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RESEARCH ARTICLE – PHYSICS

Fabrication of Silver Oxide Nanoparticles using sol gel technique and Their Role in Inhibiting Bacterial Biofilm Development

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Abstract

In this research, silver oxide nanoparticles were prepared using the sol-gel method, and measurements were made, namely XRD, FESEM, FTIR and EDX, and the nanoparticles were confirmed, and then these nanoparticles(Ag_2O) were used as a powder against the formation of the biofilm of bacteria (bacterial adhesion measurement), where five types of bacteria were used (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa* , *P. mirabilis and Stenotrophomonas maltophilia*), where these particles showed their effectiveness significantly on the formation of the biofilm of bacteria, especially bacteria *Klebsiella pneumoniae* at 0.53O.D as well as Pseudomonas 0.4O.D , while bacteria *E. coli*, *P. mirabili* and *Stenotrophomonas maltophilia* showed resistance at different concentrations.

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Keywords: Ag_2O Nanoparticles, Sol-gel, biofilm of bacteria, Structural properties.

Introduction

Nanoparticles are gaining popularity due to their useful applications in biology, medicine, and other disciplines[1],[2],[3]. Silver oxide nanoparticles are the most widely used nanomaterials in the textile, food, electronics, building materials, pharmaceutical, antibacterial, cosmetic, and paint industries[4],[5]. Current research investigations focus heavily on the environmentally friendly and economical manufacturing of silver oxide nanoparticles. Great antibacterial, antiviral, and antifungal properties are exhibited by silver oxide nanoparticles[6]. The use of nanoparticles in cancer treatment, nano medicine, nano therapeutics, and nano theranostic applications is growing quickly[7]. When human tissues and cells absorb nanomaterials or nanoparticles, oxidative stress caused by the toxicity of the nanoparticles results in cell death. The capacity to bond, physicochemical characteristics, surfactant properties, location inside cells, exocytosis, and the kinetics of uptake all affect nanoparticle size. The shape and composition of the cell affect the nanoparticle uptake kinetics as well[8],[9],[10],[11]. Silver oxide nanoparticles are employed as antiviral medications to combat certain kinds of illness-causing viruses. Main research on silver nanoparticles is done to investigate potential bacterial killing. Additionally, it has been discovered that silver oxide nanoparticles are effective against a variety of viruses. Because of their anti-viral, anti-cancer, and other surface charge qualities, synthesized nanomaterials can be used as Nano medical and nontherapeutic medications that may help treat bacterial and viral infections as well as cancer. Its potent antibacterial qualities allow it to be applied to living things, including as humans, in food, on the skin, and in the respiratory system[12]. Antimicrobial agent resistance is a major global public health concern in the twenty-first century that is only going to become worse. Infections with resistant bacteria greatly raise the death rates, treatment costs, spread of epidemics, and length of illness[13],[14].

Microbial biofilm is a surface-mounted collection of integrated populations of microbial cells that can be found on a variety of surfaces, including glass, polystyrene, methyl methacrylate, infected wounds, gallstones, and surfaces coated with pluronic material [15] ,[16].Biofilms could postpone healing of wounds, this can complicate management of a number of nosocomial diseases and disorders, such as endocarditis, otitis media, cystic fibrosis, dentists, and chronic prostate[17],[18]. In terms of structure, EPS, which is mostly made up of proteins, polysaccharides, glycoproteins, and glycolipids, and water make up more than 90% of a biofilm [19],[20],[21]. Surfaces with certain properties, such drug-tethered surfaces [22] , polysaccharide-based surfaces[23], and self-cleaning surfaces [24], can prevent the formation of biofilms. Surface functionalization—the impregnation, coating, or doping of surfaces with nanomaterials—has enormous potential for application as inhibitory instruments. There have been reports of several kinds of

nanomaterials having antibacterial and anti-biofilm qualities [25]. Biofilms serve a variety of purposes. They serve as barriers to shield germs from the immune system of the host and antibiotics [26]. Second, they maintain moisture and offer mechanical strength and stability for adhesion to surfaces [27]. Thirdly, they serve as a source of nutrients and aid in the metabolism of the bacterial constituents [28]. Lastly, they exchange genetic information amongst themselves, which enables them to adapt to hostile settings, including nutrient-poor ones [19],[29]. In nature, most bacteria don't exist alone; instead, they communicate with one another. In addition to existing inside living things like humans, they can also survive on inorganic surfaces and cause disease[30]. For instance, Pseudomonas aeruginosa is responsible for chronic sinusitis and cystic fibrosis pneumonia [31]. Endodontic infections and dental caries are attributed to acidogenic gram-positive cocci bacteria [32],[33], while endocarditis is caused by viridans streptococci[34] . Recently, there has been a lot of interest in the development of novel antimicrobials utilizing nanomaterials and the aim of this study is to prepare silver oxide nanoparticles by the colloidal solution (sol-gel) method and use these particles as antibiotics, where they were used against the formation of bacterial biofilm.

MATERIALS AND METHODS

Materials

Silver nitrate Company (SAMCHUN- DAEJUNG- KOREA), Purity %(99.8%)., potassium hydroxide (KOH)Company(SDFCAL –INDIA), Purity % (85.98%),Nutrient Broth, Company HIMEDIA INDIA, distilled water and microtiter plate are the chemicals that were employed in the preparation; they are all pure and of good worldwide origin.

Synthesis of Ag_2O nanoparticles

Silver oxide was prepared by the sol-gel method in order to obtain silver oxide nanoparticles, as follows, it began by dissolving 0.1 Mol of silver nitrate in 200 ml of deionized distilled water. Then the mixture was stirred for an hour at room temperature using a magnetic drive. Then the PH was measured, and we found out that it was 6.76. The material was exposed to a temperature of 80 degrees Celsius for two hours on a magnetic drive after adding 50 milliliters of potassium hydroxide (KOH) at a concentration of two molars. Then the material was purified using filter paper and washed about five times before being placed in the oven for twenty-four hours at 60 degrees Celsius and then placed at a temperature of 500 degrees Celsius for one hour. As for the way in which the biofilm was prepared, it is :In this test used five types of clinical bacterial isolates((*E. coli, Klebsiella pneumoniae, Pseudomonas aeroginosa , P. mirabilis and Stenotrophomonas maltophilia*), by preparing overnight broth of each bacteria, then we added 100 with five concentrations of the Nanoparticles of previous preparations, then mixed with bacterial broth , in addition to control groups (first was bacterial broth without any addition and secondly the suspension of the nanomaterials only, these test ed groups was added in a microleter in 96 microtiter plate... then all plates were incubated for 24 hours at 37 C° at the second day after 24 hours of incubation.. the pkates were washed with phosphate buffer saline then a stain of crystal violate added for a period of time, after that all wells were washed with tap water and finally the absorbency was measured by spectroscopy at 600 nm wavelength.

RESULTS AND DISCUSSION

X-ray diffraction analysis

The X-ray diffraction of all prepared samples have been measured using diffractometer of 0.156 nm wavelength. The spectra were plotted and characterized in the figure 1 .The silver oxide XRD pattern is displayed in Figure 1. The cubic phase structure, which includes pattern peaks at $2\theta = 38^{\circ}$, 44°, 64°, and 77°. may be well matched with all of the diffraction peaks of (111), (200), (220), and (311). The JCPDS card number (76-1393) [35],[36] and the structure of the information produced match each other perfectly. Additionally, with SPVinay, the researcher, and his associates and Seemab Iqbal the researcher, and his associates[8].

We used the Debye-Scherrer equation to get the average crystallite size:

$$D = K\lambda/\beta \cos\theta$$

Where D is the particle's crystallite size., θ is the glancing angle, λ is the wavelength of the X-ray Cu-Kα radiation (0.15406 nm), , K is constant equal (0.94), and β is full width at half maximum. Elastic

strain is also calculated using data from XRD analysis. According to the elastic strain data, particles smaller than 20 nm have more strain, whereas particles larger than 20 nm have less strain. This implies that smaller particles are under more strain, and larger particles are under less strain. These results matched the values found in the literature [37].

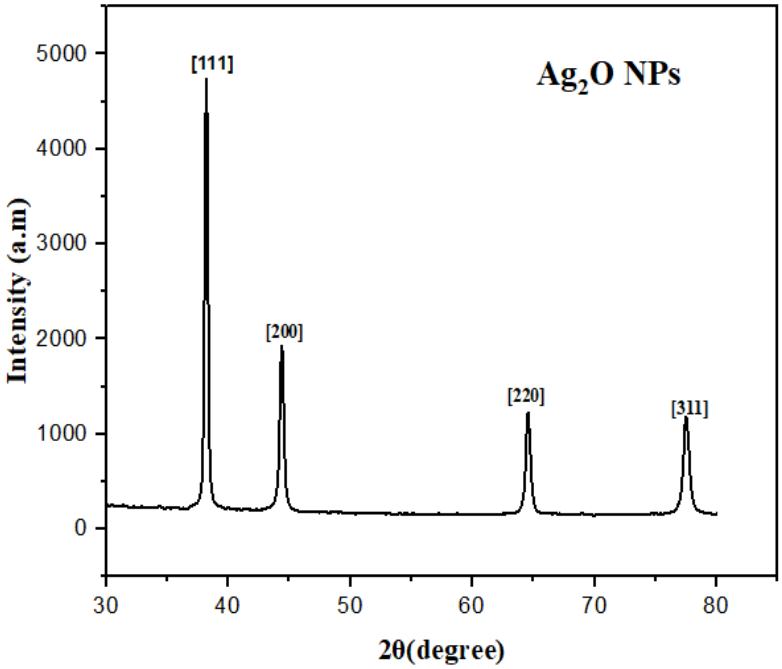

Fig. 1. X-ray diffraction (XRD) pattern for Ag_2O nanoparticles.

Table 1. Crystalliyte Size and FWHM values for Ag_2O nanoparticles.

100% Ag2O-2Θ	FWHM (radian)	Crystalliyte Size D (nm)
9.514	1.069	7.451
12.159	22.465	0.355
64.572	1.876	5.008
67.249	189.994	0.050

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

The morphology and size of the synthesised silver oxide nanoparticles created through the method of sol-gel synthesis were examined using the FESEM technique. Figure 2 exhibits the results of the experiment. An essential factor in the shape of nanoparticles is the degree of concentration of precursors use in the synthesis process. It was found that the size of silver oxide nanoparticles ranges from 50.06 to 64.52 nm, and the smallest size of nanoparticles is 50.06 nm, as shown in Figure 2, where the particles turned out to have a spherical shape, as shown in the FESEM examination[38]. X

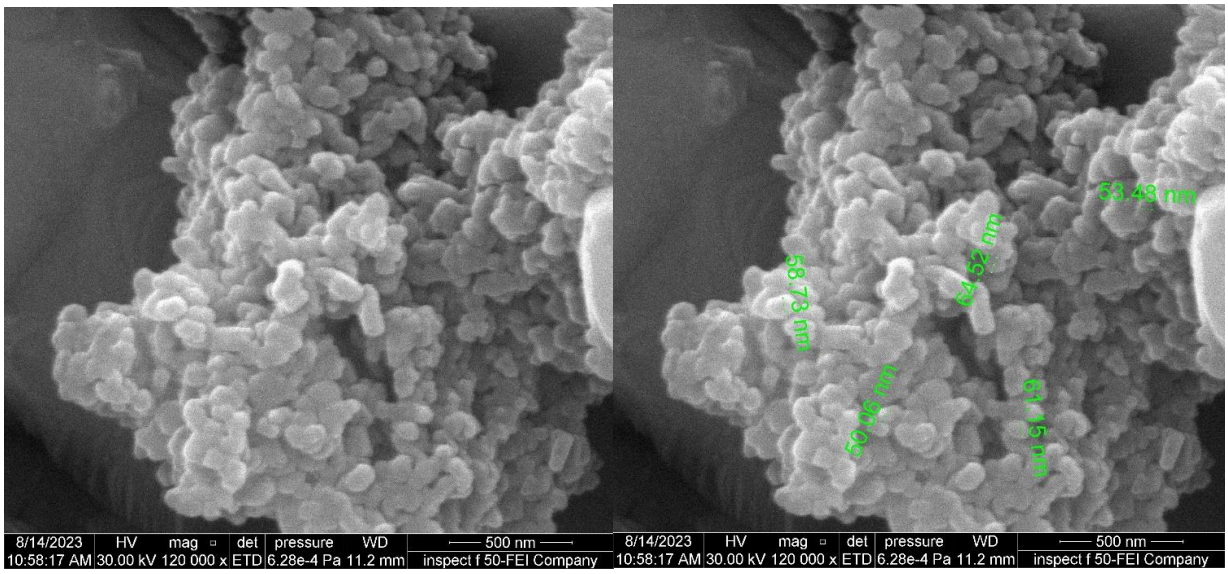

Fig. 2. FESEM images of silver oxide

Fourier transforms infrared spectroscopy (FTIR)

The Ag_2O nanoparticle FTIR spectrum where the ftir spectrum's seeming results are shown as in Fig. 3. This aids in the identification of biomolecular bound to Ag_2O nanoparticle surfaces as well. Because of the biologic molecules, it shows numerous absorption peaks that indicate it's complicated structure. Ag_2O nanoparticles show peaks at 421,570, 600, 703, 832, 982, 1403, 1628,2341,2372 and 3448 cm^{-1}. The peak Approximately 570 cm^{-1} corresponds to Ag-O vibrations[39],[40],[41].

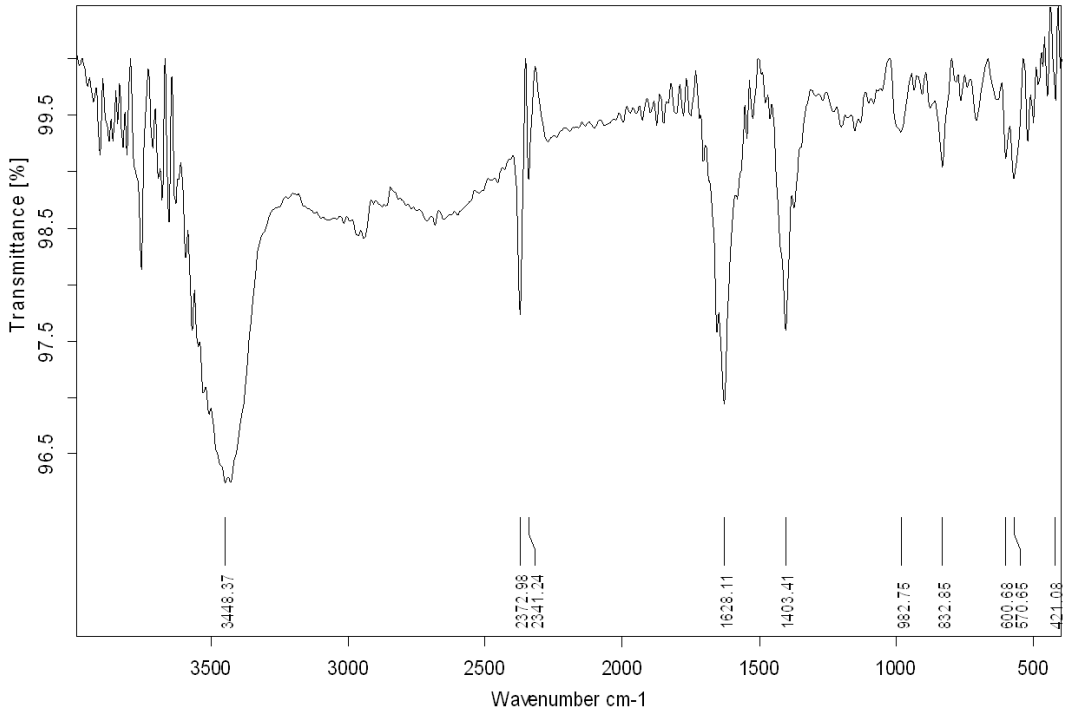

Fig. 3. FTIR spectra of Ag_2 O nanoparticles.

Energy dispersive X-ray spectroscopy (EDX)

Using X-ray scattering and a FESEM examination, the materials and percentages of each substance or element within the sample are determined. The elements in each sample, which were prepared using the sol-gel method, are shown in Figure 4. After X-rays are emitted and dispersed at particular wavelengths, an EDS analysis is performed to determine the amounts of each material and the chemical makeup of the different compounds. The sample was discovered to include silver oxide nanoparticles, as indicated by Table 2. The components present in the sample were found to be 50%

Ag, 17.2% O, and 32.8% C, as shown in Table .2., which shows the weight and atomic ratios, and also Fig.5., which shows the elemental mappings of Ag, O, and C.

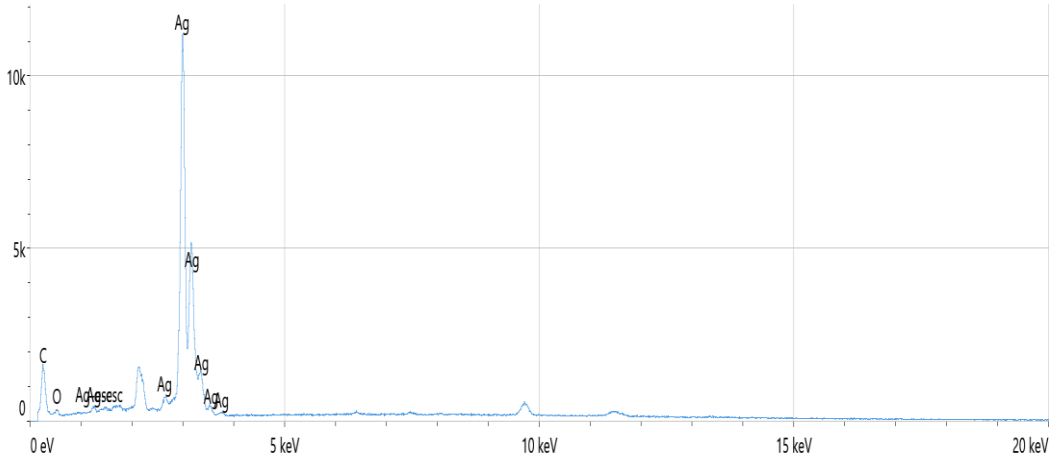

Fig.4.EDX analysis of $_{Ag2O}$ nanoparticles and certain impurities (other chemical elements).

Table .2. Ag and O nanoparticles' chemical composition together with a few other elements.

Element	Atomic %	Weight %
C	32.8	6.5
O	17.2	4.6
Ag	50	88.9
Totals	100.00	

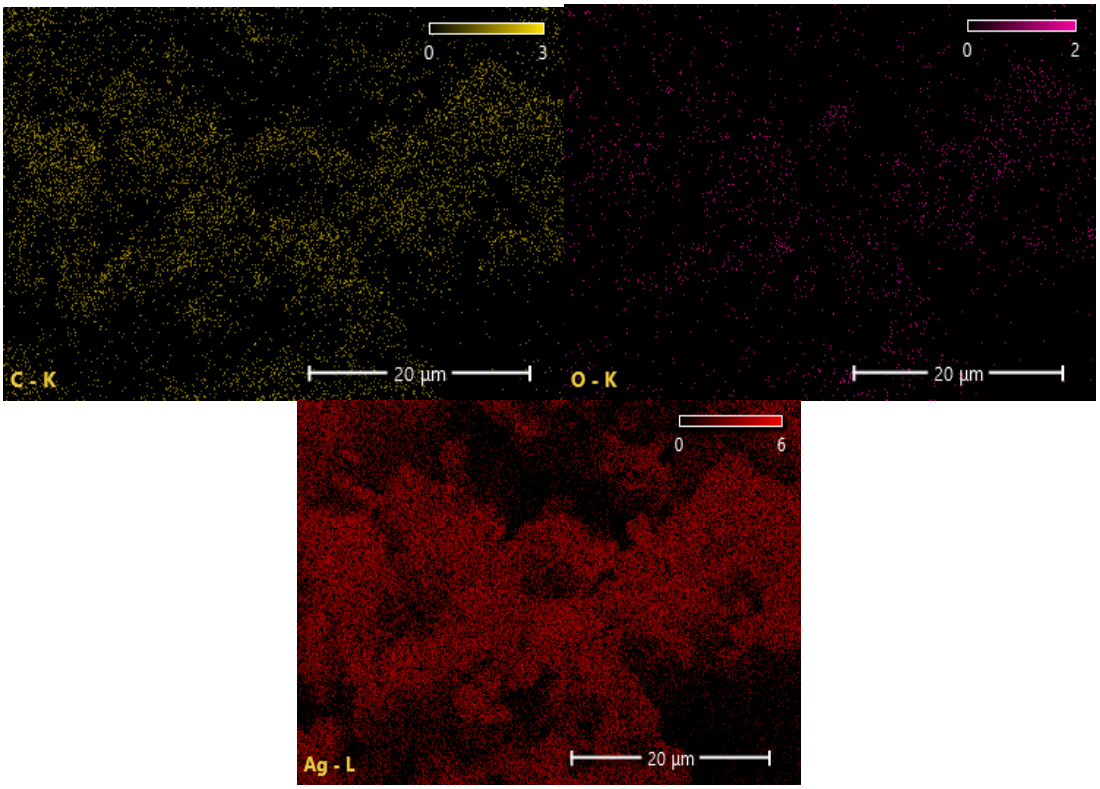

Fig.5. Elemental mapping of Ag_2O nanoparticles with some other chemical elements prepared by the sol-gel method.

Biofilm

Biofilm is the most important feature of bacteria that enhances the binding of bacteria to surfaces of surgical instruments and prostheses [42].Patients getting long-term indwelling urinary catheterization might find this particularly challenging as they run the risk of infections associated with catheters (CAUTIs). The special ability of certain bacteria to produce crystallization biofilms, which eventually cause encrusted and obstructed catheters, complicates such infections[43].

Many chemical compounds affect microbial populations in two manners either enhance or inhibit their growth. The efficacy of Ag_2ONPs, in reducing biofilm formation by *E. coli, Klebsiella pneumoniae, Pseudomonas aeroginosa , P. mirabilis and Stenotrophomonas maltophilia* were demonstrated and visually represented in Figures 6,7,8,9 and 10.

Ag_2O NPs of biofilms

Many antibiotics include target cells that are deeply rooted in the biofilm matrix, which makes treating them very challenging. Drug-resistant microbiological infections currently require no antibiotic treatments, which has led to an increase in antibiotic resistance as a major worldwide health concern. While they're effective against the biofilms because of their tiny size and high surfaces to volume ratio, nanoparticles provide an alternative and simple approach to treating PA infections. Obtained data of the current study showed that, Ag2O NPs have remarkable effect on the biofilm formation of the tested bacteria, in high concentrations especially, while another view in the case of *Klebsiella pneumoniae* which showed clear resistance O.D. 0.53 followed by *Pseudomonas aeroginosa* as O.D 0.4, while the other bacteria were resistant to the low concentrations of the nanoparticles in comparison to control group. But the interesting was that *Stenotrophomonas maltophilia* was resistant to the high concentration 0.1 and sensitive to the rest concentrations.

It had been found that silver ions have strong biocidal effects. Since ancient times, silver compounds have been applied as disinfectants. Resistant to antibiotics bacteria, fungi, and viruses can all be defeated by silver nanoparticles (Ag2NPs). Though long investigated, the antibiotic-related mechanisms of silver ion activity remain mostly unclear. Even little is known about the mechanisms by which AgNP acts on microorganisms. Instead of interacting with the phosphate groups in DNA, silver ions can interact between the bases and impact the replication capacity of DNA. It had been suggested that pouring is a key component of AgNPs' antibacterial activity mechanisms.

In comparison of the anti-bacterial effects and the inhibition of biofilm formations, it could be suggested that the main effect of silver nanoparticles had bactericidal effects and the most remarkable bacteriostatic effect.

Because AgNPs are nanoscale particles have a size of 14, 52, they are capable of changing the structure of bacterial cell walls in order to allow for penetration. Cellular lysis can occur when the organelles burst caused by breakdown of the cellular membranes. Comparing the nanoparticles in the present research (5–30 nm) to those in prior studies (65–90 nm in diameter), the lower size of the nanoparticles in this study is ascribed to their wonderful efficacy in entering cells of bacteria[44].

showcased the potential of phyto-synthesized Ag nanoparticles (AgNPs) as anti-biofilm agents and for various biomedical applications [45],on the other hand, revealed that AgNPs decreased the production of extracellular polymeric substances and led to increased cellular protein leakage due to disruption of cellular membranes. The inhibition of biofilm formation was quantified as 64% and 86% for MDR *K. pneumoniae*. AgNPs have been extensively investigated for their antimicrobial properties and their ability to mitigate biofilm formation in bacteria. Multiple mechanisms have been proposed to explain the effectiveness of AgNPs in combating biofilms, including microbial cell membrane disruption, generation of reactive oxygen species (ROS), quorum sensing inhibition, binding to microbial DNA, ion release, and metal-induced stress. These mechanisms collectively contribute to the capacity of AgNPs to reduce bacterial biofilm formation. It should be noted that the specific mechanisms and their relative importance may vary depending on the bacterial species and experimental conditions [46].

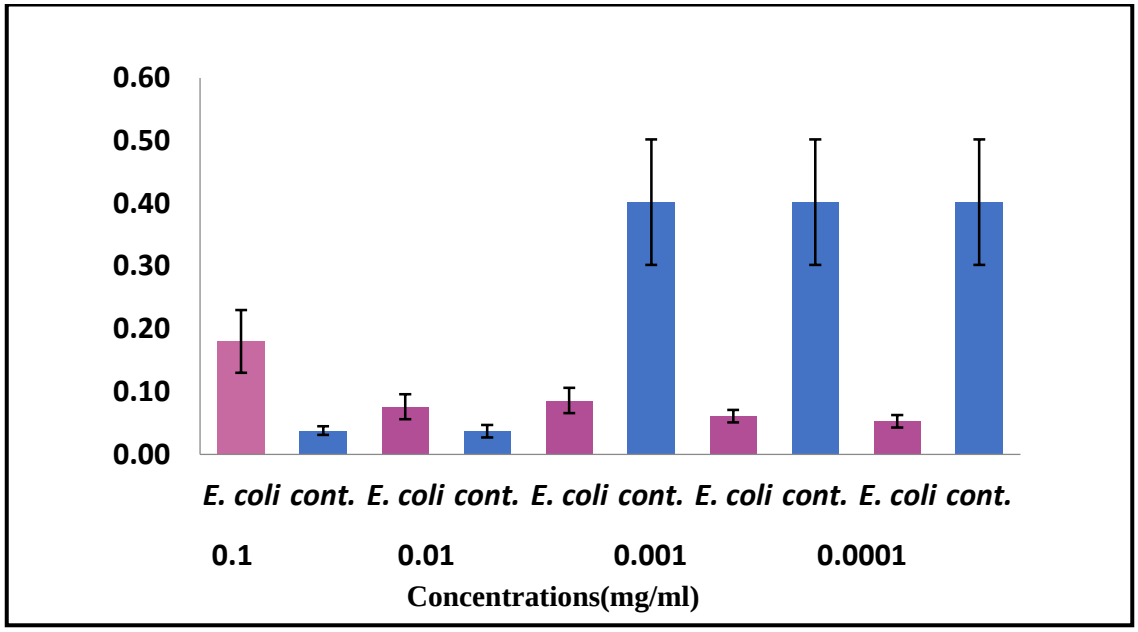

Fig .6. The ability of Ag_2O NPs to reduce the Biofilm formation of *E. coli*.

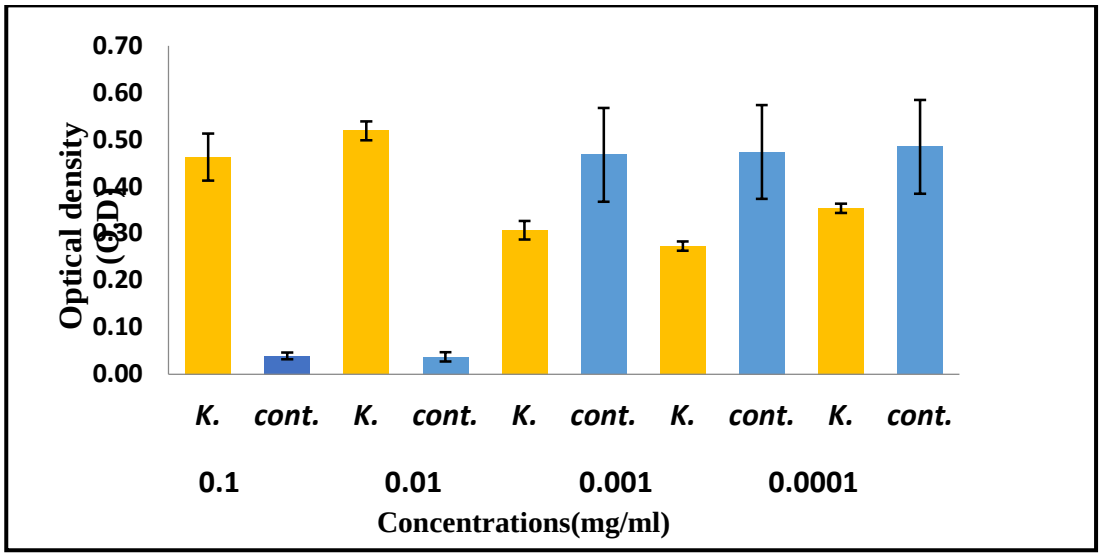

Fig .7. The ability of Ag_2O NPs to reduce the Biofilm formation of *Klebsiella*

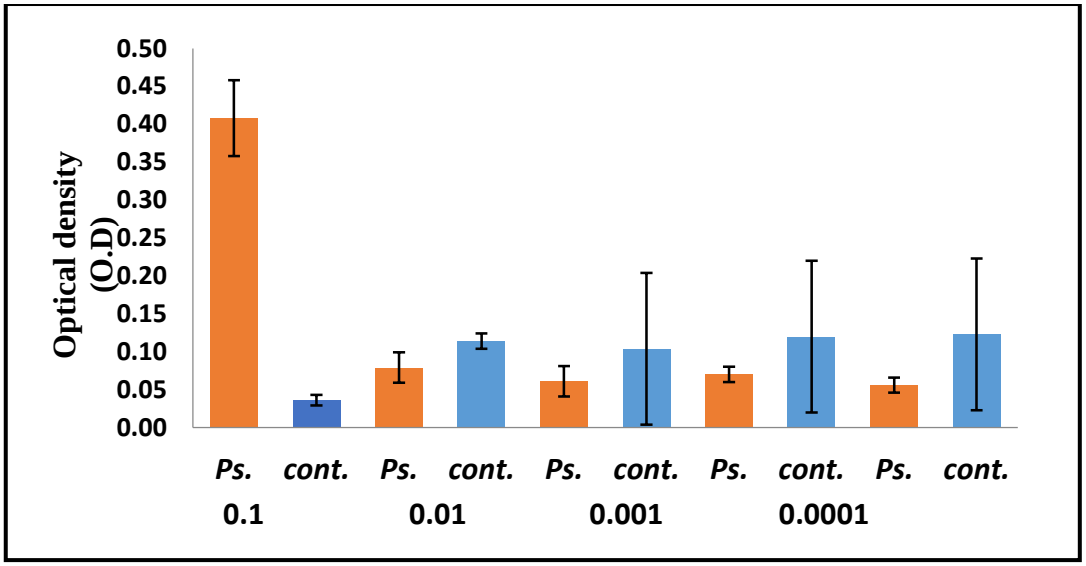

Fig .8. The ability of Ag_2O NPs to reduce the Biofilm formation of *pseudomonas*

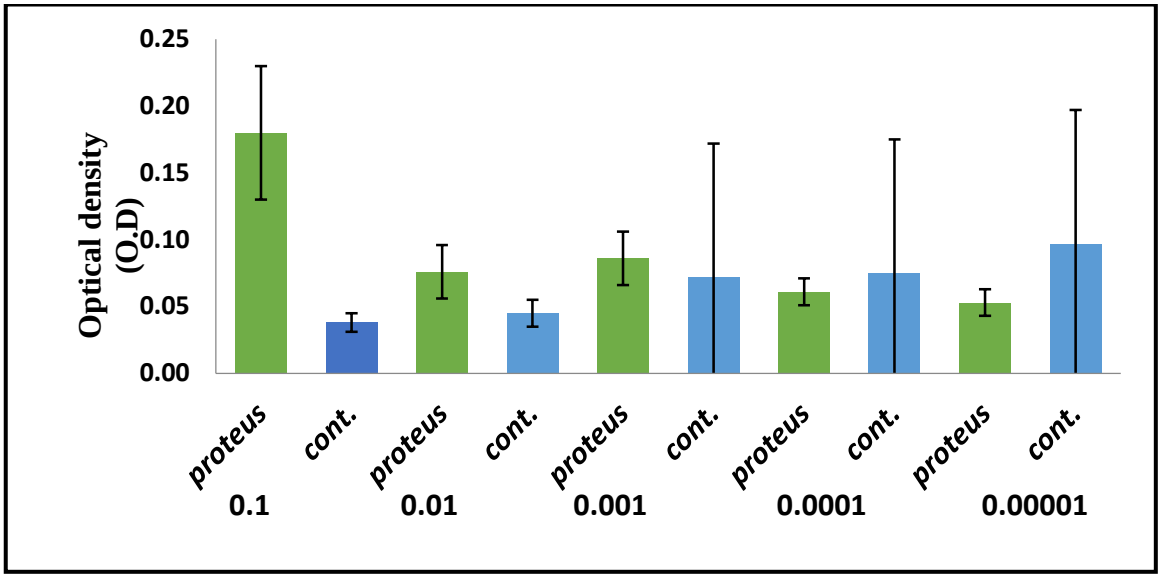

Fig .9. The ability of Ag_2O NPs to reduce the Biofilm formation of *proteus*

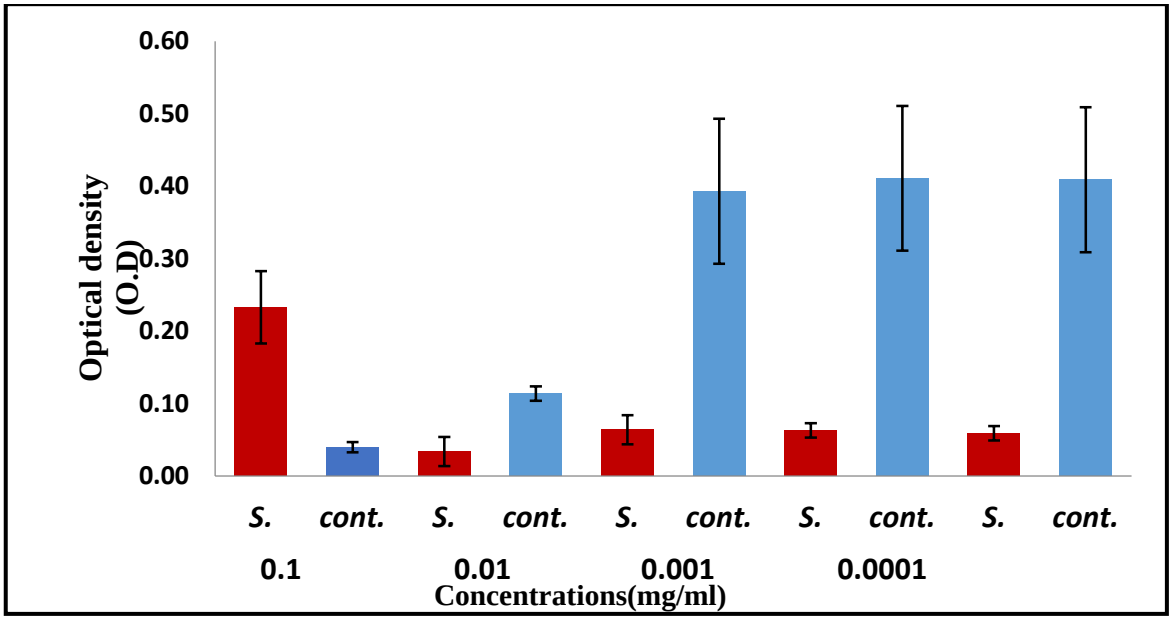

Fig .10. The ability of Ag_2O NPs to reduce the Biofilm formation of *Stenotro*

Conclusion

Obtained data of the current study showed that, Ag_2O NPs have remarkable effect on the biofilm formation of the tested bacteria, in high concentrations especially, while another view in the case of *E. coli, Klebsiella pneumoniae,* which showed clear resistance 0.53 O.D. followed by *Pseudomonas aeroginosa* as 0.4 O.D, while the other bacteria were resistant to the low concentrations of the nanoparticles in comparison to control group. But the interesting was that *Stenotrophomonas maltophilia* was resistant to the high concentration 0.1 and sensitive to the rest concentrations. It suggested that poring an important factors in the mechanisms of antibacterial action of AgNPs. In the comparison of the anti-bacterial effects and the inhibition of biofilm formations, it could be suggested that the main effect of silver nanoparticles had a bactericidal impact and the most remarkable bacteriostatic effect.Ag_2ONPs' nanoscale size makes it possible to alter the structure of bacterial cell walls, which allows them to pass through them 14, 52. Cellular destruction may ensue from the rupture of organelles caused by breakage of the cellular membranes. In contrast to other studies where the size of the nanoparticles was 65–90 nm in diameter, the smaller size of the nanoparticles in this study (5–30 nm) is ascribed to their effectiveness in entering bacterial cells.

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