concentration (31).

In the experimental study of Qani et al., Bacillus megaterium PTCC1656 was cultured in food environment and then exposed to iron nitrate. Finally, with UV, XRD and SEM techniques, the antibacterial properties of nanoparticles were investigated by disk diffusion. This study showed that the iron nanoparticles were cubic in shape and the average size of the nanoparticles was 40 nm. The MIC was measured for Staphylococcus aureus, Bacillus cereus and was estimated to be about 0.15 mg/ml. (32).

METHODS:

In Table 1-3, in Tables 2-3 and 3-3, the materials and equipment used in this research are listed.

Materials used for the biosynthesis of nanoparticles

Materials	Company	Country
Folic acid	Sigma-Aldrich	America
Iron sulfate II	Merck	Germany
Broth nutrient medium	Merck	Germany
Bacillus megatrium	Bank Microbial Collection Center of Industrial Research Organization	Iran
Whatman filter paper	GE healthcare	America
Microbial filter 0.22 micron	Superpore	Germany

Equipment used for biosynthesis and analysis of nanoparticles

Device name	Manufacturer company name	Country name
Autoclave	Tommy	Japan
Freezer temperature -80	Jaltajhiz	Iran
Shikardar Incubator	Jaltajhiz	Iran

Centrifuge	Sigma	Germany
Digital scale	Denver	Germany
Sonicator	Bandelin	Germany
UV-vis spectrophotometer	Unico	Germany
TEM microscope	Zeiss	Germany
X-ray diffraction (XRD)	GNR	Italy
Fourier transform infrared spectroscopy (FTIR)	Shimaszu	Japan
Dynamic Light Scattering (DLS)	Cordouan	France

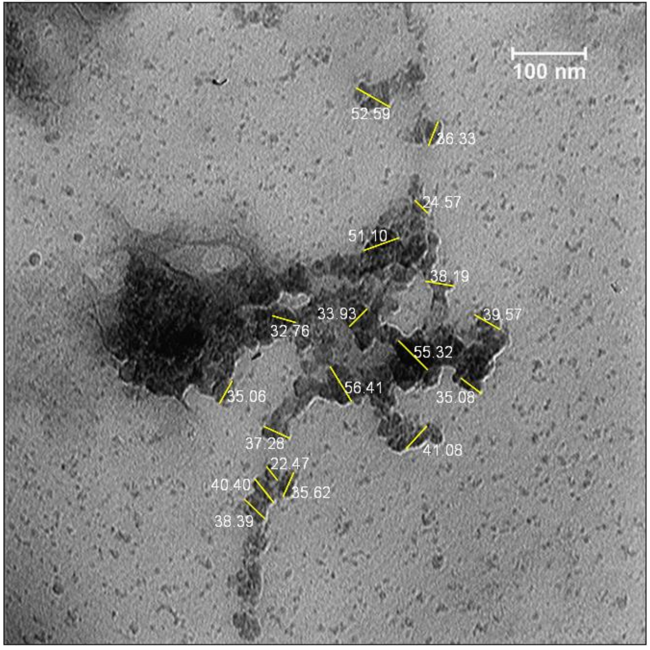
Biosynthesis:
For the biosynthesis of nanoparticles, according to the previous research done by Sajdeh Haj Ali et al., in this experimental study, Bacillus megatrium was cultured in nutrient broth and incubated for 24 hours at 32°C. After ensuring bacterial growth, the obtained bacterial suspension was combined with iron sulfate solution and nanoparticle synthesis was done at room temperature during 20 minutes. The suspension containing bacteria and nanoparticles was first passed through Whatman filter paper and then sterilized using a 0.22 micron microbial filter. After the nanoparticle synthesis, due to the sensitivity and specificity of the body's physiological environment and also the type of drug prescription, obtaining the characteristics and specifications of the synthetic nanoparticle is very important and must be carefully measured.

Determining the characteristics of the nanoparticle obtained:
After nanoparticle synthesis, in order to investigate the general and structural characteristics of nanoparticles, using transmission electron microscopy (TEM), the shape and size of synthesized nanoparticles, to investigate the intensity, peak and position of functional groups from Fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS) method was used to measure the particle size distribution. Also, the dried powder of nanoparticles was tested by X-ray diffraction (XRD) to study its crystalline properties.

RESULTS:

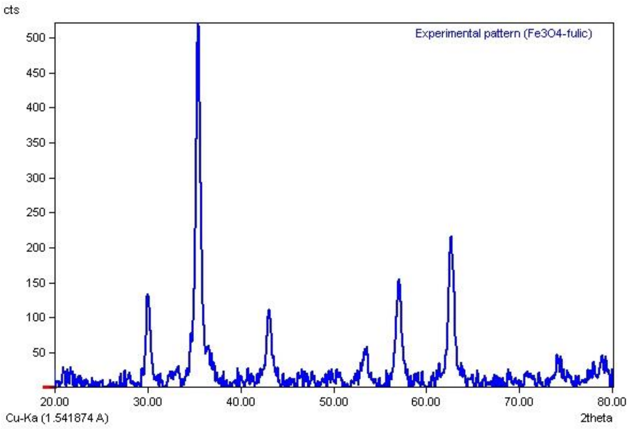
The results of the analyzes obtained from TEM, DLS, FTIR and XRD to determine the characteristics of biosynthesized nanoparticles are given in Figures as follows:

1-TEM: Image J image processing software was used to check the size of these particles and the size of 18 visible particles was labeled on each particle. It is clear that due to the lower density of the solvent compared to the density of the nanoparticles, the nanoparticles appear darker in the image and the solvent is brighter. Therefore, dark colored particles can be seen in this sample, which are nanomagnetite particles. According to this figure, magnetite particles have dimensions of about 25 to 55 nm. The average size of these particles is equal to 39.51 nm and the standard deviation is equal to 8.87 nm.



TEM micrograph of iron oxide nanoparticles coated with folic acid

2- XRD:One of the most important identification methods is the XRD test. In this method, while radiating X-rays to the sample, the reflection of this beam from the crystal plates creates diffraction patterns that are unique to each material, like fingerprints, and through which one can understand the composition of the material and its crystal structure [33]. The X-ray diffraction pattern of the examined sample is shown in Fig.



X-ray diffraction pattern of iron oxide nanoparticles coated with folic acid

X'Pert Highscore plus software was used to identify the phase in this sample. According to the figure, it is clear that only one crystal phase has been identified in this sample, and that is magnetite (Fe_3O_4) with reference code JCPDS No. 96-900-2328 has a cubic crystal structure and space group Fd-3m [34, 35]. In this diffraction pattern, the diffraction planes (220), (311), (400), (422), (511), (400) and (533) are at angles of 30.4°, 35.8°, 43.6°, 53.9°, respectively 57.4, 63.0 and 74.7 °. Also, the fixed size of the grid in this sample is equal to 8.189 angstroms.

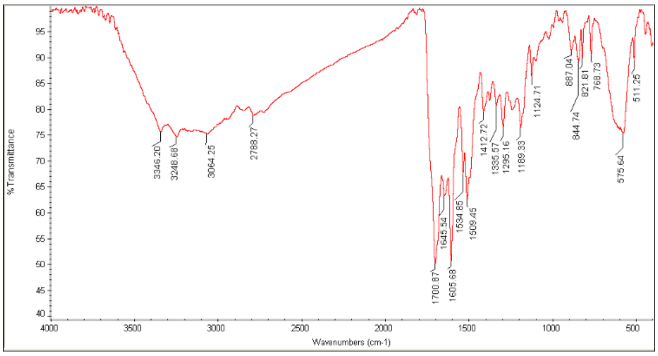
To find the size of the crystal, Scherer's relation (relation (1)) (36).

$D = K\lambda/(FWHM)\times\cos(\theta))$ 1(

In this relation, D is the size of the crystal, K is the shape factor, λ is the wavelength of the X-ray used (1.54 Angstroms), FWHM is the bandwidth at half height, and θ is the location of the peak.

Having the value of cos(θ) and FWHM as well as the constant values of λ (1.54 angstroms) and k (0.9), the value of the crystal size is obtained according to Scherer's relation, which, considering that λ was in angstroms, this value is also in angstroms. The crystal size in nanometers is calculated by dividing the obtained value by 10. This parameter for the magnetite phase in the studied sample is equal to 9.5 nm.

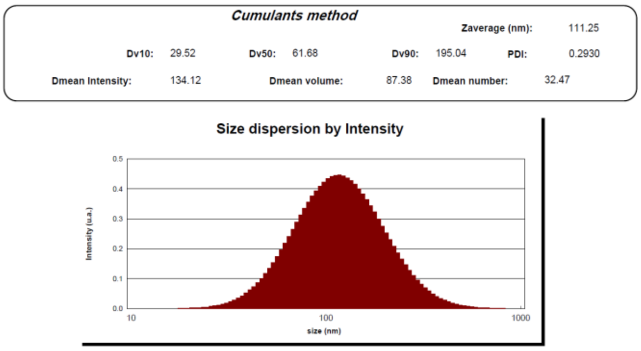
3-FTIR: Another method of material identification is FTIR test. In FT-IR spectroscopy, the energies of the infrared rays coincide with the vibrational energies of molecules, and this adaptation causes the absorption of electromagnetic radiation energy by the sample. Therefore, by changing the frequency of the radiation in a specific range (infrared), a spectrum is obtained whose transmission value is reduced in some wavelengths, or in other words, it is absorbed by the molecules of matter. Therefore, by examining the absorption frequency of each spectrum, it is possible to understand the bonds in that substance. The FT-IR spectrum of the studied sample is shown in the figure.



FT-IR spectrum of iron oxide nanoparticles coated with folic acid

In the FTIR spectrum of this sample, the peaks appearing at the wavelengths of 3346 cm-1 and 3248 cm-1 respectively are related to the stretching vibration of O-H and N-H bonds, which are present in the structure of folic acid [37] and the peak appearing found in the wave number cm-1 3064 is related to the stretching vibration of the C-H bonds in the aromatic ring [38]. The peak located at the wave number cm-1 2788 is also related to the symmetric stretching vibration of the C-H bond in aliphatic structures and the peaks located at the wave number 1700 cm-1 and 1645 cm-1 are respectively related to the stretching vibration of the C=O bond in The carbonyl group in folic acid and the bending vibration of the O-H bond in the structure of this compound [39]. The peak located at the wave number cm-1 1605 is also related to the stretching vibration of the C=C bond in benzene structures and the peaks located at the wave number 1534 cm-1 and 1509 cm-1 are respectively related to the asymmetric stretching vibration of the C-N bonds in The structure is folic acid [40,41]. Also, the peaks located at the wave number of 1412 cm-1 and 1355 cm-1 are related to the bending vibration of the C-H bond in the methyl and methylene structures of this material, respectively [42]. Also, the peaks located at 1295 cm-1, 1189 cm-1 and 1124 cm-1 wavenumbers respectively correspond to the stretching vibrations of C-OH and C-O-C bonds and the peaks located at 887 cm-1, 844 cm-1 and 768 cm-1 is related to dancing and rocking vibrations of C-H bonds in the structure of this acid [40,43]. Also, the peak related to the stretching vibration of the Fe-O bond in the magnetite structure is also located at the wave number cm-1 575 (44,45).

4-DLS: Dynamic light scattering (DLS) is a physical method used to determine the distribution of particles in solutions and suspensions. This non-destructive and fast method is used to determine the size of particles in the range of several nanometers to microns. In this method, the light scattered by the nanoparticles in the suspension changes with time, which can be related to the particle diameter. What is obtained in this test for the size of the particles is the hydrodynamic diameter of the particles, and therefore the values obtained in this test are different from the values obtained from the microscopic test [46]. The results of the dynamic light scattering test related to the examined sample are shown in the figure**.**


Cumulants method

Zaverage (nm): 111.25

Dv10: 29.52 Dv50: 61.68 Dv90: 195.04 PDI: 0.2930

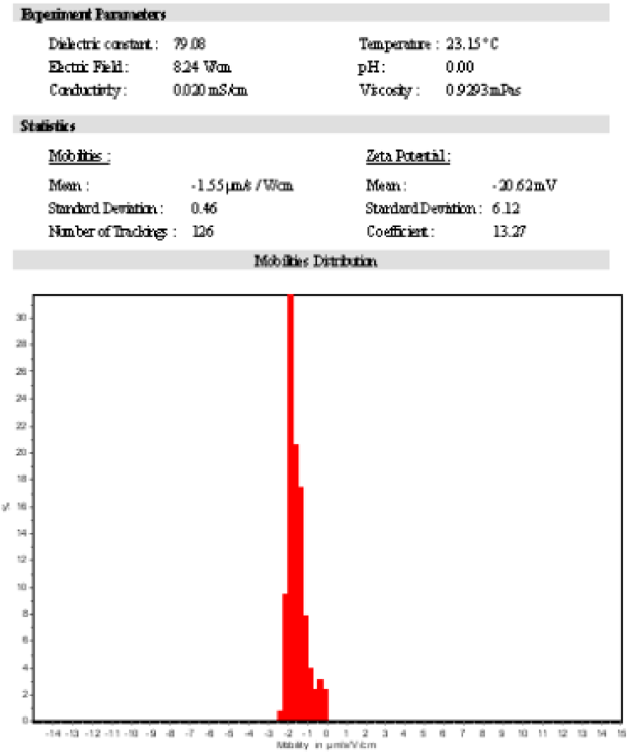
Dmean Intensity: 134.12 Dmean volume: 87.38 Dmean number: 32.47

DLS test results related to iron oxide nanoparticles coated with folic acid

According to the figure, the size distribution of hydrodynamic particles in the studied sample is completely normal and the particles start from about 20 nm and continue to about 900 nm. The average size of nanoparticles is 111.25 nm. In this figure, the parameters Dv10, Dv50 and Dv90 are respectively the diameter of particles that include less than 10%, less than 50% and less than 90% of the particles. According to the results obtained from this test, only 10% of the nanoparticles had a size less than and equal to 29.52 nm, and therefore the size of 90% of the nanoparticles was greater than this value. Also, about

50% of nanoparticles have a size less than 61.68 nm and 90% of nanoparticles have a size less than 195.04 nm. These results show that the vast majority of nanoparticles (more than 80% of nanoparticles) have dimensions between 29.52 and 195.04 nm. The value of the multicast index is obtained from the square of the standard deviation divided by the mean. The value of this index in this sample is equal to 0.2930 and the smallness of this number indicates that the particles have a small standard deviation and therefore the size of the main particles is close to the average value.

5- Zeta potential: Zeta potential is very important to understand and control the properties of colloidal suspensions. Generally, the characteristics of a suspension can be identified by understanding how colloids interact with each other. To keep the particles separate from each other and prevent their accumulation, the repulsion between the particles should reach the maximum possible value. The results of the zeta potential test related to the examined sample are shown in the figure.



Experiment Parameters

Dielectric constant : 79.08
Electric Field : 8.24 V/cm
Conductivity : 0.020 mS/cm
Temperature : 23.15 °C
pH : 0.00
Viscosity : 0.9293mPas

Statistics

Mobilities :
Mean : -1.55 µm/s /V/cm
Standard Deviation : 0.46
Number of Trackings : 126

Zeta Potential :
Mean : -20.62mV
Standard Deviation : 6.12
Coefficient : 13.27

Mobilities Distribution

Zeta potential histogram of iron oxide nanoparticles coated with folic acid

According to this histogram, it is clear that the zeta potential of the sample is -20.62 ± 6.12 mV and the electrophoretic mobility of the particles is -1.55 ± 0.46 µm2/V/cm. It has been proven that the tendency of particles of the same charge to repel each other has a direct relationship with the zeta potential, and therefore the boundary between the stability and instability of the suspension can be determined in terms of the zeta potential. In addition, it has been stated that particles whose zeta potential is greater than 30 mV or less than -30 mV are relatively stable in the spreading bed (46, 47).

Discuss:

Nanoparticles are used in various sciences and with their unique physical and chemical properties, many technological applications can be imagined for them. Nanoparticles in the field of medical science have proposed new solutions in prevention, diagnosis and treatment in the last few decades. In addition to the properties of nanoparticles used, nanoparticle coating layers can also affect their performance properties. In order to understand the effect of coatings and their efficiency, computational or experimental methods can be used. In computational methods that are performed with the help of molecular simulations, the structure and performance of nanoparticles can be predicted to some extent. This scientific field is expanding every day with the progress of computers and computing software and has had a significant impact on the science of nanotechnology.

Nanoparticles can be synthesized by top-down and bottom-up methods, each of which has its own advantages and disadvantages. Meanwhile, the synthetic method that is exactly modeled on nature and approved by researchers and scientists is called green synthesis. With the rules governing this type of synthesis, we are trying to synthesize various nanoparticles, including magnetic nanoparticles. The absence of biological and environmental hazards, low toxicity, high biocompatibility, cheap production and available resources are the advantages of this method.

Iron nanoparticles are widely used in drug and gene delivery and can be used as contrast agents in MRI and CT scan images. Fortunately, this nanoparticle has low toxicity and is well-accepted by various coatings. One of the most important and practical coatings is the diagnostic ligands of cancer cells, among which folic acid has a unique place. Folic acid receptor has a relatively high expression on cancer cells. It is possible that the nanoparticle coated with folic acid has a better and higher absorption ability by cancer cells.

In this first study, iron nanoparticles in three different formulations were designed by simulation method and then the same iron oxide nanoparticles were coated with folic acid. And they were evaluated in terms of size, volume, surface, free energy, etc. in the software environment. Also, experimentally, nanoparticles coated with folic acid were synthesized and characterized by the green synthesis method with the help of Bacillus megaterium bacteria. In the simulation method, we found that coated nanoparticles are much higher than uncoated nanoparticles and their solubility in water is also much higher. Also, in this part of the work, we found that larger nanoparticles were more stable and smaller nanoparticles were less stable. We also found that Fe_3O_4 nanoparticles are more stable than Fe_2O_3 and FeO nanoparticles.

In the experimental part, according to the results obtained from DLS dynamic light scattering test, the average size of nanoparticles is equal to 111.25 nm and the value of multiscattering index in this sample is equal to 0.2930, and the smallness of this number indicates that the particles have a small standard deviation and Therefore, the main size of the particles was close to the average value. Therefore, the results show a relatively narrow particle size distribution around 110 nm, and most of the particles are between 30 and 200 nm.

Next, in order to investigate the microstructure and size of particles using TEM microscope, oxide nanoparticles of magnetite particles with dimensions of 25 to 55 nm were synthesized. The smaller size of the particles in this test compared to the size of the particles obtained in the results of the DLS test is due to the agglomeration of the particles in the suspension state, while the TEM test was taken in the dry state and therefore the particles can be single-dispersed.

According to the histogram obtained from the results of the zeta potential test, it was found that the zeta potential value of the sample was equal to -20.62 ± 6.12 mV and the electrophoretic mobility of the particles was equal to -1.55 ± 0.46 µm2/V/cm. In fact, the zeta potential of more or less than ±30 millivolts creates an electrostatic repulsion force between the particles placed in the suspension and leads to the relative stability of these particles. Therefore, the biosynthesized magnetite nanoparticles are stable and the accumulation (agglomeration) of the particles has been prevented.

Also, by using XRD test, the existence and synthesis of magnetite nanocrystals by bacteria was confirmed. In this way, it was determined that the nanoparticles have a cubic crystal structure and space group Fd-3m. The crystal size obtained from Scherer's relation for the magnetite phase in the studied sample was found to be 9.5 nm.

In the end, by using the FTIR test and by checking the absorption frequency of each spectrum, we found out the bonds in the material. The results of the FTIR test, along with the XRD test results, confirm the presence of magnetite nanoparticles that are covered by folic acid.

With the help of the obtained results, these nanoparticles can be used in future projects for the purposes of gene therapy, drug therapy and imaging. It is recommended that in future studies, apart from Bacillus megatrium, bacteria, fungi and even viruses are also used for biosynthesis and nanoparticles synthesized by these microorganisms are compared with each other. It is also recommended that other specialized coatings and ligands be used in future studies besides folic acid, and after synthesis, their absorption rate by cancer cells should be evaluated.

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Magnetotactic bacteria in some microorganisms have this internal compass and have an organelle called magnetosome (18). Magnetotaxic behavior is also the result of the presence of magnetosome, which surrounds magnetite (Fe3O4) or greigite (Fe3S4) with its membrane (10). The formation of magnetic particles in biological membranes is an attractive topic for the production of synthetic small particles under highly controlled conditions. Small magnetic particles are produced by different physical and chemical methods. However, the particles that are made in this way are not of the same shape and are not completely crystallized, they are not homogeneous in terms of composition and make the production process problematic. Biosynthesis has advantages such as growth control and morphological properties. Magnetosomes are the key components of MTBs, which are intracellular organelles composed of magnetic iron mineral crystals that are individually surrounded by a phospholipid layer and are often located next to or near the cytoplasmic membrane, or in some cases are attached to the membrane. The number of chains and The number of magnetosomes in a chain varies from one bacterial species to another depending on environmental conditions (19). Considering the advantages mentioned, therefore, the synthesis of nanoparticles from biological sources such as bacteria, fungi, algae, yeasts and plants is considered a valuable method because it does not have the possible health and environmental risks of other methods and also uses low-cost production sources (3). Bacillus megaterium bacterium has a deep root in the Bacillus phylogeny, which has made it an important evolutionary species of bacilli. This organism was introduced as a gram-positive model organism long before Bacillus subtilis. Unlike gram-negative organisms such as Escherichia coli (Escherichia coli), Bacillus megatrium does not produce endotoxin at the same time as the growth of the outer membrane, therefore it is used as a non-pathogenic commercial host for the production of biotechnological products as well as biochemical studies. It becomes (20). In the study of Li et al., polyethylenimine (PEI) was used to synthesize magnetic iron oxide nanoparticles (FA3O4 NP) coated with folic acid for tumor imaging. This study showed that these coated nanoparticles were able to target a cancer cell model. Also, this nanostructure can be used for MRI imaging (27). In the study of Landmark et al., iron oxide nanoparticles were conjugated with poly(amidoamine), Tamra fluorescent dye, and folic acid molecules. This study showed that these coated nanoparticles are able to detect and image cells and provide the possibility of being used in targeted drug delivery (28). In the study of Huang et al., iron oxide nanoparticles coated with PEG and PEI polymers and conjugated with folic acid were made. In this study, this advanced nanostructure was able to deliver targeted drugs to cancer cells in in vitro and in vivo models and was capable of imaging with MRI (29). Krais et al showed that the targeted absorption of iron oxide nanoparticles coated with folic acid by

ovarian cancer cells can take place. Decreasing the expression of folate receptor using siRNA led to a significant decrease in cellular uptake of coated nanoparticles. This phenomenon takes place in the presence of serum (30). Fatemi et al succeeded in synthesizing MIONPs using a bacterial supernatant extracted with Lactobacillus megantrum bacteria. The produced nanoparticles were characterized using SEM, DLS, VSM, UV-VIS, FT IR and EDS. The analyzes showed that the average particle size of highly stable spherical MIIPS was about 29.3 nm. Bacterial protein profiles obtained from SDS plate analysis indicated the presence of different proteins. Cytotoxicity of nanoparticles was investigated in laboratory environment using MTT test. The results show that the toxicity of nanoparticles was low and dependent on the concentration (31). In the experimental study of Qani et al., Bacillus megaterium PTCC1656 was cultured in food environment and then exposed to iron nitrate. Finally with UV, XRD and SEM techniques. Antibacterial properties of nanoparticles were investigated by disc diffusion. This study showed that. The iron nanoparticles were cuboid in shape and the average size of the nanoparticles was 40 nm. The MIC was measured for Staphylococcus aureus, Bacillus cereus and was estimated to be about 0.15 mg/ml. (32)
The most important discussion related to the design of such nanostructures is its application in the treatment and imaging of cancer cells, which must be investigated in future studies. In this study, due to time and financial limitations, cell tests were not performed on the synthesized nanoparticles. In any way, it seems that the nanoparticles produced by the green method with the help of Bacillus megatrium have suitable synthetic characteristics and have the ability to replace conventional synthetic methods.

CONCLUSION:

In the simulation part, this study showed that the stability of coated nanoparticles is much higher than non-coated nanoparticles and their dehydration energy in water is also higher. We also found that larger nanoparticles were more stable and smaller nanoparticles were less stable. We also found that Fe_3O_4 nanoparticles are more stable than Fe_2O_3 and FeO nanoparticles. In the experimental part, with the results obtained from the DLS test, we found that the particle size distribution is relatively narrow around 110 nm, and most of the particles are between 30 and 200 nm. According to the obtained zeta potential, the biosynthesized magnetite nanoparticles are stable and the accumulation (agglomeration) of the particles has been prevented. According to the XRD results, the nanoparticles have a cubic crystal structure and space group Fd-3m. The examination of FTIR test, also with the specific bands of folic acid, indicated that this molecule was placed on the surface of nanoparticles and the coating was done well. With the help of the obtained results, these nanoparticles can be used in future projects for the purposes of gene therapy, drug therapy and targeted multi-functional imaging.

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