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ORIGINAL ARTICLE

Evaluation of the Antimicrobial Activity of Citric Acid Functionalized Magnetite Nanoparticles

[1]Ahmed M. El-Khawaga*, [2]Ayman A. Farrag, [3]Ahmed I. El-Batal, [1]Mohamed A. Elsayed

[1]Chemical Engineering Department, Military Technical College, Egyptian Armed Forces, Cairo, Egypt
[2]Professor & Chairman of Microbiology Department, Faculty of Science Al-Azhar University, Cairo, Egypt; Director of Al-Azhar Center for Fermentation Biotechnology and Applied Microbiology, Cairo, Egypt
[3]Drug Microbiology Lab., Drug Radiation Research Department, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt

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Corresponding Author:
Ahmed M. El-Khawaga
Chemical Engineering Department, Military Technical College, Cairo, Egypt.
ahmedelkhwaga15@gmail.com
Tel.: 01278707820

ABSTRACT

Background: *Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria no longer susceptible to the common medicines used to treat them.* ***Objectives:*** *To development of Fe_3O_4 NPs with specific sizes and shapes Potential as a new antibacterial agent.* ***Methodology:*** *In this study magnetite nanoparticles (Fe_3O_4 NPs) were synthesized through an aqueous co-precipitation method and functionalized with citric acid for outstanding their antimicrobial potential. Fe_3O_4 NPs were characterized by XRD, TEM, SEM, EDX and FTIR to analyze crystallinity, average particle size, morphology and functional groups, respectively. Antimicrobial activity was investigated against pathogenic bacteria as zone of inhibition (ZOI) and minimum inhibitory concentration (MIC).* ***Results:*** *Antimicrobial results showed that CA- Fe_3O_4 NPs owns maximum activity against Staphylococcus aureus and E. coli by 18.0 and 15.0mm ZOI, respectively.* ***Conclusion:*** *It should be noted that (CA-Fe_3O_4) NPs are also active upon Gram-positive than Gram- negative bacteria. The synthesized (CA-Fe_3O_4) NPs are promising for potential applications as antimicrobial agent and in drug delivery fields.*

INTRODUCTION

Serious infections caused by microorganisms resistant to commonly used antimicrobials have become a major healthcare problem worldwide in the 21st century. This is responsible for the significant increase in morbidity and mortality, longer hospitalization and increased health care costs. Keeping in view the seriousness of this problem, the World Health Organization (WHO) has selected "Antimicrobial resistance: No action today no cure tomorrow" as the theme for World Health Day 2011 as a preventive measure. Recently, the development of nanobiotechnology has led to the preparation of Fe_3O_4 NPs with specific sizes and shapes Potential as a new antibacterial agent[1-3].

The functional activity of Fe_3O_4 NPs is largely affected by their size. The magnetic, physical, chemical and effective biological properties make Fe_3O_4 NPs have valuable medical applications. Moreover, nanoparticles with smaller molecule sizes have been appeared to have antibacterial properties[4]. Numerous examinations occurred on the antimicrobial activity of Fe_3O_4 NPs on different organisms[1, 5–7].

The microbial cells are in the micrometer size while external cell layers have pores in the nanometer size. Due to the smaller size of nanoparticles contrasted with bacterial pores, they have the remarkable capacity of penetrate cell layers. The most widely recognized techniques to plan iron oxide nanoparticles are coprecipitation[8], thermal decomposition, hydrothermal[9], polyol methods[10], electrochemical methods[11], and sonication[8]. Adjustment of magnetic nanoparticles stability can occur by controlling on one of electrostatic and steric repulsion forces or both of them [12].

The density and molecular weight of the polymers affect the steric repulsive force [13, 14], while the pH and ionic strength of the solution affect electrostatic repulsion force. Hence, adjustment of these factors, stabilization of magnetic nanoparticles can be accomplished by using various stabilizers; for example, monomeric stabilizers such as carboxylates [15, 16], phosphates[17], inorganic stabilizers such as silica[18], gold[19], polymers stabilizers such as dextran[20], polyethylene glycol[21], polyvinyl alcohol[22], alginate[23], chitosan[24], poly(N-2-hydroxyethyl)-D,Laspartamide-graft-poly (butyl methacrylate [25], and folic acid[26].

The aim of the work was to prepare an citric acid coated iron oxide nanocomposite, characterize their structural, and evaluate their antimicrobial activity by comparison to bare magnetite nanopatrticle.

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METHODOLOGY

Materials

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), Sodium hydroxide (NaOH), citric acid ($C_6H_8O_7$), were purchased from E-Merck Products. All reagents were with analytical grade and used without further purification.

Isolation of Microorganisms

Escherichia coli, *Staphylococcus aureus* isolated from human urinary tract infections were received from the culture collections in Drug Microbiology Lab., Drug Radiation Research Dep., NCRRT, Cairo, Egypt.

Synthesis of magnetite nano particles (Fe_3O_4) by chemical co precipitation method

Aqueous solutions of Fe^{2+} and Fe^{3+} in their molar ratio (1:2) were prepared in deionised water. Solutions were mixed in a separate flask and kept on ultrasonic stirrer for 10-15 minutes at room temperature to get homogenised solution. After that (1 M) NaOH solution was drop wise added to the mixture of salts solution at a fixed rate and elevated temperature (90 degree) on ultrasonic stirrer. Co-precipitation occurs in alkaline medium between pH 8-14. Mixing and stirring continues till all salts were precipitated in black colour magnetite nanoparticles (Fe_3O_4). Reaction was completed in two hours and further half an hour was required for aging. The black precipitate of magnetite nano particles were separated by using external magnetic field and washed thrice with deionised water till pH is near neutral (6.5 -7). Finally, the magnetic Fe_3O_4 nanoparticles were obtained after drying in a vacuum oven. The chemical reaction during the co-precipitation of ferrous and ferric salts occurs as follows:

$$Fe^{2+}+Fe^{3+}+8OH^- \rightarrow Fe_3O_4+4H_2O \quad (1)$$

In the present study instead of maintaining inert atmosphere to check the oxidation of ferrous ions (Fe^{2+}), co-precipitation was allowed at high temperature (80 - 90^{0}C).

Synthesis of citric acid-coated magnetic Fe_3O_4 nanoparticles

The surface of Fe_3O_4 NPs was modified with citric acid by a direct addition method [28], to obtain modified Fe_3O_4 NPs with carboxylic groups. Briefly, the Fe_3O_4 NPs was mixed with a CA aqueous solution (0.02 g/ml), and left to react at 60 °C during 90 min to obtain citric acid coated magnetite. To yield uncoated magnetite, the black precipitated was washed several times and resuspended in water at a pH close to neutral one (7- 7.4). The pH at which CA was adsorbed to the magnetite surface was varied from 4.58 to 7.08. Ammonia Solution (0.25 % w/w) was used to adjust the suspension pH to close to 7. Then the suspension was again placed in a permanent magnet during 10h. Fe_3O_4 NPs stabilized with citric acid are abbreviated as (CA-Fe_3O_4) NPs.

Characterization of materials

Crystal structures of the samples were determined using an XRD-6000 diffractometer (Shimadzu, Tokyo, Japan) with CuKα radiation (λ 1.5406 Å) at 30 kV and 30 mA to the over a range of 20°–70°. The functional groups of the materials were determined using Fourier transform infrared (FTIR) by Nexus, Smart Orbit spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) over a range of 400–4,000 cm-1 by KBr disk method. The surface structure and homogeneity of the synthesized ferrite NPs were determined by scanning electron microscopy (SEM) ZEISS, EVO-MA10 equipped with Energy-Dispersive X-ray spectra (EDX) for elemental analysis.

Finally, the images of TEM were taken by a JEM-1010 (Jeol, Japan) operated at 200 kV of an accelerating voltage.

Antimicrobial activity and minimal inhibitory concentration (MIC)

The synthesized (CA- Fe_3O_4) NPs. Nanocomposite (20.0 µg/ml) were tried for their antimicrobial performance producing the agar-disc distribution process[27, 28]. Bacterial strains were *Escherichia coli* and *Staphylococcus aureus*. Conventional antibiotic discs (AX; 20µg/ml; 6.0 mm diameter), was chosen to determine the performance of the considered magnetic nanocomposite. The serial dilutions method of Luria-Bertani (LB) medium was applied to evaluate the (MIC) of the tested samples with the highest antimicrobial activity[29]. For these determinations, the medium broth was used as a negative control, the examined pathogenic microbes, bare Fe_3O_4 NPs, citric acid and (CA- Fe_3O_4) NPs (beginning with concentration= 20.0 µg/ml) were applied as positive control. MIC was determined next 24 h. incubation at 36.0 ± 1.0°C [30].

Statistical analysis

The mathematical analysis of the effects was conducted by applying the ONE WAY ANOVA (at $P < 0.05$), the least significant differences (LSD), and Duncan's multiple systems[31]. The effects and data were examined and decided through SPSS software (version 15).

RESULTS AND DISCUSSION

Characterization of prepared nanoparticles

X-ray diffraction

The XRD patterns of (CA- Fe_3O_4) NPs show the crystalline structure of nanoparticle as depicted in Fig. 1. For Fe_3O_4 the peak were obtained 30.2° (2 2 0), 35.6° (3 3 1), 43.2° (4 0 0) 53.4° (4 2 2) 57.2° (5 1 1), and 62.7° (4 4 0) at 2 degree indicated the cubic spinal structure of magnetite[32]. The similar peaks were found for (CA- Fe_3O_4) NPs, which reveals that citric acid coating does not result in the phase change of bare Fe_3O_4[33, 34].

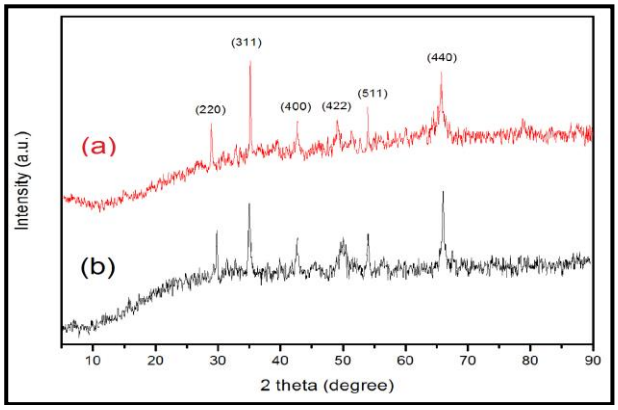

Fig. 1: XRD pattern of magnetic nanoparticles (a) bare magnetic nanoparticles (Fe_3O_4), (b) citric acid coated magnetic nanoparticles (CA- Fe_3O_4) NPs.

FTIR

The FTIR spectrum range (4000–400 cm^{-1}) of citric acid coated Fe_3O_4 dispersed in KBr revealed bands from the iron citrate spectrum. A large and intense band at 3450 cm^{-1} that could be assigned to the structural OH groups as well as to the traces of molecular water and citric acid. Whereas the 1700 cm^{-1} peak assignable to the C O vibration (symmetric stretching) from the COOH group of citric-acid (CA) shifts to an intense band at about 1600 cm^{-1} for the Fe_3O_4 coated with citric acid (Fe_3O_4-Cit) revealing the binding of a CA radical to the magnetite surface showing in Fig. 2. Furthermore, the vibrational modes appearing at 1253 cm^{-1} and 1075 cm^{-1} in (CA- Fe_3O_4) NPs correspond to the symmetric stretching of COO^- and OH group of citric acid. It has been proposed that citric acid binds to the magnetite surface by chemi-sorption of the carboxylate, that is, citrate ions[34]. The low-intensity bands between 400 and 600 cm^{-1} are attributed to the stretching vibration mode associated to the metal-oxygen Fe–O bonds in the crystalline lattice of Fe_3O_4.

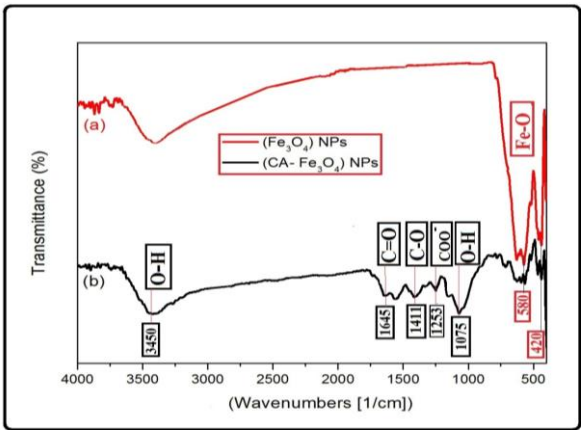

Fig. 2: FTIR spectra of magnetic nanoparticles **(a)** bare magnetic nanoparticles (Fe_3O_4)**, (b)** citric acid coated magnetic nanoparticles (CA-Fe_3O_4).

Scanning electron microscopy and transmission electron microscopy analysis

Morphological analysis was studied with electron microscopic images. In the SEM images of Fe3O4 nanoparticles, it can be seen clearly that the particles are uniformly aggregated, spherical shaped with size 8–25 nm (figure 3). SEM images of magnetic uncoated-Fe3O4 nanoparticles and CA-Fe3O4 are shown in figure 3. The results suggested that all nanoparticles are most spherical in shape and the size of CA-Fe3O4 nanoparticles was in the range from 8–17 nm. The results suggest that the core/shell particles remain single crystals with average diameter of 8 nm,
and the thickness of citric acid shells is approximately 9 nm. The results may demonstrate that the citric acid layer is consistently deposited on Fe_3O_4 nanoparticles, as shown in figures 3(b).

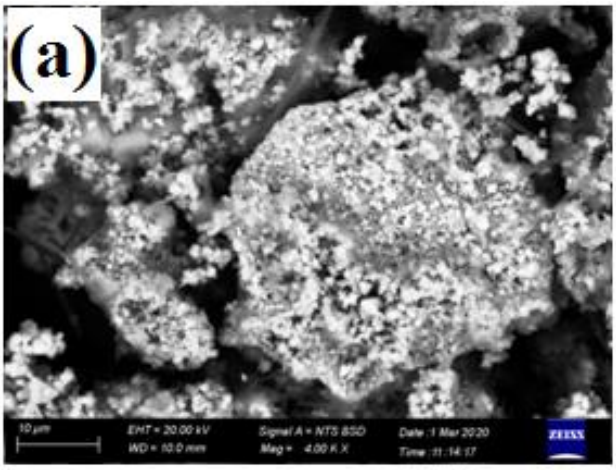

Fig. 3a: SEM images of uncoated Fe3O4.

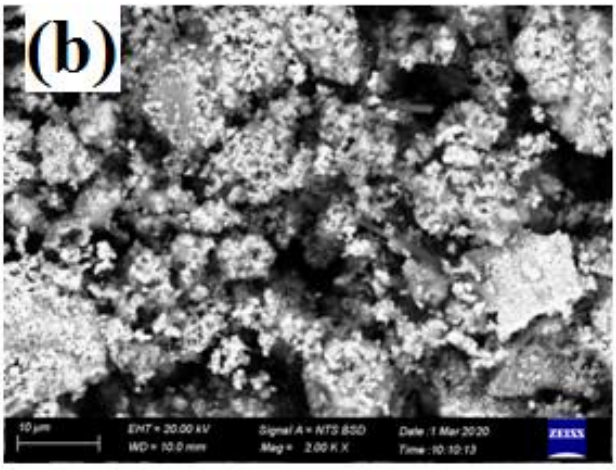

Fig. 3b: SEM images of CA-Fe3O4 nanoparticles.

TEM images demonstrate that the bare Fe_3O_4 and CA-Fe_3O_4 magnetic nanoparticles are spherical particles with average size of 13–17 nm as in figure 4. Accordingly, the citric acid prevents the aggregation between the particles. These data may prove the appearance of the bonds on surface of magnetic Fe_3O_4 nanoparticles.

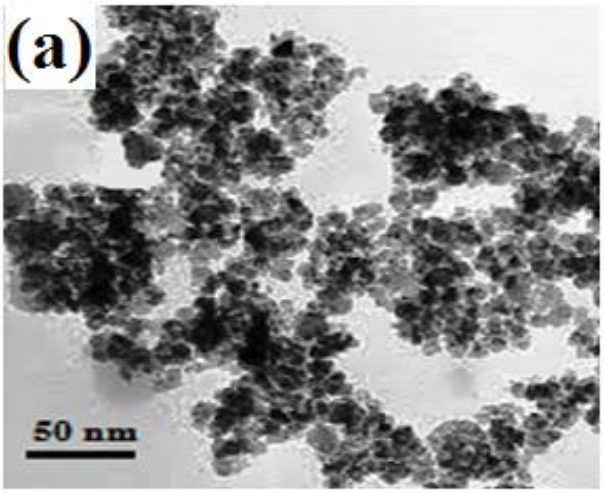

Fig. 4a: TEM images of uncoated Fe_3O_4

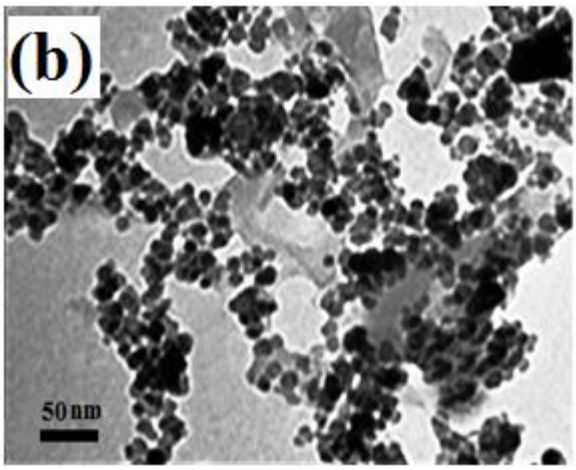

Fig. 4b: TEM images of CA-Fe_3O_4, nanoparticles

***In vitro* antimicrobial activity of the synthesized (CA-Fe_3O_4) NPs, Citric acid and Fe_3O_4 NPs**

It is remarked from the disc agar distribution method (as a screening procedure) that the citric acid and Fe_3O_4 NPs represented a qualitative antimicrobial potential toward the tested bacteria. The *in-vitro* ZOI result verified that (CA-Fe_3O_4) NPs exhibited its encouraged antibacterial activity against *S. aureus* (18.0 mm ZOI; Fig. 5a), and *E. coli* (15.0 mm ZOI; Fig. 5b) as displayed in table 1.

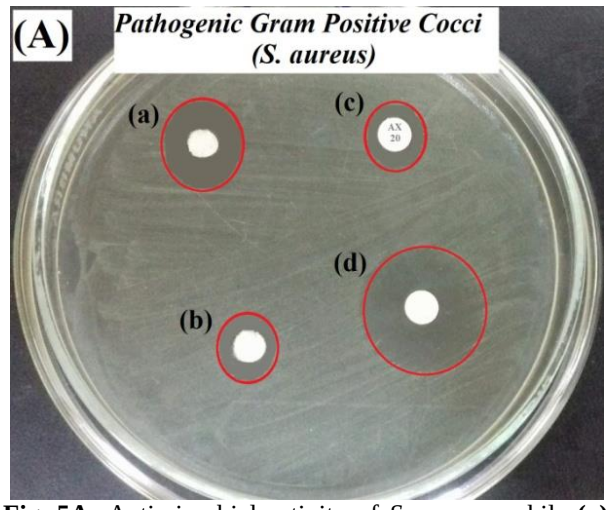

Fig. 5A: Antimicrobial activity of *S. aureus*, while **(a)** bare Fe_3O_4 NPs, **(b)** Citric Acid, **(c)** standard Amoxicillin and **(d)** CA- Fe_3O_4 in each plate.

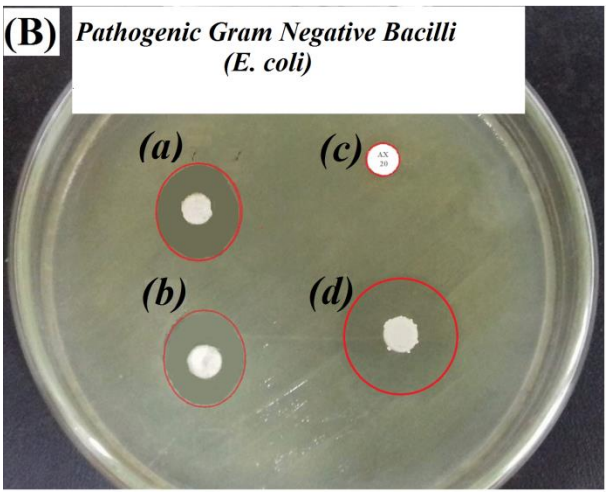

Fig. 5B: Antimicrobial activity of *E. coli*, while **(a)** bare Fe_3O_4 NPs, **(b)** Citric Acid, **(c)** standard Amoxicillin and **(d)** CA- Fe_3O_4 in each plate.

It worth considering that the antibacterial potency of (CA-Fe_3O_4) NPs was significantly more powerful than MgF NPs, CA and standard antibacterial agents (Amoxicillin; AX). It is necessary to recognize that; the (CA-Fe_3O_4) nanocomposite were active upon Gram positive (*S. aureus*) further than Gram-negative (*E. coli*) bacteria (18.0 and 15.0 mm ZOI respectively) as recorded in table 1.

Table 1: Antibacterial activity of CA- Fe_3O_4 NPs, Fe_3O_4 NPs and Citric Acid (CA) against pathogenic *E. coli* and *S. aureus* measured as ZOI (mm) and MIC (µg/ml)

Assay / Pathogens	Zone Of Inhibition (ZOI)				Minimal Inhibitory Concentration (MIC)	
	Fe_3O_4	Citric acid	CA-Fe_3O_4	AX	Fe_3O_4	CA-Fe_3O_4
E. coli	10.0	10.0	15.0	-ve	10^{-1}	10^{-3}
S. aureus	16.0	10.0	18.0	10.0	10^{-1}	10^{-4}

The cell walls constituents in Gram-negative bacteria contain principally little layers of lipopolysaccharide, lipid, and peptidoglycan. On the other hand, the cell walls of Gram-positive incorporate very solid peptidoglycan forms[35]. The MIC results of (CA-Fe_3O_4) NPs against *S. aureus* and *E. coli* were 10^{-4} and 10^{-3} µg/ml as mentioned in table 1. (CA-Fe_3O_4) NPs possess promising MIC of 10^{-4} against *S. aureus*.

CONCLUSION

Fe_3O_4 NPs have been synthesized by a chemical co-precipitation method and characterized by structural and optical tools. The surface of Fe_3O_4 NPs was stabilized with citric acid form CA-Fe_3O_4 NPs. Furthermore, their antibacterial behavior has been examined against different pathogenic bacteria which separated from urine samples of UTI-patients. It should be noted that (CA-Fe_3O_4) NPs are also active upon Gram-positive than Gram- negative bacteria. The synthesized (CA-Fe_3O_4) NPs are promising for potential applications as antimicrobial agent and in drug delivery fields.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Rai J, Randhawa GK, Kaur M: Recent advances in antibacterial drugs. International Journal of Applied and Basic Medical Research 2013, 3(1):3.
2. Zazo H, Colino CI, Lanao JM: Current applications of nanoparticles in infectious diseases. Journal of Controlled Release 2016, 224:86-102.
3. Paladini F, Pollini M, Sannino A, Ambrosio L: Metal-based antibacterial substrates for biomedical applications. Biomacromolecules 2015, 16(7):1873-1885.
4. Qiao R, Yang C, Gao M: Superparamagnetic iron oxide nanoparticles: from preparations to in vivo MRI applications. Journal of Materials Chemistry 2009, 19(35):6274-6293.
5. Sahoo Y, Pizem H, Fried T, Golodnitsky D, Burstein L, Sukenik CN, Markovich G: Alkyl phosphonate/phosphate coating on magnetite nanoparticles: a comparison with fatty acids. Langmuir 2001, 17(25):7907-7911.
6. Arias LS, Pessan JP, Vieira APM, Lima TMTd, Delbem ACB, Monteiro DR: Iron oxide nanoparticles for biomedical applications: a perspective on synthesis, drugs, antimicrobial activity, and toxicity. Antibiotics 2018, 7(2):46.
7. Niemirowicz K, Durnaś B, Tokajuk G, Głuszek K, Wilczewska AZ, Misztalewska I, Mystkowska J, Michalak G, Sodo A, Wątek M: Magnetic nanoparticles as a drug delivery system that enhance fungicidal activity of polyene antibiotics. Nanomedicine: Nanotechnology, Biology and Medicine 2016, 12(8):2395-2404.
8. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A: Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. International journal of nanomedicine 2012, 7:6003.
9. Gupta AK, Gupta M: Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. biomaterials 2005, 26(18):3995-4021.
10. Hachani R, Lowdell M, Birchall M, Hervault A, Mertz D, Begin-Colin S, Thanh NTK: Polyol synthesis, functionalisation, and biocompatibility studies of superparamagnetic iron oxide nanoparticles as potential MRI contrast agents. Nanoscale 2016, 8(6):3278-3287.
11. Ramimoghadam D, Bagheri S, Abd Hamid SB: Progress in electrochemical synthesis of magnetic iron oxide nanoparticles. Journal of Magnetism and Magnetic Materials 2014, 368:207-229.
12. Lu AH, Salabas EeL, Schüth F: Magnetic nanoparticles: synthesis, protection, functionalization, and application. Angewandte

Chemie International Edition 2007, 46(8):1222-1244.

13. Ayala V, Herrera AP, Latorre-Esteves M, Torres-Lugo M, Rinaldi C: Effect of surface charge on the colloidal stability and in vitro uptake of carboxymethyl dextran-coated iron oxide nanoparticles. Journal of nanoparticle research 2013, 15(8):1874.
14. Gupta AK, Naregalkar RR, Vaidya VD, Gupta M: Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. 2007.
15. Kharisov BI, Dias HR, Kharissova OV, Vazquez A, Pena Y, Gomez I: Solubilization, dispersion and stabilization of magnetic nanoparticles in water and non-aqueous solvents: recent trends. RSC Advances 2014, 4(85):45354-45381.
16. Karimzadeh I, Aghazadeh M, Doroudi T, Reza Ganjali M, Hossein Kolivand P, Gharailou D: Superparamagnetic iron oxide nanoparticles modified with alanine and leucine for biomedical applications: development of a novel efficient preparation method. Current Nanoscience 2017, 13(3):274-280.
17. Sahoo Y, Pizem H, Fried T, Golodnitsky D, Burstein L, Sukenik CN, Markovich G: Alkyl phosphonate/phosphate coating on magnetite nanoparticles: a comparison with fatty acids. Langmuir 2001, 17(25):7907-7911.
18. Alcala M, Real C: Synthesis based on the wet impregnation method and characterization of iron and iron oxide-silica nanocomposites. Solid State Ionics 2006, 177(9-10):955-960.
19. Lin J, Zhou W, Kumbhar A, Wiemann J, Fang J, Carpenter E, O'connor C: Gold-coated iron (Fe@ Au) nanoparticles: synthesis, characterization, and magnetic field-induced self-assembly. Journal of Solid State Chemistry 2001, 159(1):26-31.
20. Lacava L, Lacava Z, Da Silva M, Silva O, Chaves S, Azevedo RBd, Pelegrini F, Gansau C, Buske N, Sabolovic D: Magnetic resonance of a dextran-coated magnetic fluid intravenously administered in mice. Biophysical Journal 2001, 80(5):2483-2486.
21. Xu H, Yan F, Monson EE, Kopelman R: Room-temperature preparation and characterization of poly (ethylene glycol)-coated silica nanoparticles for biomedical applications. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials 2003, 66(4):870-879.
22. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN: Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chemical reviews 2008, 108(6):2064-2110.
23. Ma H-l, Qi X-r, Maitani Y, Nagai T: Preparation and characterization of superparamagnetic iron oxide nanoparticles stabilized by alginate. International journal of pharmaceutics 2007, 333(1-2):177-186.
24. Zhu L, Ma J, Jia N, Zhao Y, Shen H: Chitosan-coated magnetic nanoparticles as carriers of 5-fluorouracil: preparation, characterization and cytotoxicity studies. Colloids and Surfaces B: Biointerfaces 2009, 68(1):1-6.
25. Licciardi M, Scialabba C, Fiorica C, Cavallaro G, Cassata G, Giammona G: Polymeric nanocarriers for magnetic targeted drug delivery: preparation, characterization, and in vitro and in vivo evaluation. Molecular pharmaceutics 2013, 10(12):4397-4407.
26. Licciardi M, Scialabba C, Cavallaro G, Sangregorio C, Fantechi E, Giammona G: Cell uptake enhancement of folate targeted polymer coated magnetic nanoparticles. Journal of biomedical nanotechnology 2013, 9(6):949-964.
27. Jamshidi D, Sazegar MR: Antibacterial Activity of a Novel Biocomposite Chitosan/Graphite Based on Zinc-Grafted Mesoporous Silica Nanoparticles. International Journal of Nanomedicine 2020, 15:871.
28. Kourmouli A, Valenti M, van Rijn E, Beaumont HJ, Kalantzi O-I, Schmidt-Ott A, Biskos G: Can disc diffusion susceptibility tests assess the antimicrobial activity of engineered nanoparticles? Journal of Nanoparticle Research 2018, 20(3):62.
29. Mosallam FM, El-Sayyad GS, Fathy RM, El-Batal AI: Biomolecules-mediated synthesis of selenium nanoparticles using Aspergillus oryzae fermented Lupin extract and gamma radiation for hindering the growth of some multidrug-resistant bacteria and pathogenic fungi. Microbial pathogenesis 2018, 122:108-116.
30. El-Batal AI, Balabel NM, Attia MS, El-Sayyad GS: Antibacterial and antibiofilm potential of mono-dispersed stable copper oxide nanoparticles-streptomycin nano-drug: implications for some potato plant bacterial pathogen treatment. Journal of Cluster Science 2019:1-20.
31. Maksoud MA, El-Sayyad GS, Ashour A, El-Batal AI, Elsayed MA, Gobara M, El-Khawaga AM, Abdel-Khalek E, El-Okr M: Antibacterial, antibiofilm, and photocatalytic activities of metals-substituted spinel cobalt ferrite nanoparticles. Microbial pathogenesis 2019, 127:144-158.

32. Silva VAJ, Andrade PL, Silva MPC, Bustamante D A, De Los Santos Valladares L, Albino Aguiar J: Synthesis and characterization of Fe3O4 nanoparticles coated with fucan polysaccharides. Journal of Magnetism and Magnetic Materials 2013, 343:138-143.
33. Beltran CHP: Magnetic nanoparticles for biosensing and immunoprecipitation. 2019.
34. Singh D, Gautam RK, Kumar R, Shukla BK, Shankar V, Krishna V: Citric acid coated magnetic nanoparticles: synthesis, characterization and application in removal of Cd (II) ions from aqueous solution. Journal of water process engineering 2014, 4:233-241.
35. Walter A, Mayer C: Peptidoglycan Structure, Biosynthesis, and Dynamics During Bacterial Growth. In Extracellular Sugar-Based Biopolymers Matrices. Springer; 2019: 237-299.