Original Research Article

Antimicrobial activity of iron oxide nanoparticles stabilized by alginate

Abstract

Increase in microbial resistance of commonly used antibiotics is a major health concern globally. This study aimed at exploring the use of iron oxide nanoparticles (IONPs) stabilized by alginate as sources of antimicrobials. Antimicrobial activity of synthesized magnetic nanoparticles was tested against six organisms (*Bacillus subtilis, Staphylococcus aureus, Candida albicans, Vibrio cholerae, Proteus vulgaris* and *Micrococcus luteus*) using agar diffusion method. Results showed that IONPs exhibited a strong antimicrobial activity against most of the tested clinical isolates. *M. luteus* had highest antimicrobial activity (21 mm), followed by *B. subtilis* (20 mm), *S. aureus* (20 mm) and *V. cholerae* (20 mm) while *P. vulgaris* and *C. albicans* had intermediate activities against IONPs. The minimum inhibitory concentration (MIC) results showed that IONPs was most effective against *B. subtilis* and *S. aureus,* followed by *M. luteus* and least activity was noticed against *V. cholerae*. Also, minimum bactericidal concentration (MBC) result revealed that IONPs had MBC of 40 mg/ml against both *B. subtilis* and *S. aureus*, and 60 mg/ml against *M. luteus* and *V. cholerae*. These findings revealed that alginate stabilized IONPs have great potentials for inhibiting clinical isolates; thus, their use as an alternative means for new drug discovery should be encouraged.

Keywords: Antimicrobial activity**;** Nanoparticles; Drug discovery; Bacterial and fungal species

Introduction

The treatment of infectious diseases has become more challenging as a result of emergence of new transmissible diseases and the rapid rise of antibiotic resistance to pathogenic microorganisms has greatly affected the rate of treatment. Increase in morbidity and mortality rate is of great concern due to antimicrobial resistance which poses a lot of threat to human health [1]**.** The use of nanoparticles as alternative to antibiotics has also been an aspect of research interest due to the multidrug resistance of antibiotics to bacterial infections. The recent advancement in nanotechnology has also shown that these nanoparticles possess

antimicrobial activity [2]. The use of nanoparticles were employed by researchers due to the unique physiochemical characteristics of nanoparticles [3]. These unique properties have led to their applications in different areas of biomedical sciences such as sensing application, gene delivery, biomolecules detection and clinical diagnostics [4].

The application of nanoparticles as antimicrobial agent has rapidly gained significant attention in medical therapies because they are small in size and they also exhibit large surface area which improve their interaction with microorganisms and results in effective antimicrobial activities [5]. Thus, reports have shown that the antimicrobial activity of nanoparticles is attributed to the small size, volume ratio and their large surface area [6]. Magnetic nanoparticles possess unique advantages which include (i) cheap and ease of preparation, (ii) biocompatibility, (iii) physical and chemical stability, environmentally safe, and (iv) ease of separation of biological components [7].

Applications of magnetic nanoparticles in biomedical field include molecular detection [8], bio-separation [9,10], drug delivery [11–13], hyperthermia [14,15] and MRI [16,17]. Moreso, magnetic nanoparticles have also shown significant antimicrobial activity through the generation of reactive oxygen species (ROS) which is capable of causing both physical and mechanical damages to the microorganisms [18,19]. In the present study, magnetic nanoparticles were successfully synthesized and characterized by Transmission electron microscopy (TEM), Energy dispersive x-ray spectroscopy (EDS) and XRD. The application of magnetic nanoparticles as an antimicrobial agent was tested against six selected organisms and their antimicrobial activity was evaluated.

Materials and Methods

Chemicals and reagents

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), sodium alginate ($C_6H_9NaO_7$ technical grade) were purchased from Sigma Aldrich, others include Ammonia solution (NH_4OH, 28%), Ethanol and Deionized water. All reagents were prepared and used according to manufacturer's instruction.

Preparation of alginate stabilized iron oxide nanoparticles

Iron oxide nanoparticles (IONPs) stabilized by alginate were prepared through previously reported conventional co-precipitation method using aqueous solution of ferric (and ferrous ions at 2:1 mole ratio of Fe^{3+}: Fe^{2+}. Typically, $FeCl_3.6H_2O$ (64.8 mmol) and $FeCl_2.4H_2O$ (32.4 mmol) were dissolved in 300 ml of absolute ethanol and water (v: v; 1:2). The mixture was stirred and heated at 80^{oC} for 30 mins. Then 15 ml of Ammonium hydroxide was added to the mixture drop wise and the reaction was stirred for another 30 min. Afterwards 0.5% (w/w) of sodium alginate was directly added and the reaction was allowed to proceed for 30 min after the addition of sodium alginate. The black crude magnetic nanoparticles were recovered magnetically, washed with deionized water and ethanol for three times, and then dried in the oven for 24 h.

Media Preparation

Nutrient agar medium was prepared according to manufacturer's specification. The medium was homogenized by boiling to fully dissolve all components. It was then autoclaved at 121^oC for 15 min. After autoclaving, the medium was allowed to cool to about 45^oC and poured into Petri-dish. The plates were allowed to set/solidify before inoculation.

Test organisms

Six different clinical isolates were obtained from Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko. The morphological and biochemical characteristics of these isolates were confirmed using cultural, morphological and biochemical characterization. The isolates include *Bacillus subtilis, Staphylococcus aureus, Candida albicans, Vibrio cholerae, Proteus vulgaris* and *Micrococcus luteus.* The bacterial cultures were maintained in nutrient broth and stored at 4^oC throughout the study period.

Determination of antimicrobial activity

Bacterial cultures were maintained on nutrient broth. Inoculum size containing $1.5x10^8$ CFU/ml (0.5 McFarland standard) of each bacterium was used to seed already solidified Petri plates of Mueller-Hinton agar using spread plate technique. The bacterial plates were incubated at 37^oC for 24 hours. Antimicrobial activity of the compound (IONPs) was determined using agar well diffusion method. A sterile 6 mm cork borer was used to make 3 wells on already solidified agar plate and one of the well was filled with the compound (IONPs**)** the second well with positive control (ampiclox) and the third well was filled with water (negative control). The plates were allowed to stand for 2 hours to allow absorption of

the samples into the medium after which they were incubated at 37^{o}C for 24 hours. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using a method described by Akinyemi *et al.* [26] with MIC concentrations of 80 mg/ml, 60 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml and 1 mg/ml. The results were observed and recorded. MIC was determined as the lowest concentration of compound permitting no visible growth (no turbidity). For MBC determination, the test MIC dilutions were cultured on fresh agar medium and incubated at 37^{o}C for 24 hours. The lowest concentration of MIC tubes with no visible growth was taken as MBC.

Characterization of alginate stabilized magnetic nanoparticles

XRD patterns of iron oxide nanoparticles (IONPs**)**

The XRD diffractograms of IONPs is presented in Figure 1. The diffraction peaks at 2θ equal to 30.4^{o}, 35.8^{o}, 43.4^{o}, 57.8^{o}, and 63.2^{o} were observed, which is an indicative of a cubic spinel structure of the magnetite. The XRD diffractogram clearly revealed the formation of the IONPs. The diffraction peaks show the characteristic of magnetite which were indexed for the following Miller Indices (220), (311), (400), (511) and (440)
[20,21].

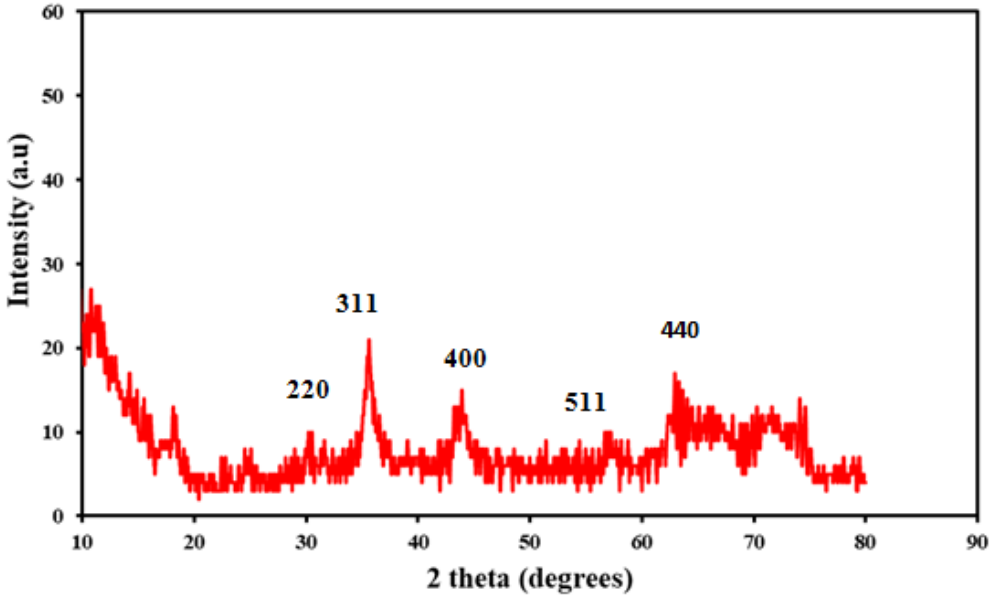

Figure 1: XRD diffractogram of iron oxide nanoparticles (IONPs)

Energy dispersive x-ray spectroscopy (EDS) of iron oxide nanoparticles

EDS is an elemental analysis which was used to characterize the synthesized magnetic nanoparticles. The result obtained shows the presence of all the expected elements which is an indication that the magnetic nanoparticles was successfully synthesized (Figure 2).

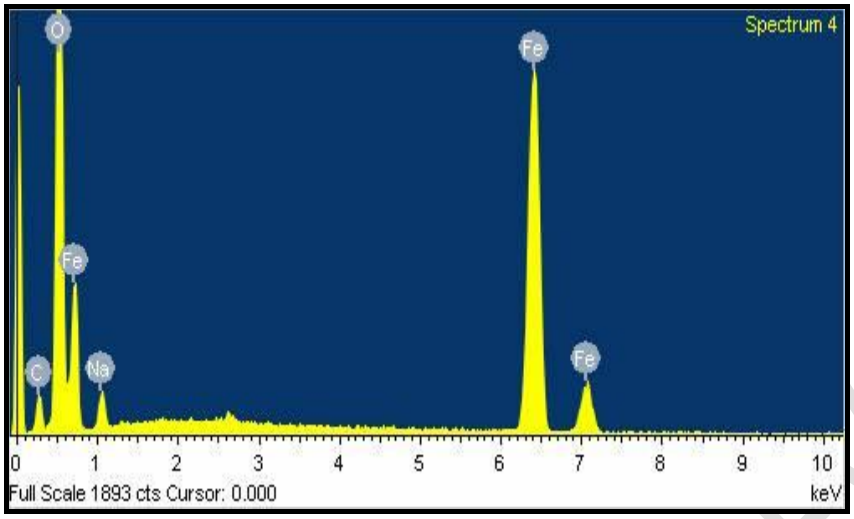

Figure 2: EDS spectrum of iron oxide nanoparticles

Transmission electron microscopy (TEM) image of iron oxide nanoparticles

TEM image of the nanoparticles revealed the morphology of the iron oxide nanoparticles. The TEM image revealed the morphology of the magnetic nanoparticles which shows that the synthesized IONPs were spherical in shape (Figure 3).

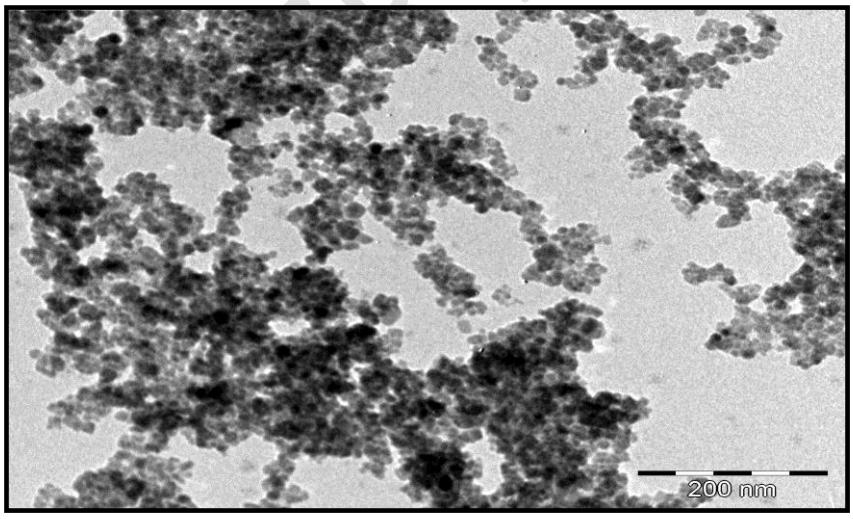

Figure 3: TEM image of iron oxide nanoparticles

Antimicrobial susceptibility test (AST)

The results from this study revealed that *B. subtilis*, *S. aureus*, *M. luteus* and *V. cholera* were susceptible to magnetic nanoparticles while *P. vulgaris* and *C. albicans* had intermediate activity against IONPs (Table 1). The MIC results showed that the IONPs was most effective against *B. subtilis* and *S. aureus*, even at concentration (10 mg/ml), followed by *M. luteus* (20 mg/ml) and the least activity was noticed at a concentration of 40 mg/ml against *V. cholerae* (Table 2). The result of the MBC indicated that IONPs had MBC of 40 mg/ml against both *Bacillus subtilis* and *Staphylococcus aureus*, and 60 mg/ml against *M. luteus* and *V. cholerae* (Table 3).

Table 1: Antimicrobial susceptibility test of IONPs against some bacterial isolates

Organism	Diameter of inhibition zone (mm)
Bacillus subtilis	20±1.0
Staphylococcus aureus	20±0
Candida albicans	15±0.5
Vibrio cholerae	20±0.5
Proteus vulgaris	17±0.5
Micrococcus luteus	21±0.5

Key: 1-14 = Resistant; 15-19 = Intermediate; 20 and above = Susceptible. Values presented with standard errors of the means (±SEM).

Table 2: Minimum inhibitory concentration (MIC) of IONPs against bacterial isolates

Organism	80 mg/ml	60 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml	1 mg/ml
Bacillus subtilis	+	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	+	-
Vibrio cholera	+	+	+	-	-	-
Micrococcus luteus	+	+	+	+	-	-

Key: + = growth; - = no growth

Table 3 Minimum bactericidal concentration (MBC) of IONPs against bacterial isolates

Organism	80mg/ml	60mg/ml	40mg/ml	20mg/ml	10mg/ml
Bacillus subtilis	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-
Vibrio cholera	+	+	-	-	-
Micrococcus luteus	+	+	-	-	-

Key: + = growth; - = no growth

Discussion

Iron oxide nanoparticles have shown superior antibacterial activity against various pathogenic bacteria [22]. The use of iron oxide nanoparticles as an alternative to antibiotics has become an area of interest in research. Antimicrobial activity of IONPs was tested against six clinical isolates. The compound exhibited an effective activity against four out of the six test organisms with *B. subtilis, S. aureus, V. cholerae, M. luteus* among the susceptible organisms. Similar findings were observed by Niemirowicz-Laskowska *et al.* [23], who reported bactericidal among immunomodulatory properties of magnetic nanoparticles functionalized by 1,4-dihydropyridines and Arakha *et al.* [24], that reported antimicrobial activity against *B. subtilis* and *Escherichia coli* at relatively high concentration while exploring the role of interaction pattern of iron oxide nanoparticles (IONPs) on some bacteria.

Moreover, the study revealed that *C. albicans* had intermediate activity against IONPs. *C. albicans* is a eukaryotic microorganism (yeast), which has a complex structure [25]. It is a commensal organism in respiratory, gastrointestinal, and genitourinary tracts. *C. albicans* is a harmless fungus that may become an opportunistic organism in immunocompromised individuals. Also, from the toxicity point of view, the synthesized IONPs, under the same experimental conditions, selectively kills bacterial species (*B. subtilis, S. aureus, V. cholerae, M. luteus*), and displayed significantly little or lower level of toxicity towards *C. albicans*. Similarly, Lazic *et al*. [25] who investigated the use of AgNps supported by functionalized Fe_3O_4 with 5-aminosalicyclic acid against *E. coli*, *S. aureus* and *C. albicans*. The activity towards IONPs could be attributed to the fact that the synthesized IONPs generate reactive oxygen species (ROS) in bacterial cells which gradually led to reducing ability of the bacteria to survive.

Conclusion

Alginate stabilized IONPs were successfully synthesized and characterized. The results obtained from the XRD, TEM and EDS was an indication of the successful synthesis of the IONPs. It was observed that *B. subtilis, S. aureus, V. cholerae* and *M. luteus* were susceptible to the synthesized IONPs. These findings suggest that IONPs are promising lower-cost alternatives that can inhibit the growth of some microorganisms and can provide alternative means of overcoming the challenge of multi drug resistance to antibiotics. Also, the applications of magnetic nanoparticles may lead to valuable findings in various fields such as medical devices and antimicrobial system.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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