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Spectroscopic Characterization of Iron Oxide Nanoparticles Functionalized with Chitosan Biosynthesis by a Clean one Pot Method

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ABSTRACT

Co-precipitation is the simplest way to obtain iron oxides in different forms such as magnetite (Fe_3O_4) or maghemite (Fe_2O_3). Although this method is a classic ones but is economic, and versatile as well. It consists of mixing ferric (Fe-III) and ferrous ions (Fe-II) in strong basic conditions. PH of the medium, ionic strength and ratio of salts are the factors which affect the quality of nanoparticles prepared. This method has been manipulated here to synthesis iron oxide nanoparticles functionalized with chitosan. The precursor of chitosan in this study is the fungal species *Aspergillus deflectus* and *Penicillium pinophilum*. The synthesized nanoparticles were characterized by FTIR and TEM. The results show that the mycelia of *P.pinophilum* was better to synthesis iron oxide nanoparticles considering particle size and dispersity. Nanoparticles sizes were 75 and 20 nm by *Aspergillus deflectus* and *Penicillium pinophilum respectively.*

Key words: *Fungal mycellia, Aspergillus deflectus, Penicillium pinophilum,* spectroscopic and microscopic characterization.

Introduction

Magnetic nanoparticles (MNP) synthesis can be performed via different chemical routs, including microemulsions, co-recipitation, sol-gel syntheses, aerosol, template assisted, sonochemical, laser exposer, wet-chemical synthesis, thermal decomposition, plasma synthesis, sonochemical reactions, hydrothermal reactions, hydrolysis and the rmolysis of precursors, flow injection syntheses and electrospray syntheses (Jain *et al.,* 2013). However, these conventional methods needs multiple conditions, pH, temperature and pressure, much expensive equipment, and toxic chemicals. Furthermore, such techniques also generate different toxic to ecosystems byproducts therefore, there is a growing requirement to replace them by a low-cost, ecofriendly methods. In addition, the synthesis of superparamagnetic nanoparticles is complicated because of their colloidal nature (Mazumdar and Haloi, 2011). Monodisperse colloid is the essential chemical consideration. Microorganisms growing in metal-rich regions such as soil exert metal absorption or adsorption of metals and their chelation by extra- or intracellular proteins (Pócsi, 2011). Therefore, fungus isolated from native metal-rich soil conditions can be a better source for bio- synthesis metal nanoparticles as an indigenous microbial ecotype results from the long-term adaptation to soil with extreme properties.

The functional groups on the surface of the nanoparticles are used for covalent bonding with biomolecules, drugs and for catching ions or molecules in detection processes. One of the major applications of the MNP is magnetic resonance imaging where they are used as contrasting agents. The functionalization strategies used can affect the magnetic properties of resulting functionalized MNP, therefore, care should be considered in choosing the functionalization strategies while targeting such applications. Apart from biomedical applications iron oxide possess various other applications which include use functionalized iron oxides for constructions of detector systems, as a pigments and as probes or adsorbates for removal specific substances from the mixtures, catalysts, or catalytic supports and fillers for nanocomposites preparations. There are many reports where magnetic iron oxides bearing various groups, functionalized with polymers, amino acids, and biomolecules (Jadhav and Bongiovanni, 2012; Anamaria *et al.,* 2012).

Molecular modeling as well as molecular spectroscopy is well known methods for identification of natural systems with and/or without nano modifications (Ibrahim *et al.,* 2006; Ibrahim *et al.,* 2010; Morsy et al., 2014; El-Khodary *et al.,* 2014). Both are supporting each other's not only in nano scale but also in other systems and application in biological and different branches of science (Okasha *et al.,* 2015; Ibrahim, 2010; Ibrahim and Osman, 2010).

The complicated natural bottom-up chelating process inspired the present trial of synthesizing iron oxide nanoparticle from two different fungal species identified previously as *Aspergillus deflectus* and *Penicillium pinophilum*. Transmission electron microscopy was employed to characterize the shape and size of the

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synthesized magnetic nanoparticles and the molecular structure changes of the fungal mycelia was observed by Fourier transform infrared to determine the functional groups on the cell wall that have performed the bonding with nanoparticles.

Materials and Methods

Materials:

Iron (II) chloride -hexahydrate ($FeCl_3$. 6H2O), Ammonium iron(II) sulfate hexahydrate $(NH_4)2Fe(SO_4)_2 \cdot 6H_2O$ were purchased from Merck (Germany) and used as received without any purifications.

Production of biomass

Aspergillus deflectus and *Penicillium pinophilum* were isolated from soil samples which collected from local areas in Egypt. Flasks containing potato dextrose broth were incubated with tested fungal isolates at 25^{o}C, under shaking condition (180 rpm).

Identification of isolates

The culture media were performed at the genus level depending on their morphological characters. In addition to, the microscopic examination for conidia and hyphae (Barnett *et al.,* 1987; Pitt, 1979; Domsch *et al.,* 1980). The media consists of potato dextrose agar and Czapeks-dox agar media. The identification of fungal isolates was done in Mycology Lab of Botany and Microbiology Department, Faculty of Science, Helwan University.

Intracellular synthesis of iron oxide nanoparticles.

Under asepsis conditions, the fungal mycelia dried and then weighted were filtered from the mediacautiously. In a clean Erlenmeyer flask, the 5mM $FeCl_3$ and 2.5 mM$Fe(SO_4)_2$ were mixed in 50 ml sterile distilled water and the pH adjusted to 12.5 under vigorous shaking. When the color changes to dark brown the mycelia were re-suspended in the solution with vigorous shaking. After 24 hours, the solution were filtered, the mycelia were dried, weighted and examined by FTIR. The nanopaticles were then characterized.

Characterization of magnetic Nanoparticles

Fourier transform infrared spectroscopy (FTIR) analysis was performed to study the molecular groups responsible for capping and functionalization of iron oxide nanoparticles. The dried biomass (before and after synthesis) were mixed with Potassium Bromide (KBr) at a ratio of 1:100 and the spectra were recorded with a Vertex 70 Bruker Transform Infrared Spectrophotometer at resolution 1cm^{-1} in the range between 4000 to 400 cm^{-1}.

Average particle size and size distribution were determined by Transmission electron microscope of the aqueous suspension of iron oxide nanoparticles, prepared by placing a drop of the suspension on carbon-coated copper grids and anabling the water to evaporate. The morphology and structure of samples was performed by electronmicroscope JEOL (JEM-1400 TEM) at the convenient magnification. Images weretaken by CCD camera model AMT, optronics camera with 1632 x 1632 pixel format as side mount configuration. This camera work by a 1394 fire wire boarded for acquision.

Results and Discussion

TEM measurements were used to study the morphological confirmation of iron nanoparticles. Figure 1 showed well distribution of iron oxide nanoparticles. Furthermore, the upper part of the figure show the square and triangle shape of the nanoparticles synthesized by *A.deflectus,* moreover, the particle size was between 75 and 100 nm. on the other hand, the lower part of the figure show the highly dispersed, homogenous sized and spherical iron oxide nanopaticles synthesized by *P.pinophilum.*The figure also show that the particle size of iron oxide synthesized by *P.pinophilum* range was 10-20 nm. This figure show that the conditions of synthesis in this work, was convenient for the synthesis of iron oxide nanopatricles from *P.pinophilum,* whereas, this condition of preparation should be modified for the A. deflectus

The FTIR absorption spectra of *Aspergillus deflectus* and *Pencillium pinophilium* mycelia are shown in figure 2. The figure reveals the assignment of chitin present in the cell wall of the two species before and after the MNP biosynthesis, their corresponding deconvolution (in the range 1800-700 cm^{-1}) is in the lower part of the figure (Osman *et al.,* 2015) .Seven bands for *Aspergillus deflectus* and *Penicillium pinophilum* has been assigned in the biosynthesis of Ag/CS NP (Ahmad *et al.,* 2011; Govindan *et al.,* 2012; Lin *et al.,* 2011). Chitin is one of the main compound of fungal cell wall (Pochanavanich and Suntornsuk,2002) , these peaks (Lillo, and Matsuhiro, 1997; Ninganagouda *et al.,* 2014) demonstrate the MNP/Chitosan nanoparticle (MNP/CS NPs) synthesis.The absorption band at 3437 cm^{-1}can be attributed to N-H or O-H stretching, the 2916 cm^{-1} band is assigned to C-H stretching, The FTIR spectrum of chitosan shows two bands of exopolysaccharides at 1654.80

and1596.94 cm^{-1} due to a C=O stretching vibration of an N-acetyl group and N-H stretching of a primary amine group characteristic bands. Additionally, the C-O deformation of a secondary alcoholic group is found at 1417 cm^{-1} (Lillo *et al.,* 2007).The sharp peak at 1038 cm^{-1}could be due to that the monosaccharide in EPS has a pyran structure. The absorption band at 877 cm^{-1} (C-N finger print band of chitosan)reveals that the glucoside bond in the exopolysaccharides is β-linkage.The *Aspergillus deflectus* after synthesis show a significant decrease in the intensity of following bandsamide I, amide II, asymmetric vibrations of CO (1150-1000 cm^{-1}). This result indicates the involvement of N-H, C=O and C-O in the bonding with iron oxide nanoparticles. On the other hand, *Penicillium pinophilum* after synthesis show the partial disappearance of N-H band and the significant decrease in the band at 1158 cm^{-1} (attributed to νas(C-O-C)) in expense of that at 1038 cm^{-1}attributed to νs(C-O-C). These results reveal that, the involvement of the mentioned groups in the bonding of chitosan to the iron oxide nanoparticles.

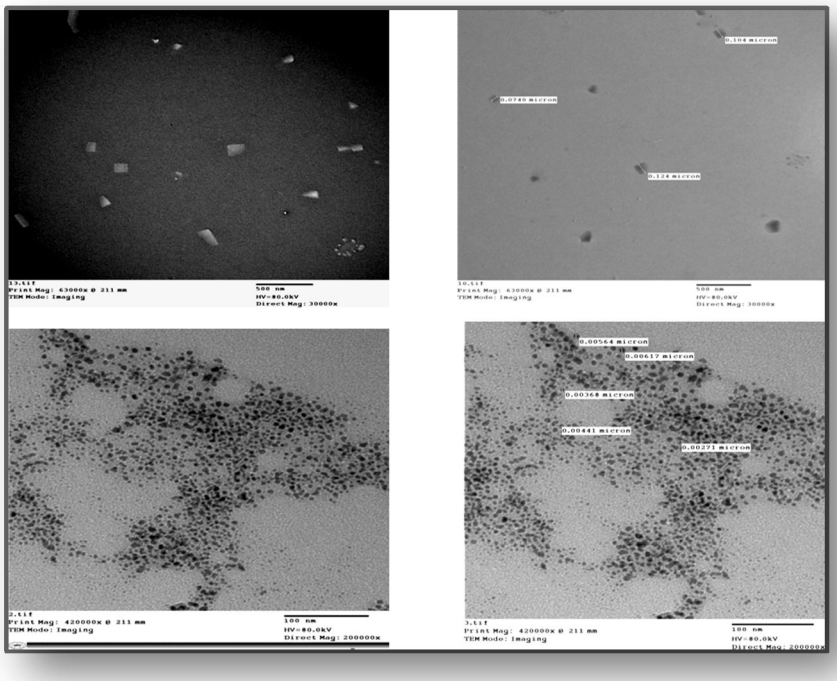

Fig. 1: TEM of iron oxide nanoparticles synthesised by *A.deflectus*(upper)and*P.pinophilum*(lower).

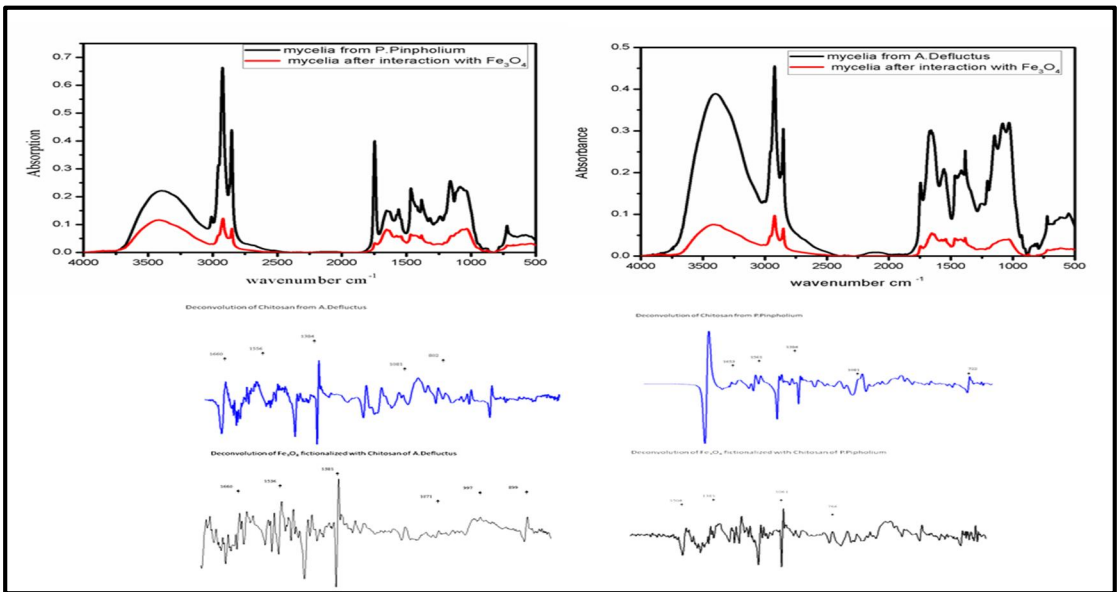

Fig. 2: FTIR spectrum of *A.deflectus*(left)and*P.pinophilum*(right) mycelia before (black) and after (red) the synthesis of iron oxide nanoparticles. In the lower part their corresponding deconvolution, blue for the mycelia before synthesis and black for the mycelia after synthesis.

Figure 3 shows the FTIR spectrum of Fe_3O_4 nanoparticles biosynthesized by A.defluctus and P.pinpholium. Both spectrums assign the bands at 3423, 2907, 2845, 1636, 1447, 1382, 1057, and 873 cm^{-1}. These bands confirm the decoration of the iron oxide nanoparticles by chitosan and the involvement of the N-H bond stretching in the interaction between chitosan and the Fe_3O_4nanopaticles

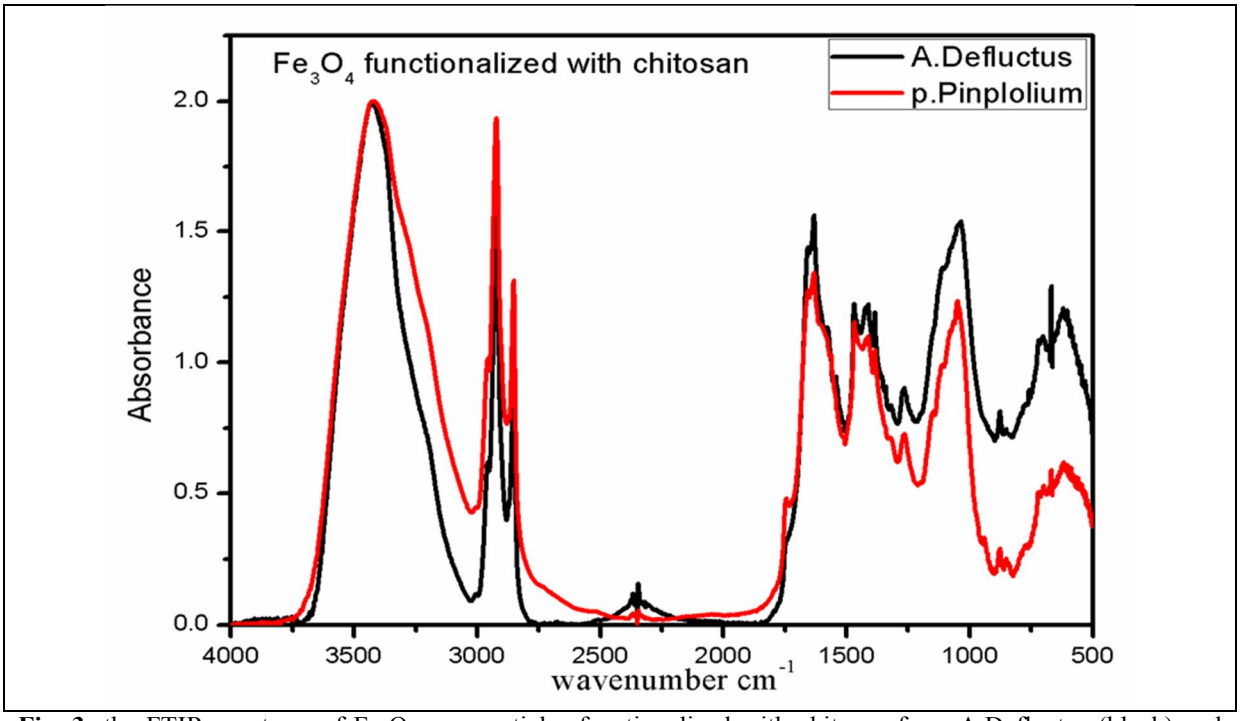

Fig. 3: the FTIR spectrum of Fe_3O_4 nanoparticles functionalized with chitosan from A.Defluctus (black) and P.Pinpholium(red).

Conclusion:

Aspergillus deflectus and *Penicillium pinophilum* has been employed in the co-precipitation method for the biosynthesis of iron oxide nanoparticles functionalized with chitosan. The data reveals that the *Penicillium pinophilum* the nanoparticles shape and sizes resulted from the biosynthesis varies completely although the same condition of preparation has been applied. The iron oxide nanopaticles resulted from the *Aspergillus deflectus* were square and triangle in shape and the sizes various from 75-100 nm. Whereas, *Penicillium pinophilum* particles show spherical shape and the sizes varies from 20-55 nm. On the other hand, the FTIR results show the functionalization of the produced nanoparticles with chitosan functional groups. This method of preparation prove to be facile, simple and less costive than other preparation methods. further work is needed to characterize the particles magnetic properties and to examine different preparation conditions in controlling particle size and shape, in addition, the medical application of this particles in photodynamic therapy has to be followed in the next paper.

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References

Jain, N., A. Bhargava, J. C. Tarafdar, S. K. Singh and J. Panwar, 2013. A biomimetic approach towards synthesis of zinc oxide nanoparticles. Applied microbiology and biotechnology, 97(2), 859-869.

Mazumdar, H., and N. Haloi, 2011. A study on biosynthesis of iron nanoparticles by Pleurotus sp. J. Microbiol. Biotechnol. Res, 1(3), 39-49.

Pócsi, I., 2011. Toxic metal/metalloid tolerance in fungi—a biotechnology-oriented approach. In Cellular Effects of Heavy Metals (pp. 31-58). Springer Netherlands.

Jadhav, S. A., and R. Bongiovanni, 2012. Synthesis and organic functionalization approaches for magnetite (Fe3O4) nanoparticles. Adv Mat Lett, 3(5), 356-361.

Anamaria, D.A., P. Mariana, and C. S. Bogdan, 2012. Tailored andFunctionalized Magnetite Particles for Biomedical and Industrial Applications, Materials Science andTechnology, Prof. SabarHutagalung (Ed.), ISBN: 978-953-51-0193-2, InTech.

Ibrahim, M. Alaam, M. El-Haes, A. F. Jalbout, and A.D. Leon, 2006.Analysis of the Structure and Vibrational Spectra of Glucose and Fructose. Ecl. Quim., Sao Paulo, 31: 15-21.

Ibrahim, M. Mahmoud, A.A. Osman, O. Refaat, A. and M. E. El-Sayed, 2010.Molecular Spectroscopic Analyses of Nano Chitosan Blend as Biosensor, Spectrochimica Acta A.77, 802-806.

Morsy, M. Helal, M. El-Okr, M. and M. Ibrahim, 2014. Preparation, Purification and Characterization of High Purity Multi-Wall Carbon Nano Tube, Spectrochimica Acta A. 132, 594‑598.

El-Khodary, S. A. El-Enany, G. M. El-Okr, M. and M. Medhat, 2014 .Preparation and Characterization of Microwave Reduced Graphite Oxide for High-Performance Supercapacitors", Electrochimica Acta. 150, 269–278.

Okasha, A. Gomaa, F. Elhaes, H. Morsy, M. El-Khodary, S. Fakhry, A. and M. Ibrahim, 2015. Spectroscopic Analyses of the Photocatalytic Behavior of Nano Titanium Dioxide, Spectrochim. Acta A. 136, 504-509

Ibrahim, M., 2010. Molecular Spectroscopic Study of Acid Treated Fenugreek Seeds, Spectrochimica Acta A. 77, 1034–1038.

Ibrahim, M. and O Osman, 2010. Spectroscopic Analyses of Cellulose: Fourier Transform Infrared and Molecular Modelling Study, J. Comput. Theor. Nanosci. 6, 1054-1058.

Barnett, H. L., and B. Hunter Barry, 1987. Illustrated Genera of Imperfect Fungi,'' 4th Edition, Macmillan Publishing coy, New York, Collier Macmillan Publishers, London.

Pitt, J. I., 1979. The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces.

Domsch, K. H., W. Gams and T. H. Anderson, 1980. Compendium of soil fungi (Vol. 1, No. 2). London: Academic press.

Osman, M. E., M. M. Eid, O. H. Khattab, S. M. El-Hallouty, D.A. Mahmoud, 2015. Spectroscopic Characterization of the Effect of Gamma Radiation on the Physical Parameters of eco-friendly synthesized Silver/Chitosan Nano-particles and their Antimicrobial Activity. Quantum matter, In Press.

Ahmad, M. B., M. Y. Tay, K. Shameli, M. Z. Hussein, and J. J. Lim, 2011. Green synthesis and characterization of silver/chitosan/polyethylene glycol nanocomposites without any reducing agent. International journal of molecular sciences, 12(8), 4872-4884.

Govindan, S., E. A. K. Nivethaa, R.S aravanan, V. Narayanan and A. Stephen, 2012. Synthesis and characterization of chitosan–sil ver nanocomposite.Applied Nano science, 2(3), 299-303.

Lin, Y., W. Jing, P. Kang, Z. Xiaoming, W. Zhouping and X. Wenshui, 2011. Preparation and Characterization of Core/Shell-type Ag/Chitosan Nanoparticles with Antibacterial Activity. Bulletin of The Korean Chemical Society, 32(4), 1277-1281.

Pochanavanich, P., and W. Suntornsuk, 2002. Fungal chitosan production and its characterization. Letters in applied microbiology, 35(1), 17-21.

Lillo, L. E., and B. Matsuhiro, 1997. Chemical modifications of carboxylated chitosan. Carbohydrate Polymers, 34(4), 397-401.LilloLE MatsuhiroB 2003Carbohydr. Polym. 51 317 – 325.

Ninganagouda, S., V. Rathod and D. Singh, 2014. Characterization and biosynthesis of Silver nanoparticles using a fungus Aspergillusniger. International Letters of Natural Sciences, 10.

Lillo, L., J. Alarcón, G. Cabelloamp and S. Águila, 2007. Structural studies of native Paecilomyces sp. exopolysaccharide. Zeitschrift für NaturforschungC,62(9-10), 623-626