A New DNA-Mediated Antibacterial Agent Magnetic (Fe_3O_4) Nanoparticles with Gold and Silver Functionalization

https://doi.org/10.31185/wjps.VolX.IssX.XX

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Abstract- By using a hydrothermal process, magnetite (Fe_3O_4) nanoparticles with gold and silver caps (Au, Ag) were created, and their antibacterial activity was tested against *S. aureus* and *E. coli* .By using X-ray diffraction (XRD) and Scanning electron microscopy (SEM), the functionalized nanoparticles were evaluated. The functionalized Fe_3O_4-Au had an average size of (8-22) nm, whereas the Fe3O4-Ag had an average size in the range of (4-16) nm. At room temperature, the Fe_3O_4-Au @ Fe_3O_4-Ag displayed superparamagnetic and strong saturation magnetization. Using the agar well-diffusion technique, the antibacterial activity of the Fe_3O_4-Au and Fe_3O_4-Ag was assessed against *Escherichia coli* and *Staphylococcus aureus.* The release technique was used to track alterations in the morphology of the microorganisms under investigation. The outcomes demonstrated the effectiveness of magnetic (Fe_3O_4) nanoparticles functionalized with gold and silver as a new DNA-mediated antibacterial agent. By breaking through the bacterial membrane's cytoplasm and nucleic acid, Fe_3O_4-Au @ Fe_3O_4-Ag nanoparticles were shown to kill bacteria by causing cell-wall integrity to be lost, increased cell wall permeability and damage to nucleic acids.

Keywords: Fe_3O_4-Ag; Fe_3O_4-Au; Hydrothermal synthesis; Release method; Antibacterial activity; well-diffusion.

1. Introduction

There is research on the use of surface functionalized magnetic nanoparticles in biological applications [1, 2, 3]. Fe_3O_4 is beneficial in some catalytic and bio-separation processes due to its biodegradability. Because of their high biocompatibility, low toxicity to humans, and superparamagnetic properties, Fe3O4 nanoparticles have been extensively studied [4,5]. Furthermore, they have a high surface energy, which allows them to combine easily, albeit doing so may reduce their magnetic and adsorption effectiveness. Therefore, an organic or inorganic coating placed on top of these nanoparticles avoids agglomeration. The goal of coating is to increase the magnetite nanoparticles' chances of further functionalization while also stabilizing them. There are several methods for creating gold and

silver-coated magnetic nanoparticles [6, 8] The shell structure, where the magnetic nanoparticle is coated with the gold and silver shell [9, 10]. It has been shown that uncoated magnetite nanoparticles are vulnerable to leaching in acidic environments. The gold and silver shell coating improves the nanoparticles' hydrophobicity and biocompatibility [11,12]. Additionally, The coating material (core/shell structure) possesses a number of alluring qualities, including great thermal and chemical stability as well as an exceptional capacity for absorption [13,14]. The functionalized magnetite nanoparticles (Fe_3O_4) operate as accessible binding sites for drugs, proteins, and enzymes thanks to the active sites on their gold and silver surfaces [15], and the Fe_3O_4 also serves as a delivery system for the loaded drugs' controlled release into the desired cells [16,17]. Furthermore, due to its decreased toxicity, Fe_3O_4-Au @ Fe_3O_4-Ag may be more useful for biotechnological applications that are more successful [18]. Superparamagnetic magnetite nanoparticles have frequently been made using hydrothermal, sol-gel,microemulsion, and co-precipitation processes, while the hydrothermal approach is the quickest and most straightforward [19, 20]. Because of the possible health and environmental harm, the use of nanoparticles for therapeutic reasons has also drawn criticism. In studies that have examined the toxicity of Fe_3O_4 nanoparticles on eukaryotic cells [21], Fe_3O_4 nanoparticles functionalized with surfactants have demonstrated a minimal degree of toxicity. There is further research on the toxicity of Fe_3O_4 nanoparticles to a few bacterials species. In order to properly comprehend their biological functions across a variety of bacterial species, functionalized Fe_3O_4 nanoparticles must be tested for toxicity on a wider range of bacteria. Different Fe_3O_4 nanoparticles were created in this work with various surface functionalities. Either adding gold and silver to the nanoparticles' surface or leaving the surface bare allowed for this.

utilizing a study of their effect on the shape of the bacteria's cell wall, as observed using the well diffusion method, their biological activity was also examined in relation to both *S. aureus* and *E. coli* [22]. Release of cellular was employed to monitor the harm done by the bacterial DNA. The possibility of employing these functionalized Fe_3O_4 nanoparticles as a strategy for treating illnesses brought on by bacterial species other than those mentioned in this paper has to be further investigated.

2. Experimental

2.2 Chemicals and materials

Beijing Chemicals supplied the following materials: sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), ethylene glycol (EG), ethanolamine (ETA), Sodium borohydride ($NaBH_4$), Sodium citrate ($Na_3C_6H_5$), ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), anhydrous sodium acetate (NaOAc), ($HAucl_4$) and ethanol were purchased from (Beijing, China). Every chemical agent is of analytical quality and may be utilized right away without any additional purification.

2.2 Synthesis of bare Fe_3O_4 MNPs

We used a hydrothermal technique to create the raw Fe_3O_4 MNPs. To create the orange-colored stable solution, 1.5 g of ($FeCl_3$•$6H_2O$) was first dissolved in 40 ml of solvent (20 ml) each of EG and ETA. Additionally, we

continuously magnetically mixed 4.0 g of NaOAc add to the orange-colored the solution to create a homogenous mixture. We then transferred the homogeneous mixture to a Teflon-lined stainless steel autoclave (100 ml), sealed it, and heated it at 200 °C. We cooled the autoclave to the surrounding air's normal temperature once the 10-hour heating process had finished. Then, after washing magnetic nanoparticles in ethanol and deionized water (DW), we dried them at 60 °C for an overnight period.

2.3 Core-shell Fe_3O_4-Au MNP Synthesis

Iron oxide nanoparticles were continuously stirred into a $HAuCl_4$ solution to create Fe_3O_4-Au. With sodium citrate (2.29 g/ml) with heat at 90°C, we made 100 ml. Therefore, we immediately added 40 mg from Fe_3O_4 NPs add to the solution. Further addition of about 5ml of $HAuCI_4$ solution (0.01 mol/l) was heated for 15 minutes before cooling at room temperature with constant stirring for 15 to 20 minutes. We used a magnetic field to isolate the resulting colloidal solution. The Fe_3O_4-Au NPs were also cleaned, magnetically separated, and the suspended of 20 ml of double-deionized water.

2.4 Core-shell Fe_3O_4-Ag MNP Synthesis.

By reducing Ag ions in the presence of Fe_3O_4 nanoparticles, we were able to create Fe_3O_4-Ag nanoparticles. One gram of Fe_3O_4 nanoparticles was suspended in 70 ml of 0.064 mM $AgNO_3$ before 50 ml of 0.32 mM $NaBH_4$ (a reducing agent) was gradually added to the mixture for five minutes. with perplexity. $NaBH_4$ at room temperature was added, and the mixture was mixed for an additional 10 minutes. It progressively transforms the mixture from black to a grayish green tint when Fe_3O_4 nanoparticles enclosed in Ag are formed. After being magnetically washed, the separated Fe_3O_4-Ag NPs were resuspended in 20 ml of doubly deionized water.

3. Characterization of (Fe_3O_4-Ag@ Fe_3O_4-Au) MNPs

Where the prepared magnatic nanoparticles were characterized using structural and optical methods, the Shimadzu XRD 6000 was used with Cu-K radiation source, and angle 2θ = 10°–80° to study the powder diffraction mode.The morphological and size characteristics in the MNPs were studied using Scanning Electron Microscopy (SEM; Philips) to study the morphological qualities of the MNPs.

4. Test for antibacterial activity

4.1 Using agar well diffusion

In this study, an agar well diffusion experiment was used to test the antibacterial activity of (Fe3O4-Ag@ Fe3O4-Au) MNPs against two distinct bacterial strains: E. coli and S. aureus. 20 ml of Mueller-Hinton (M-H) was aseptically applied to sterile Petri plates prior to culture. The bacteria were removed from their stock cultures using a sterile wire loop. Using a sterile point, 6 mm-diameter wells were drilled into the agar plates after the organisms had been cultured. The use concentration of the (Fe_3O_4-Ag@ Fe_3O_4-Au) MNPs (100 g/ml) were injected into the bore wells. Overnight at 37°C, the cultivated plates containing the test organisms and NPs were incubated before measuring and noting the average width of the bacterial inhibition zones that the corresponding concentrations of nanoparticles created. The tests were carried out three times. DW served as the adverse control.

4.2 Release of cellular materials

Sterilized peptone water (0.75 g/50 ml) was used in this procedure, which took 15 minutes at 121 °C and 15 Pa of pressure to complete. Then each bacterial strain was introduced into the medium. Each tube was filled with the produced solutions of (Fe_3O_4-Ag@ Fe_3O_4-Au) MNPs at a concentration of 100 g/ml following a 24-hour incubation period. Cells were centrifuged at 3500 rpm after 0, 30, 60, and 120 minutes of treatment to measure the absorbance of the spectrum at 260 nm. The results were expressed as the ratio of the absorbing materials at 260 nm of each interval to the time [23].

Statistical analysis

To compare the two groups, the unpaired t-test was applied. A p-value of <0.05 or less was considered significant [24].

5. Results and Discussion

5.1 Properties of (Fe_3O_4-Ag@ Fe_3O_4-Au) MNPs structures

The XRD patterns of two generated the samples are explain in Figures (1.1,1.2). All of the detected diffraction peaks' locations and relative intensities were remarkably close to those of magnetite recognized by the JCPDS card number(11-0614), and the acquired crystalline single-phase cubic inverse spinal Fe3O4 structure made up the samples.There was no impurity-related peak seen. The characteristic peaks of the coated NPs displayed a little broadening rather than a shift in position, suggesting that the crystal size of the Fe_3O_4-Ag MNPs was lower than that of Fe_3O_4-Au MNPs materials. Because Ag was more extensively coated on the surface of the MNPs made using previous technique, the peak intensity of the Fe_3O_4-A MNPs manufactured was likewise lower than that of the MNPs prepared using Fe_3O_4-Au. The half-height breadth of the crystal was measured to determine the crystallographic size **(**311) peak, which is the strongest reflection plane, and using the Scherer's relation (D=0.9 λ/β

cos(θ)), where (β) is the full width at half maximum (FWHM) of the 311 peak. The diameters of the (Fe_3O_4-Ag@ Fe_3O_4-Au) were calculated to be 7.3 nm and 13.7 nm, respectively [25].

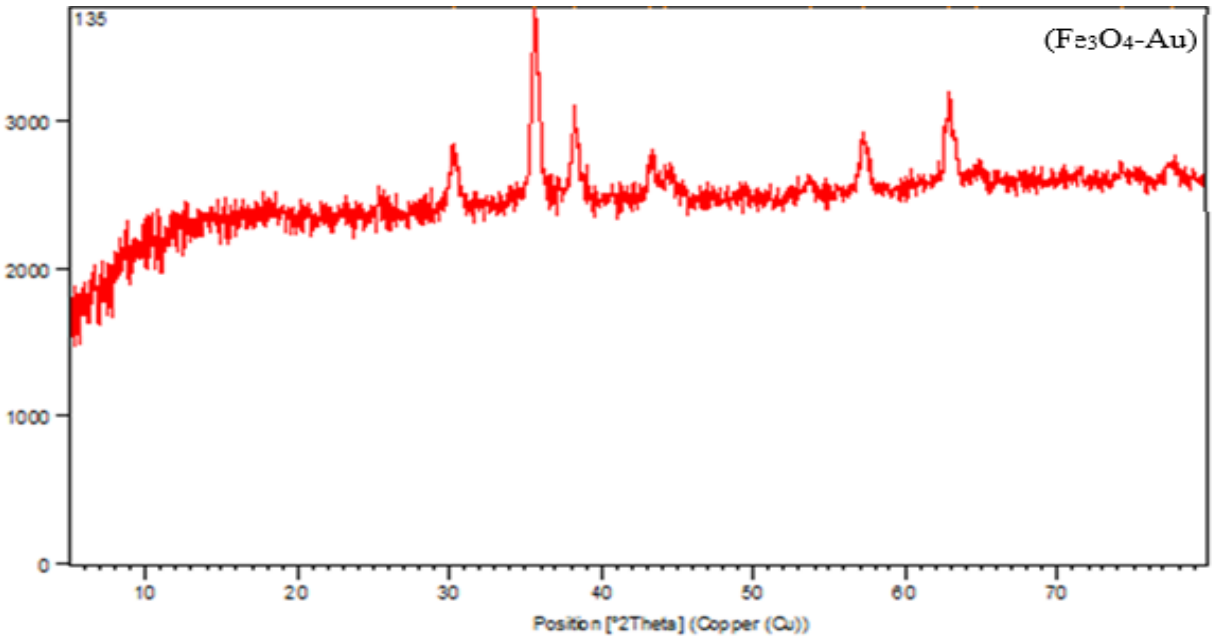

Fig. (1.1) XRD pattern of (Fe_3O_4-Au) MNPs

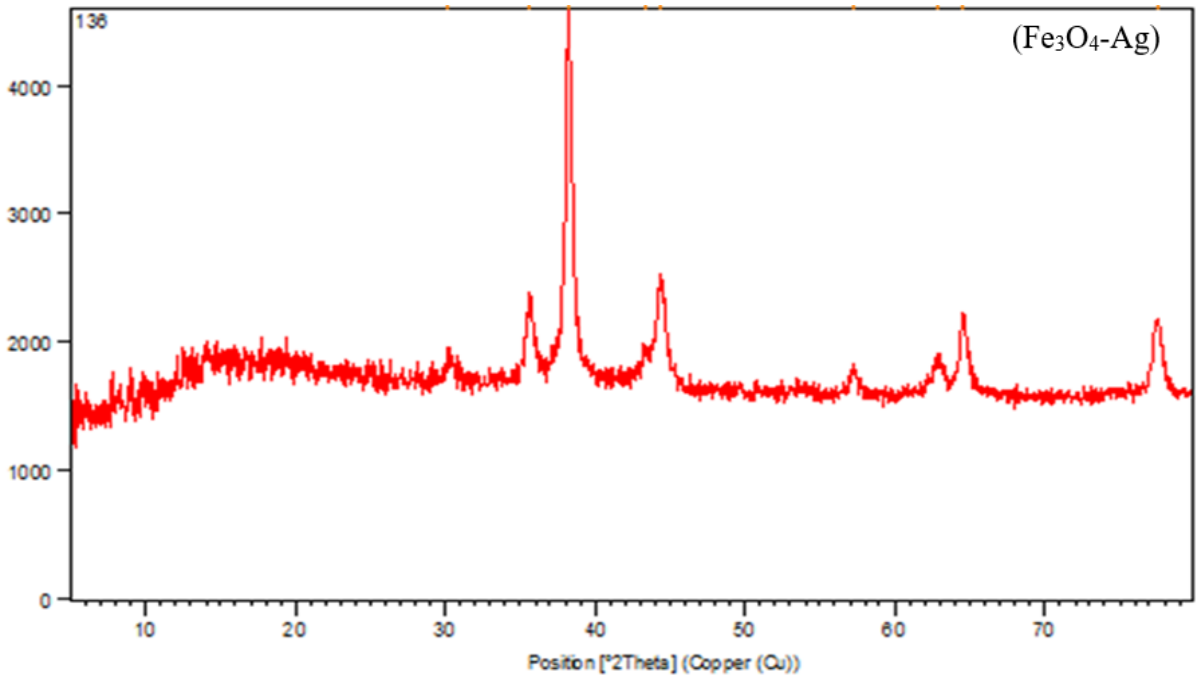

Fig. (1.2) XRD pattern of (Fe_3O_4-Ag) MNPs

5.2 Scanning electron microscopy

Scanning electron microscopy was used to examine the produced NPs' size and morphological characteristics in further detail. Figures (2.1,2.2) shows a sample from the same set of samples under a scanning microscope. Figures 2 show that a significant number of uniform MNPs (Fe_3O_4-Ag@ Fe_3O_4-Au) are obtained, with mean sizes of around 4-16 nm for Fe_3O_4-Ag and 8-22 nm for Fe_3O_4-Au. These findings showed that the created NPs were well within the preferred size for the efficient delivery of the medicines housed, suggesting a potential selective absorption of the gold and silver surfactant onto the particle's advantageous facets and a potential suppression of Fe_3O_4 free development[26].

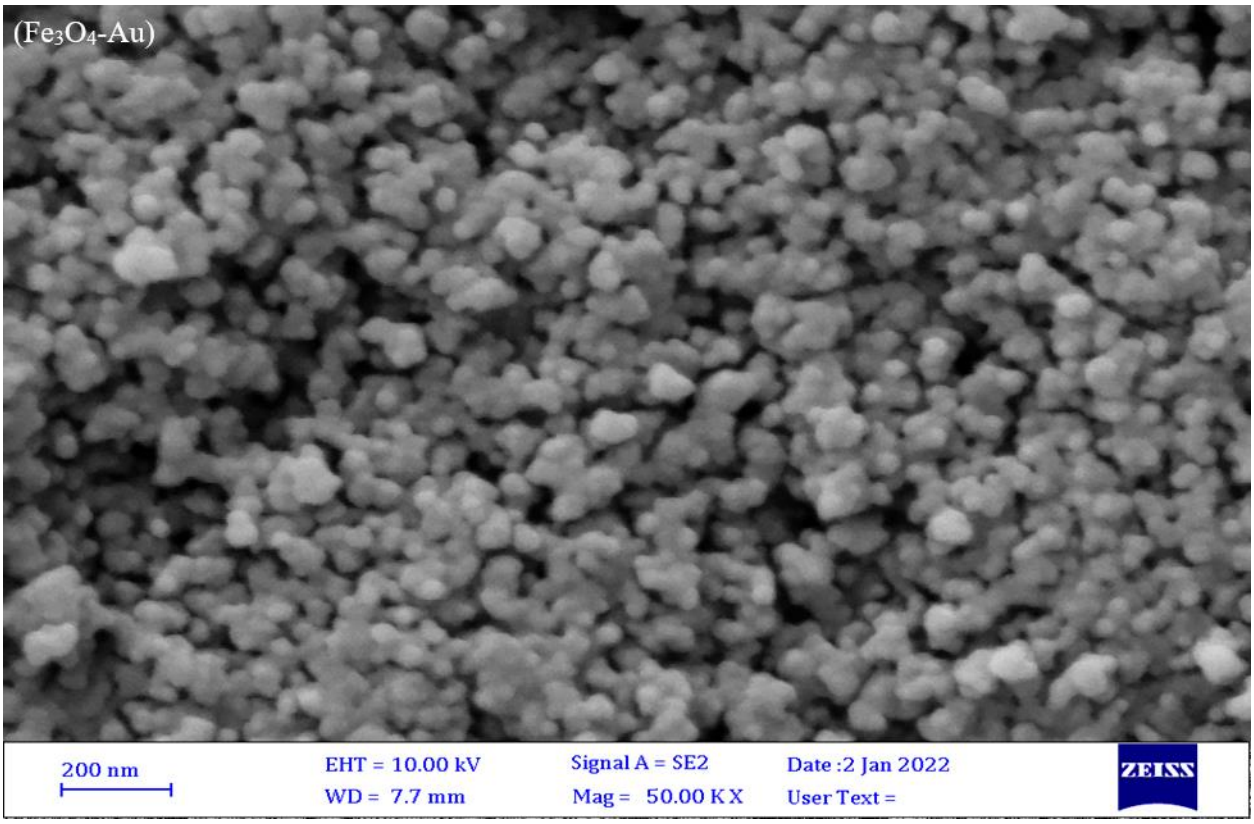

Fig.(2.1) SEM pattern of (Fe_3O_4-Au) MNPs

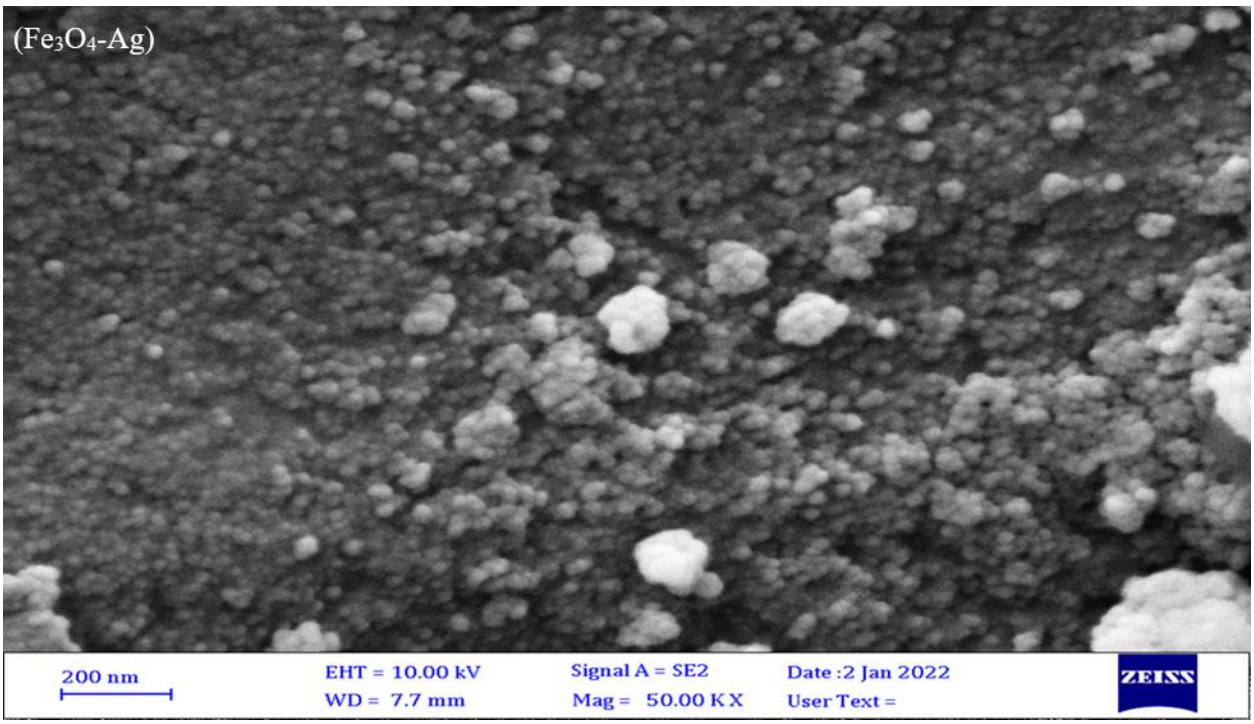

Fig.(2.2) SEM pattern of (Fe_3O_4-Ag) MNPs

6. Antibacterial activity of (Fe_3O_4-Ag@ Fe_3O_4-Au)MNPs

The *E. coli* and *S. aureus* were used to test the antibacterial activity of (Fe_3O_4-Ag@ Fe_3O_4-Au) nanoparticles. Figure (3) depicts the inhibition zones caused by different doses of (Fe_3O_4-Ag@ Fe_3O_4-Au) **.**According to the findings,(Au@ Ag) was superior to regular Fe3O4 in preventing bacterial growth. At a concentration of 100 g/ml, Fe_3O_4-Ag generated an Fe3O4-Au produced an inhibitory zone diameter of (17 mm) against *S. aureus* and (11 mm) against *E. coli*, with inhibitory zones of (21 mm) against *S. aureus* and (16 mm) against *E. coli*, at a concentration of 100 g/ml [27]. These outcomes matched those of further research.

Fe_3O_4-Ag has more antibacterial activity against the species under study than Fe_3O_4-Au Figure (3).The concentration of the nanoparticles had an impact on the organisms under study. The existence of an outer membranes in the bacterials structures aer what gives microorganisms their resistance to external chemicals [28]. Silver is a frequently used coating material for a variety of biomedical applications, boosting cellular NP absorption, delaying NP aggregation, and prolonging the plasma half-life of magnetic nanoparticles (NPs) in the blood.

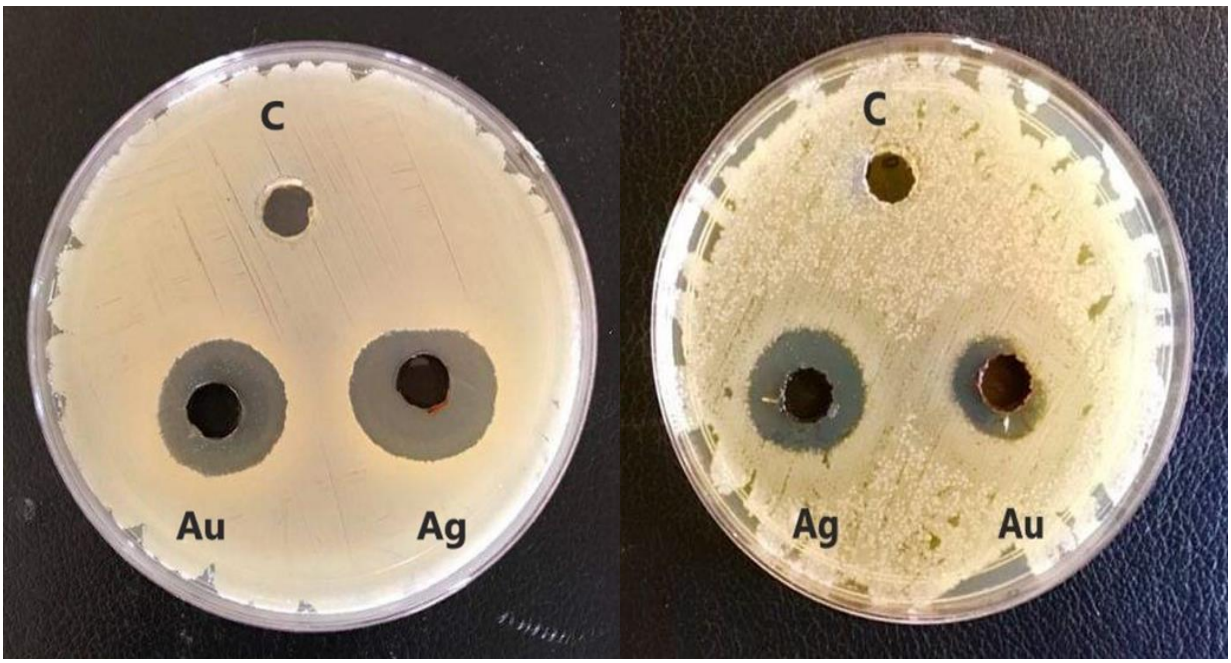

Fig. 3 (Fe_3O_4-Ag@ Fe_3O_4-Au) magnetic nanoparticles have antibacterial action against *S. aureus* and *E. coli.*

6.2 Release of cellular materials

Figure (4) displays The treated organisms emitted the absorbance of biological compounds at 260 nm. This method connects the passing of time to the 260 nm OD of the culture medium. Figure (4) shows that Fe_3O_4-Ag was more effective in damaging the cell membrane of the species under investigation than Fe_3O_4-Au. The results demonstrate that both (Fe_3O_4-Ag and Fe_3O_4-Au) MNPs increased bacterial cytoplasmic membrane permeability. It is important to note that the cytoplasmic membrane of bacteria serves as a stopgap for ion leakage [28-29].

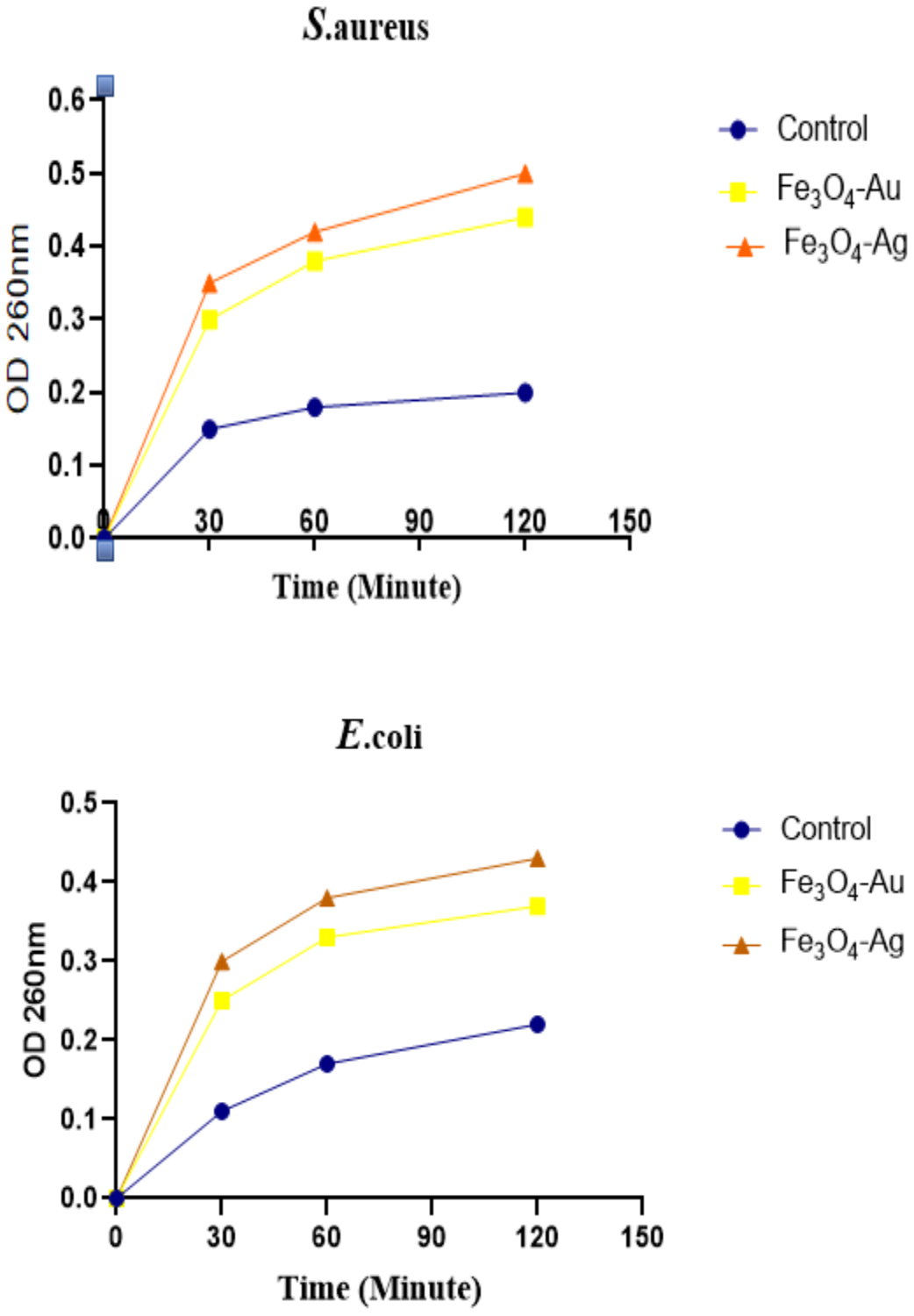

Fig. 4 Effects of (Fe_3O_4-Ag@ Fe_3O_4-Au) MNPs on the release of bacterial cellular components.

7. Conclusion

This work investigated the antibacterial properties of hydrothermally produced functionalized magnetite nanoparticles. The functionalized nanoparticles Fe_3O_4-Ag showed more inhibitory effects against the investigated microorganisms than the Fe_3O_4-Au ,according to the findings. In addition, the molecules outperformed *Escherichia coli* in addition to their ability to fight *Staphylococcus aureus* even more. The results show that the Fe_3O_4-Au and functionalized nanoparticles Fe_3O_4-Ag were successfully prepared and characterized and showed promising antibacterial activity against *S.aureus* and *E.coli*.

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