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Comprehensive Characterization and Antibacterial Evaluation of Synthesized Magnetite Nanoparticles for Enhanced Biosensor Applications: A Multi-Instrumental Study Using FESEM, FTIR, And XRD Analyses

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Abstract: In recent years, the development of advanced nanomaterials has gained significant attention due to their unique physicochemical properties and potential applications in various fields, including biotechnology and medicine. Magnetite nanoparticles (MNPs) have emerged as a promising candidate for biosensor applications owing to their magnetic properties and tunable surface functionalities. This study focuses on the comprehensive characterization of prepared magnetite nanoparticles and explores their potential antibacterial activity for biosensor development. The characterization involves the utilization of Field Emission Scanning Electron Microscopy (FESEM), Fourier-Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) instrumental techniques to assess the size, smoothness, distribution, and crystal structure of the MNPs.

Keywords: MNPs–Magnetite or Iron oxide Nanoparticles, XRD–X-Ray Diffraction Spectrophotometer, FESEM–Finite-Element Scanning Electron Microscopy, FT-IR –Fourier Transform Infra- red Spectroscopy.

1. Introduction

In the realm of nanotechnology and materials science, the development of advanced nanomaterials has opened new avenues for enhancing the performance of biosensors, a pivotal domain in healthcare diagnostics, environmental monitoring, and biomedical research. Among these nanomaterials, magnetite nanoparticles have gained significant attention due to their unique magnetic properties,biocompatibility, and diverse applications in biosensing. This paper presents a comprehensive investigation into the synthesis, characterization, and antibacterial evaluation of magnetite nanoparticles, with a specific focus on their potential for enhancing biosensor applications.

Biosensors play a critical role in the rapid and sensitive detection of various analytes, such as biomolecules and pathogens, by converting biochemical signals into measurable electrical or optical outputs. The efficiency of biosensors largely depends on the choice of nanomaterials used for signal transduction. Magnetite

nanoparticles, composed of iron (II) and iron (III) ions, have emerged as promising candidates for biosensor development, primarily due to their superparamagnetic behavior, high surface area, and tunable surface chemistry. These characteristics enable the immobilization of biomolecules on their surfaces and the efficient capture of target analytes, resulting in enhanced sensitivity and selectivity.

This study employs a multi-instrumental approach, incorporating Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) analyses to comprehensively investigate the physicochemical properties, surface functionalization, and crystalline structure of the synthesized magnetite nanoparticles. Such a multifaceted analysis is essential to gain insights into the nanoparticle's morphology, chemical composition, and crystallinity, which are pivotal in tailoring their performance for biosensor applications.

Furthermore, the evaluation of the synthesized magnetite nanoparticles' antibacterial properties holds significant importance, as biosensors often find application in the detection and monitoring of bacterial infections. The interaction between these nanoparticles and bacteria may elucidate their potential in pathogen detection and eradication.

2. Characterization of MNPs

This synthesis method allows for the precise control of particle size, shape, and surface properties by adjusting various parameters, such as reactant concentrations, temperature, and reaction time. The as-synthesized magnetite nanoparticles were then subjected to a comprehensive characterization process to understand their physicochemical properties, as detailed in subsequent sections of this paper.

2.1 Field Emission Scanning Electron Microscopy (FESEM) Analysis:

FESEM analysis was employed to investigate the morphology and size distribution of the magnetite nanoparticles. High-resolution images were captured at various magnifications to assess the shape and surface characteristics of the nanoparticles. The FESEM images provided valuable insights into the size uniformity and agglomeration tendencies of the particles.

2.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis:

FTIR spectroscopy was conducted to examine the surface functional groups and chemical bonding of the magnetite nanoparticles. By recording the infrared absorption spectra, we could identify the presence of functional groups and ligands on the nanoparticle surfaces. The FTIR analysis facilitated the assessment of surface modifications and potential biomolecule immobilization sites, critical for biosensor applications.

2.3 X-ray Diffraction (XRD) Analysis:

XRD analysis was performed to elucidate the crystalline structure and phase purity of the synthesized magnetite nanoparticles. The X-ray diffraction pattern was analyzed to determine the crystallographic phases and the average crystallite size. This analysis confirmed the formation of magnetite (Fe3O4) and provided information on the nanoparticles' degree of crystallinity, which is vital for their magnetic and electronic properties.

In summary, the combination of FESEM, FTIR and XRD analyses offers a comprehensive understanding of the physical, chemical, and structural properties of the synthesized magnetite nanoparticles. This detailed characterization is essential for tailoring the nanoparticles for enhanced biosensor applications, where their physicochemical properties play a critical role in the sensor's performance and sensitivity.

2.4 Antibacterial Activity of Magnetite Nanoparticles:

The assessment of the antibacterial properties of the synthesized magnetite nanoparticles is of paramount importance, as it directly relates to their potential in biosensor applications, particularly in the detection and eradication of bacterial pathogens.

Experimental Procedure was conducted using **Disk Diffusion Assay** in which magnetite nanoparticles were incorporated into filter paper disks. The disks were then placed onto agar plates inoculated with the respective bacterial strains. The inhibitory zones, if any, were measured as an indicator of the nanoparticles antibacterial activity.

3. Results And Discussion

The surface properties and chemical makeup of the nanoparticles were examined using FTIR. To determine the potentialfunctional group in iron oxide nanoparticles that is responsible for capping and stabilizing nanoparticles, FTIR spectra of produced magnetite nanoparticles were conducted. It is possible to see the absorption and emission brought on by molecule rotation and vibration inthe infrared area of electromagnetic waves. It identifies the component content of the sample (quantification) according to band intensity and qualitatively exposes the unknown composition according to the characteristic frequency of the bands. Additionally, it can identify isomers, expose the molecular structure (including functional groups and bonds), and ascertain complex structures. By dissecting the band change, one can also comprehend the way molecules interact. This method can be used to measure samples in the state of gas, liquid and solid. There are also extremely sensitive changes in molecular structure called "fingerprint zone" (1300~400 cm^{-1}), which provides a reliable determination to analyze unknown chemical composition and structure (Perkin 1987).

3.1 FTIR Analysis:

The chemically synthesized NPs of iron oxide were analysed using FTIR to identify the nature of biomolecules involved in the synthesis and stabilisation. Figure 1 depicts the FTIR spectrum which shown certain characteristic peaks at 3386.1, 1590.3, 578.4cm^{-1}associated with magnetite nanoparticles. The peaks 3340-3670, have been assigned to the C-Hstretching (Casillus 2012). The peak at 1472 is attributed to the stretching vibration of carboxyl (C-O-C) group. The magnetite nanoparticles canbe seen by a strong absorption band at around 578.4cm^{-1} attributed to the Fe-O stretching band of bulk iron oxide (Fe_3O_4). These results revealed that the C=O groups were bonded on the surface of iron oxide particle. The absorption bands 983.50 cm^{-1} and 844.88cm^{-1} are due to the stretching vibrations of hydroxyl (OH) onthe magnetite particle surface. Moreover, the two broad bands at 3386.1cm^{-1} and 1590.3cm^{-1} can be attributed to the amine (N–H) stretching vibration (Mohamed et al 2011) and hence results in the formation of magnetitenanoparticles. It has been observed that the absorption band at a high wave number region are due to the OH stretching which suggests that the surfaces of the Fe_3O_4 nanoparticles are covered with a number of hydroxyl groups as is clear and well reported when they are preparedin the aqueous phase as suggested by Awwad and Salem (2013).

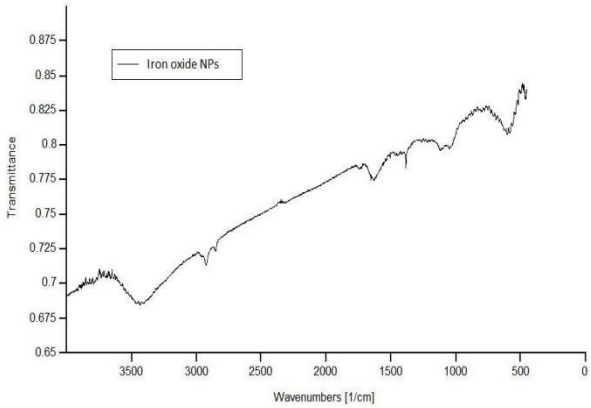

Fig 1: FT-IR Spectra of Synthesized IronOxide Nanoparticles

In the case of magnetite, infrared spectroscopy is extremely helpful because it results from the interaction of divalent and trivalent cations with electromagnetic radiation. This interaction involves the excitation of molecules in their ground electronic state forvibration or rotation, and it is also linked to stretching deformation of the interatomic bonds and bending deformation of the interbond angles (Casillus et al. 2012).

Hence FTIR was used toidentify the functional group present in the prepared iron oxide

nanoparticles. The main groups are Fe-O and Fe-OH.

3.2 XRD Analysis:

Rigaku, a Japanese company, has been used to collect XRD data on the magnetite NPs. One can learn about a material's composition, structure (including the atoms' three-dimensionalcoordinates, chemical bonds, molecular conformation, and three-dimensionalconformation), and information on how molecules interact from the XRD data. One of the most crucial techniques for characterizingthe structure of crystalline material is XRD. It may ascertain the particle size in addition to the sample phase and phase composition. The diffraction line broadening happens when the grain size is less than 100 nm. The Debye-Scherrer equation, which provides a link between peak broadening in XRD and particle size, can also be used to quantitatively assess theparticle sizes from the XRD data.

$$d = (k\lambda/\beta\cos\theta)$$

where d is the particles size, k is the Debye– Scherrer constant (0.89), λ is the X-ray wavelength (0.15406 nm) and β is the full width at half maximum, θ is the Bragg angle (Sun et al 2006).

Figure 2 displays the XRD patternsof the magnetite nanoparticles produced via co precipitation. Sharp diffraction patterns signify the synthesized sample's tiny size, great purity, and crystallinity (Sun et al. 2010). The magnetite nanoparticles' XRD pattern shows twodistinct peaks at 35.73 o and 62.96 o. The nanoparticles synthesized showed crystallinenature with 2θ peaks lying at 35.73o, which matches the crystallographic plane (311) and 62.96o which matches the crystallographic plane (440) of magnetite phase., indicating that thesample has a cubic spinel structure which could confirm that the prepared nanoparticles, was made of magnetite and the diffraction data were in good agreement with COD (Crystallography Open Database) DB Card number 9003781. For the chemically created MNPs, this formula yields a crystallite or grain size of 83.7 nm.

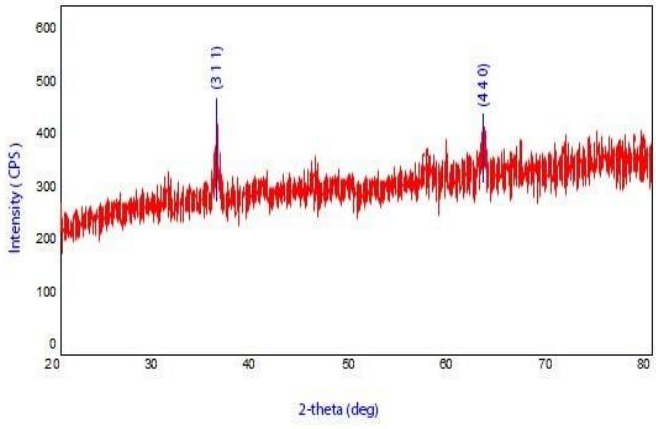

Fig 2: XRD Pattern of Prepared MagnetiteNanoparticles (MNPs)

According to the findings of X-ray power diffraction (XRD) on nanoparticles, magnetite (Fe3O4) is the material that makes up produced nanoparticles (Marquez et al. 2011). The most popular method for figuring out a material's crystalline structure is X-raydiffraction. It is challenging to determine synthetic magnetite using XRD since the crystalline structure of magnetite is comparable to that of maghemite, which features interstitial spaces (Palanisamy et al 2013).The distinction between the two minerals is that the maghemite has atomic holes because some of its interstitial atomic locations aren't completely filled in(Casillus et al. 2012).

3.3 FESEM Analysis:

With the use of a Field Emission Scanning Electron Microscope (Germany's Carl Zeiss), the size and form of the magnetite NPs were determined. A field-emission cathode in a scanning electron microscope's electron gun produces narrower-beam probing probes at both low and high electron energies, improvingspatial resolution and reducing sample charging and damage. The analysis of semiconductor device cross sections for gate oxide widths and thicknesses, film thicknesses, and construction details, as well as the determination of uniform

structure, coating thickness, and features of geometry and elemental composition on small contamination, are benefits of SEM (Hajipour et al. 2013). As a result, the FE-SEM is a crucial instrument for high-resolution surface imagingin the field of nanoscience (Yao and Kimura).

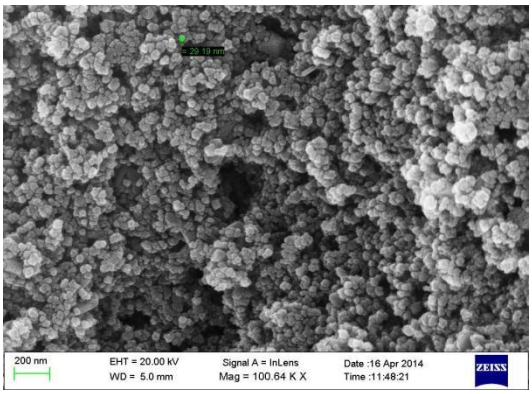

Fig 3: FE-SEM Image of synthesized MNPs

The FE-SEM images of artificial iron oxide nanoparticles (NPs) are shown in Figure 3. The creation of magnetite NPs is readily visible in the image, and as canbe seen in figure 3, the particles were found tobe uniformly dispersed with little agglomeration.Additionally, it displays a high-resolution FE- SEM image of the synthesized NPs that shows that the majority of them have spherical forms. By randomly choosing 10 particles from the FE-SEM images and averaging their sizes, the average particle size was determined to be 29.19nm.

3.4 Antibacterial Activity :

According to Huang's paper from 2010, the Iron oxide nanoparticles synthesized through co-precipitation have a size of 12 nm on the FE-SEM image. Using the sol gel technique, Hema et al. (2013) estimated the size of the magnetite particles to be roughly 86 nm.

Figure 4 shows the bacterial activity of synthesized MNPs against different bacteria such as E.coli, S. aureus, Bacillus subtilis and Klebsiella sp. . As it showed a clear inhibition zone (Table 1), thesynthesized MNPs were highly effective in their activity against these bacteria's. Sterile distilled waterserved as negative control. Surfaces of MNPs affect / interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria.

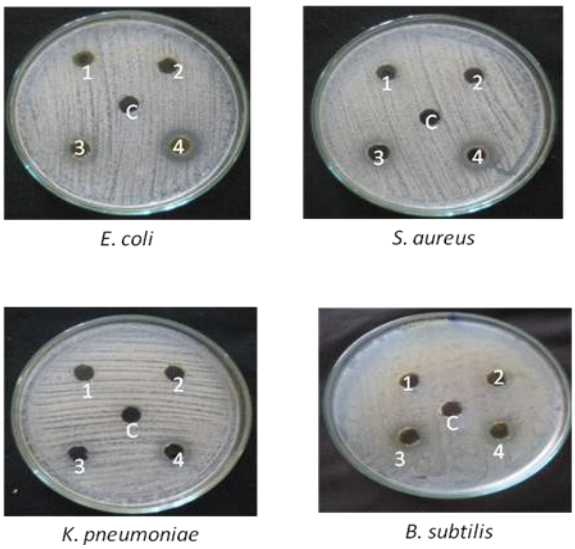

Fig 4: Bacterial activity of synthesized MNPs

Table 1: Inhibition zone of synthesized MNPs against different bacteria

S.No	Concentration(µg)	Zone of inhibition (mm)			
		B. subtilis	E. coli	S. aureus	K. pneumoniae
1	250	-	-	-	-
2	500	-	11	-	-
3	750	9	12	11	11
4	1000	10	16	14	12

4. Conclusion

Magnetite nanoparticles are often synthesized through various chemical routes, and in this study, we employed a well-established co-precipitation method to produce these nanoparticles. This method is chosen for its simplicity, reproducibility, and the ability to yield monodisperse nanoparticles with controlled properties. The FTIR spectra displayed specific peaks at 3386.1, 1590.3, and 578.4 cm-1 that were indicative of magnetite nanoparticles. By randomly choosing 10 particles from the FE-SEM images and averaging their sizes, the average particle size was determined to be 29.19 nm. The magnetitenanoparticles XRD pattern shows two distinct peaks at 35.73º and 62.96º. The nanoparticles synthesizedshowed crystalline nature with 2θ peaks lying at 35.73º, which matches the crystallographic plane (311) and 62.96º which matches the crystallographic plane (440) of magnetite phase., indicating that the sample has a cubic spinel structure which could confirm that the prepared nanoparticles was made of magnetite and the diffraction data were in good agreement with COD (Crystallography Open Database) DB Card number 9003781. Using this formula, a crystallite or grain size of 83 is produced. This study provides a comprehensive investigation into the synthesis, characterization, and antibacterial activity of magnetite nanoparticles. The FESEM, FTIR, and XRD techniques collectively offer valuable insights into the physical and chemical properties of the MNPs, paving the way for their integration into biosensor platforms. The observed antibacterial activity further extends their potential applications, highlighting their versatility and significance in biotechnological advancements. Future studies could focus on the immobilization of biomolecules on the surface of these nanoparticles to develop functional biosensor platforms for rapid and sensitive detection in various fields, including medical diagnostics and environmental monitoring.

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