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RESEARCH PAPER

Evaluation of Antibacterial Properties of γ-Al_2O_3:Eu Nanoparticles on Various Microorganisms

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ABSTRACT

In this study, γ-Al_2O_3: Eu nanoparticles synthesized by sol-gel method were manufactured at 800 °C using X-ray diffraction pattern analysis (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). X-ray diffraction energy (EDX) and Fourier transform infrared spectrum (FTIR), the formation and construction of these nanoparticles are investigated. The XRD and SEM images confirmed the correct formation of the material structure and the uniformity of the formed nanoparticles, respectively, images of TEM showed the the average crystallite size is obtained to be about 10 nm, EDX violated the impure presence of these nanoparticles, created an infrared absorption spectrum, and examined the binding of nanoparticle bonds. Antibacterial properties of γ-Al_2O_3: Eu nanoparticles led to resistance to activity of 11 types of microorganisms. Results demonstrated that prepared Al_2O_3:Eu NPs have maximum antibacterial activity against microorganisms (15.63 µg/ml) and Al_2O_3:Eu have inhibitory effect against tested microorganisms. For Al_2O_3;Eu NPs, the disk diffusion test proved that the highest growth inhibition zone was related to Staphylococcus aureus and Escherichia coli.

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INTRODUCTION

Extensive research has been done on Nanotechnology and Nanoscience. The nanoparticle's small size changes their chemical and physical properties, which is the basis for new phenomena. The size range of nanoparticles is from 1 to 100 nanometers and they have different properties with their balk sample. Nanotechnology is the basis for the development of tools, as well as the extension and change in biological and medical systems such as cancer treatment, cell photography, and drug delivery [1, 2].

Nanoparticles have different applications; one of them is the increase in antibacterial activity, in which parameters such as size, shape, morphology, concentration, stability, and surface efficiency can affect the antibacterial properties of nanoparticles. Due to the improvement in sustainability and nature friendliness, non-organic nanoparticles are more significant [3].

One of the metal oxides that have antibacterial properties is Al_2O_3, which has excellent resistance to gram-negative, gram-positive bacteria, fungi, and yeasts [4]. Another reason for using alumina nanoparticles is their acidity and surface properties [5]. Some of the other unique features of Al_2O_3 are high mechanical strength, high surface area, high hardness, and good stability

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[6]. Alumina nanoparticles can be synthesized by various methods such as ball milling [7], sol-gel [8], pyrolysis [9], sputtering [10], hydrothermal [11], and laser ablation [12].

Ansari et al. studied the toxicity of alumina and its antibacterial properties in E. coli bacteria. Using SEM analysis, they photographed alumina nanoparticles inside and on the surface of the bacterium, and the effect of these nanoparticles was observed in preventing the growth of bacterial cells [13]. Priyakshree Borthakur et al. studied the adsorption capacity of alumina nanoparticles, which were prepared by the mechanical method of ball milling, and the absorption of Escherichia coli gram-negative bacteria, increased by reducing the size of alumina nanoparticles [5]. A.S. Lozhkomeov et al. examined the antibacterial properties of Al_2O_3: Ag nanoparticles using wires electronic explosion. The antibacterial properties of these nanoparticles, was different from gram negative and positive bacteria and better results than pure Ag nanoparticles were reported by this group [14, 15].

The production of reactive oxygen species (ROS) depends on specific surface groups, which leads to the formation of active sites (thiol groups, carboxyl groups, carbonyl groups, surfactants, SDS, DMSO), that the donor and receptor electrons deal with oxygen and produce radical superoxide, the activated oxygen is then extracted through the ROS process [16, 17]. Reactive oxygen species are unable to detect healthy cells and cancer cells, which cause toxicity near the body's cells. This is where the role of antioxidants comes into play, alumina nanoparticles act like antioxidants. They prevent damage to the healthy cells of the body and stop the production of active oxygen species near the healthy cells of the human body [18-20]. To the best of our knowledge, however, no attention has so far been attention to the antibacterial activity of Al_2O_3:Eu nanoparticles.

consider three possible mechanisms for antibacterials: Ions lead to the production of reactive oxygen species, in which oxygen radicals react with the membrane and cell wall components of the bacterium and other cellular components (such as mitochondria), leading to Irreversible changes. They are indestructible and cause the death of bacterial cells. The ions act on intracellular ATP and penetrate into the bacterial cell, disrupting DNA replication. Ions accumulate in the bacterial cell membrane and in permeability they prevent the transfer of proteins, as a result of which the cell membrane is destroyed and causes bacterial cell death. Antibacterials prevent the cell wall of bacteria from forming and alter the function of the cell membrane. Antibacterials inhibit the growth of microorganisms, increase the permeability of the cell membrane and destroy the membrane, and they also change the Cytosol redox cycle, also break down intracellular radicals, and disrupts proton stimulus in ATP synthesis [21, 22]. The mechanism used for nanoparticles is that the nanoparticles adhere to the outer surface of the bacterium and metal ions penetrate the bacterial cell and destroy their biomolecules such as mitochondria and DNA, and then the cytotoxicity increases due to the presence of oxygen radicals. The result of the antibiogram obtained from the disk penetration test is reported as the diameter of the growth inhibition zone.

In this paper, Al_2O_3: Eu nanoparticles were synthesized by the sol-gel method, and the structural structure of these nanoparticles was confirmed using SEM, TEM, FTIR and XRD analyzes. For the first time, nanoparticles γ- Al_2O_3: Eu were used as an antibacterial agent that had a significant effect on microorganisms, also, the minimum inhibitory concentration and the minimum bactericide concentration at different dilutions for gram-positive, gram-negative bacteria, fungi, and yeasts were investigated.

MATERIALS AND METHODS

Materials and Equipment

In this study an X-ray diffraction device (XRD) with a Philips x'pert pro MMP model and a nickel-filtered CuKα diffraction pattern to determine the quantities of the crystal structure and identify the crystal phase was used. Other devices such as scanning electron microscope (SEM) with Mira 3-XMU TESCAN-SEM model for morphology and microstructure analysis, transmission electron microscope (TEM) with Zeiss EM900 model for morphology, and nanoparticle size determination Fourier transform infrared spectroscopy (FTIR) device with MagnalR550 model was used to determine the most probable functional groups by examining the frequency range of specific radiation.

Synthesis procudre

Al_2O_3:Eu nanoparticles were synthesized using a sol-gel method. In this regard, aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$], oleic acid ($C_{18}H_{34}O_2$), and Europium nitrate [Eu ($NO_3)_3$] were purchased from Merck, and used without further purification. 1.875 g of aluminum nitrate was mixed with 1 mole% of Europium nitrate in deionized water, and the resultant solution was placed on a magnetic stirrer to homogenize it during 60 min at room temperature, thus obtaining the sol. In the following, oleic acid with a volume ratio of 1:1 was added to the solution of the mixture, and it was placed on a magnetic stirrer (600 rpm) for 1 h at 25°C. To evaporate the remaining water, the solution was heated to the temperature of 180°C for 8 h, thereby transforming it into a black gel. Finally, the black gel was calcined at temperatures 800C° to form Al_2O_3: Eu powder.

RESULTS AND DISCUSSION

X-ray diffraction pattern (XRD)

X-ray diffraction pattern related to γ-Al_2O_3: Eu nanoparticles synthesized by sol-gel at 800 °C, in the Fig. 1, it can be seen with the card number 00-001-1303 with radiation (λ=0.154060 nm) CuKα ranged from 2θ from 10 to 80 degree, crystallite size from the Scherer equation in the (211) direction and an angle of 67.27° it reached in the limit of 10 nm. According to this spectrum, the formation of γ-Al_2O_3: Eu was approved with cubic structure and 0.34100 nm crystal lattice constant which is also consistent with the articles [23].

The Scherer equation shows the dependence of crystallite size on the overspread of diffraction lines, the constant k is related to the device, λ wavelength of the incident beam, β full width at maximum half on the main peak hkl page handle, θ angular beam angle, and D is the size of the crystallite.

$$D_{hkl} = \frac{K\lambda}{\beta \cos\theta}$$

Scanning electron microscope (SEM) analysis

Fig. 2 shows the SEM image of material made of γ-Al_2O_3: Eu at 800 °C. At this temperature, the effect of exceptional grain diameter contraction and crystalline property of the system has increased at this temperature, and the angles of the edge of the particles are perfectly polished, the result is a tiny particle size of γ-Al_2O_3: Eu [24].

Transmission electron microscope (TEM) analysis

The morphology and crystalline properties of nanoparticles are measured by TEM analysis. TEM images show the density of nanoparticles that is increased in constant crystal surface. The Fig. 3 shows showed the average crystallite size is obtained to be about 10 nm. The results of TEM

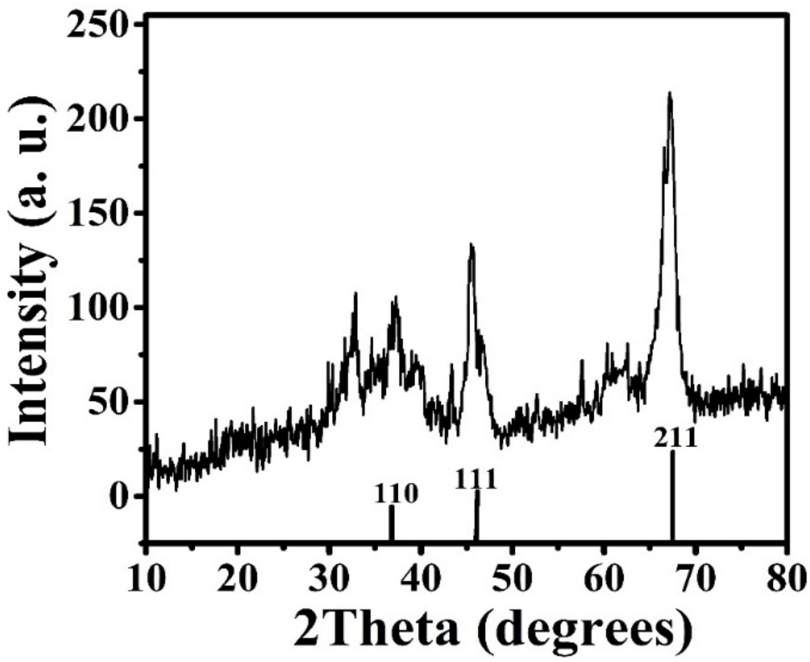


Fig. 1. XRD pattern of γ-Al_2O_3: Eu nanoparticles at the temperature of 800 °C is made by sol-gel method.

analysis are consistent with SEM analysis.

X-ray energy analysis (EDX)

EDX analysis shows the purity and atomic percentage of the ingredients and the reason for the existence of nanoparticles Al, O, and Eu. In order to use this analysis, no impurities were found in the substance γ-Al_2O_3: Eu (Fig 4).

Fourier transform infrared spectroscopy (FT-IR) analysis

FTIR is used to determine the structure and

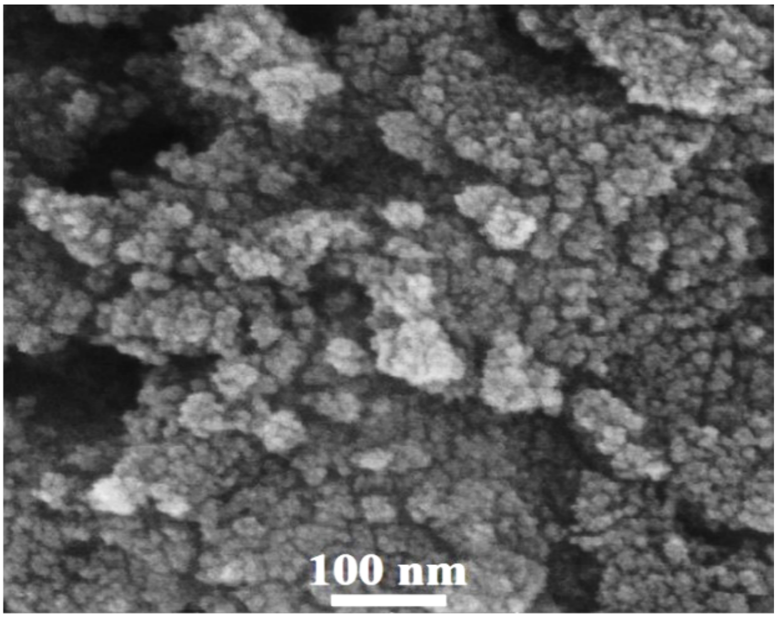


Fig. 2. SEM image of γ-Al_2O_3: Eu nanoparticles at the temperature of 800 °C is made by sol-gel method.

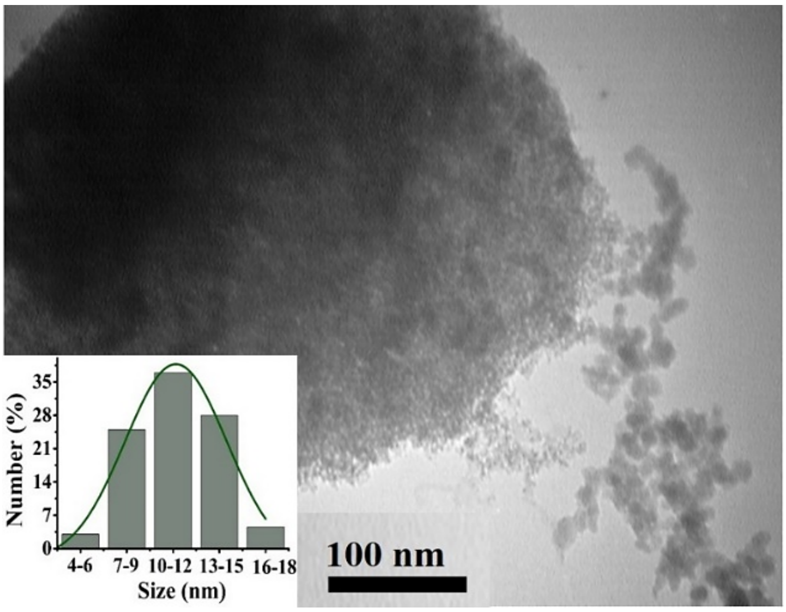


Fig 3. TEM image of γ-Al_2O_3: Eu nanoparticles at the temperature of 800 °C is made by sol-gel method.

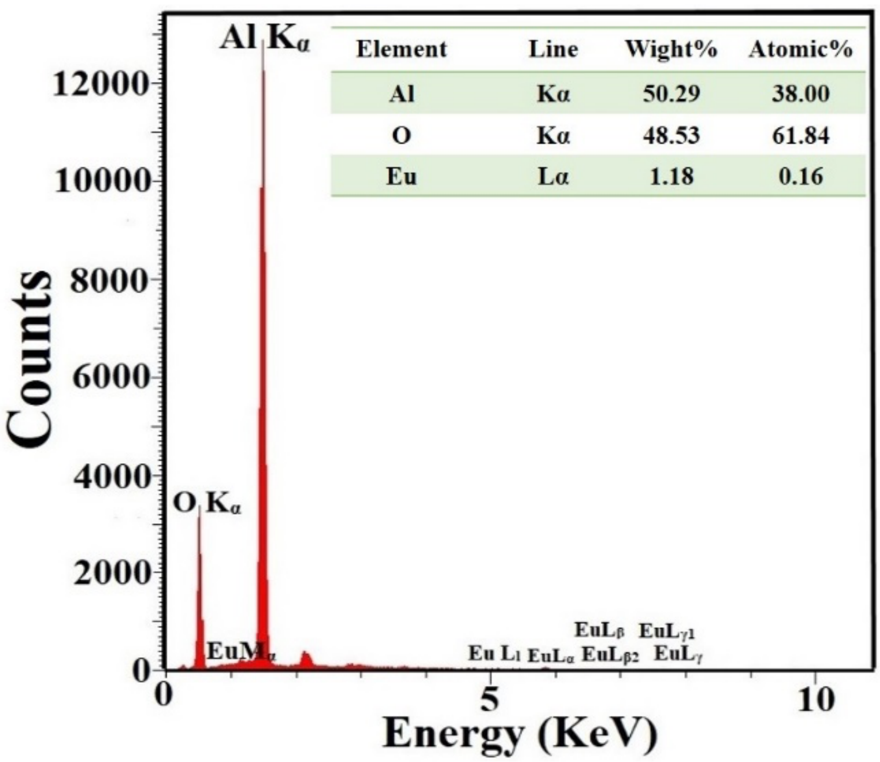
measurement of chemical species. In this method, the mutation vibrations of the particle bands are examined. In the Fig. 5, the wavelength range is 400 to 4000 cm^{-1}. In the 560 cm^{-1} wave number Al-O bonds, in the 1628 cm^{-1} H_2O bands, and in 3442 cm^{-1} O-H bands are the peaks liable [25].

Antimicrobial activity

Antimicrobial activity of nanoparticles by measuring the minimum inhibitory concentration (MIC), the minimum bactericide concentration (MBC) of microorganisms with micro-well dilution method, and the halo of non-growth on bacteria was investigated by the Diffusion Disc (DC) method for 11 types of microorganisms. To determine MIC micro-pages of 96 sterile homes were provided. For each of the plates, 95 µl of culture medium, five µl of bacterial suspension with 0.5 µm McFarland dilution and 100 µl of different nanoparticle dilutions were added, the plate was then heated in an incubator at 37 °C for 24 hours. The first microdilution step was performed at the highest concentration of nanoparticles γ-Al_2O_3: Eu and the range of changes in concentration from 1000 to 15.63 micrograms per milliliter, and each dilution was half the previous dilution (1000-500-250-125-62.5-31.25-15.63).

To determine the MBC after 24 hours of heating, five microliters from each of the microwell plates where there was no growth, were inoculated into the nutrient agar environment and exposed to heat for 24 hours at 37 °C. The concentrations used in the first stage of control antibiotics in each well for Rifampin were five micrograms per disc, Gentamicin 10 micrograms per disc, and Nystatin. The lowest concentrations of nanoparticles with no turbidity (no microorganisms) in their wells were 15.63 micrograms per milliliter of MIC, which had more effect on gram-positive and negative bacteria resistant to nystatin antibiotics. Also, the results of various dilutions on the microorganisms used in this study can be seen in the Table 1. [26, 27].

As mentioned, the halo of non-growth on



bacteria by the diffusion disc (DC) method is another way to measure the antibacterial activity of substances. The diameter of the halo indicates the effectiveness of the microorganisms. The γ-Al_2O_3: Eu nanoparticles had a significant effect on microorganisms at the lowest concentrations.

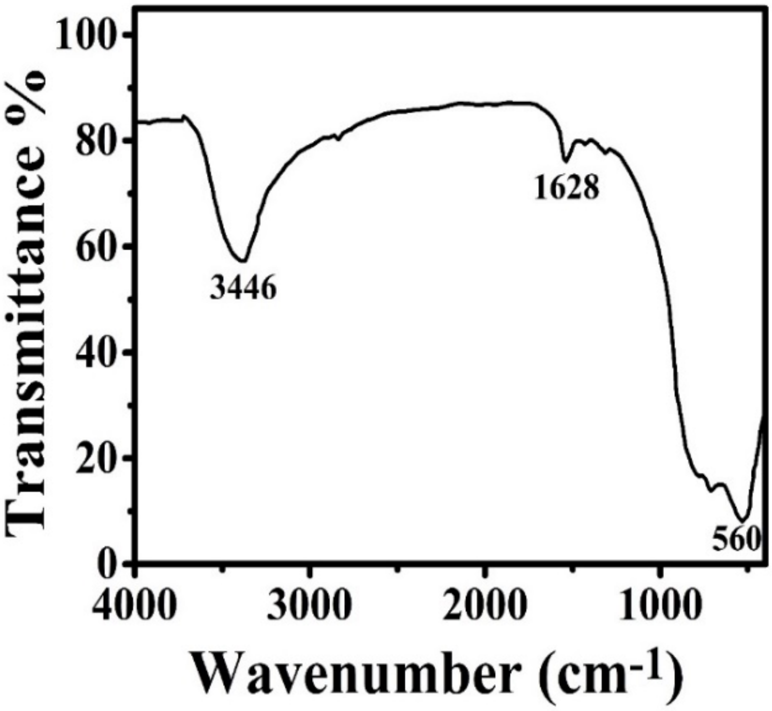


Fig 5. FTIR of γ-Al_2O_3: Eu nanoparticles at the temperature of 800 °C made by sol-gel method.

Table 1. Results from the antimicrobial activity of γ-Al_2O_3: Eu nanoparticles

Microorganisms	Al_2O_3: Eu		Rifampin		Gentamicin		Nystatin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram posetive bacteria								
Bacillus subtilis	15.63	>1000	31.25		3.90		NA	
Staphylococcus epidermidis	15.63	125	1.95		1.95		NA	
Staphylococcus aureus	15.63	125	1.95		1.95		NA	
Gram negative bacteria								
Escherichia coli	15.63<	15.63<	3.90		3.90		NA	
Klebsiella pneumonia	15.63<	15.63<	15.63		3.90		NA	
Shigella dysenteriae	15.63<	15.63<	15.63		3.90		NA	
Salmonella paratyphi-A serotype	15.63<	15.63<	15.63		3.90		NA	
Pseudomonas aeruginosa	15.63<	15.63<	31.25		7.81		NA	
Streptococcus pyogenes	15.63<	15.63<	0.975		0.975		NA	
Fungi								
Aspergillus niger	-	-	-	-	-	-	31.2	
Yeast								
Candida albicans	15.63<	15.63<	NA		NA		125	

Fig 6. Antimicrobial activity of γ-Al_2O_3: Eu nanoparticles using diffusion disk method. A) Escherichia coli B) Staphylococcus aureus.

Antibacterial activity of γ-Al_2O_3: Eu nanoparticles prepared by sol-gel method were

observed in contrast to the activity of gram-negative, gram-positive bacteria, fungi, and yeasts. The effect of nanoparticles γ-Al_2O_3: Eu on bacteria increases with increasing concentration of nanoparticles, also this effect in increasing the aura diameter of Escherichia coli and Staphylococcus aureus bacteria can be seen in the Fig. 6.

CONCLUSION

In this study, the nanoparticles γ-Al_2O_3: Eu were well developed by the sol-gel method, which confirmed the analyzes of XRD, TEM, SEM, EDX, and FTIR made by these nanoparticles. Test results of antibacterial activity of nanoparticles γ-Al_2O_3: Eu in low concentrations against gram-positive and gram-negative bacteria showed that they perform better than Nystatin's strong antibiotic. In antibacterial measurements, these nanoparticles with a MIC of 15.63 micrograms per milliliter of growth inhibited the growth of 11 types of microorganisms. The increase in the diameter of the halo on Escherichia coli and Staphylococcus aureus bacteria in the diffusion disc method was the reason for the non-growth of these bacteria. These results will be promising in the field of medical activities and nature-friendly applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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