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Susceptibility of Biosynthesis Silver Nanoparticles on the Antimicrobial Potential of Poly N-vinylpyrrolidone

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Abstract: Silver nanoparticles were synthesized by chemical reduction method from pomegranate Peel with and without of surfactant sodium dodecylsulphat (SDS). In the present studies polyN-vinylpyrrolidone (PNVPy) nanocomposite was polymerized in situ in presence of various compositions of AgNPs. Thin films of these nanocomposites were characterized by X-ray diffraction and Transmission electron microscopy TEM. X-ray diffraction showed the presence of the peaks at 2θ values of 38°, 44°, 64° and 77° corresponding to clusters phase of silver metal. TEM photographs revealed the presence of Ag nanoparticles of sizes varying from 20 - 50 nm. Antimicrobial potential of PNVPy/AgNPs nanocomposites were studied. Silver nanoparticles are the newly used method mitigating most of the resistant bacteria. Nuisance bacteria could disturb the efficacy of different industrial plants when related to fouling problems. The present study investigates the potentials of some vinylpyrrolidone compounds with biosynthesized silver nanoparticles for mitigation of microbial foulants. Marine microorganisms like *Bacillus subtilis, Staphylococcus sp., Pseudomonas sp.,* and *Escherichia sp.* where considered as the primary causatives of micro biofouling mechanism. They were exposed to ten different concentration of the formerly mentioned compounds W/WO addition of surface active materials. The obtained optimum mixture was the tested polymer with 0.4 AgNPs with the addition of the tested surfactant.

Keywords: Antimicrobial Activity, Microbiofouling, Polyvinylpyrrolidone, Silver Nanoparticles, and Sodium Dodecyl Sulphate

1. Introduction

Today, nanometal particles, especially silver, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of electronics, intercalation material for electrical batteries, solar energy absorption, optical receptors, antimicrobial and therapeutics at the nanoscale [1-4]. However, there is still need for cost-effective, commercially feasible as well environmentally clean synthesis route to synthesize silver nanoparticles. A number of approaches are available for the synthesis of silver nanoparticles and recently via green chemistry route [5-7]. The synthesis of silver nanoparticles by using environmentally benevolent materials like plant leaf extract [8], bacteria [9], fungi [10], soluble starch [11] and enzymes [12] offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they use non-toxic chemicals for the synthesis protocol.

Nowadays, the use of nanoparticles as a green alternative to chemicals and antibiotics are wide spread possessing a high potential to solve the problem of the emergence of bacterial resistivity towards different treatments [13]. Particularly speaking, silver nanoparticles have been proved to possess the potentials of production of some sorts of antimicrobials [14, 15–25]. Cytotoxicity of Silver promotes its antimicrobial activity towards various diseases as an antiseptic and antimicrobial against Gram-positive and

Gram-negative bacteria [26–28] due to its low cytotoxicity [29]. Although the highly antibacterial effect of AgNPs has been widely described, their mechanism of action is yet to be fully elucidated. Its broad-spectrum antibacterial efficacy and broad-spectrum activity against different microorganisms might be referred to their interaction with microbes. Moreover, their particular structure and the different modes of establishing an interaction with bacterial surfaces may offer a unique and under probed antibacterial mechanism to exploit. From a structural point of view, AgNPs have at least one dimension in the range from 1 to 100 nm and more importantly, as particle size decreases, the surface area-to-volume ratio greatly increases. As a consequence, the physical, chemical and biological properties are markedly different from those of the bulk material of origin. Non-traditional antibacterial agents are thus of great interest to overcome resistance that develops from several pathogenic microorganisms against most of the commonly used antibiotics [14]. Marine microorganisms like *Escherichia sp., Staphylococcus sp., and Pseudomonas sp.* are known to be effective in the biofouling process [30]. Moreover, *Pseudomonas sp*., the most prevalent in the water and seawater industries, has been implicated in the corrosion process of stainless and mild steels, and aluminium alloys in marine habitats. In industrial settings of cooling-water towers, water pipelines, membrane unit, and food-processing plants, the unwanted biofilms of *Staphylococcus aureus and Staphylococcus epidermidis* are responsible for fouling [31-32]. Polyvinylpyrrolidone (PNVPy) showed good antibacterial activity towards *S. aureus, E. coli, P. aeruginosa, Bacillus subtilis*, and good fungicidal activity against various yeasts and molds [33]. In the course of their 40 year history, vinylpyrrolidone polymers is water-soluble, non-charged, non-toxic and have been extensively used as, especially in the pharmaceutical, cosmetic, and food industry as well as for numerous technical application [34]. Poly (N-vinylpyrrolidone) was grafted onto modified nanoparticles by surface- initiated radical polymerization. The structure, particle size, and composition of PNVPy-functionalized magnetite nanoparticles were examined. Recently, the most widely used constant material for the stabilization of silver nanoparticles is polymer such as poly [N-vinylpyrrolidone (PNVPy)] with metal nanoparticles. The PNVPy can also control the reduction rate of the silver ions and the aggregation process of metal atoms. The PNVPy control the aggregation of the silver atoms in solution [35].

In the present study, an eco-friendly process for the synthesis of silver nanoparticles was prepared through the reduction of aqueous Ag^+ (1mM) ions by the Pomegranate fruits. The reduction of metal ions is fairly rapid, occurs readily in solution, and results in a high density of extremely stable silver nanoparticles. We carried out chemical synthesis and characterization of PNVPy-silver nanoparticles. We investigate the potentials of some polyvinylpyrrolidone compounds with biosynthesized silver nanoparticles for mitigation of microbial foulants. Marine microorganisms like *Bacillus subtilis, Staphylococcus sp., Pseudomonas sp., and Escherichia sp.* where considered as the primary causatives of micro biofouling mechanism. They were exposed to ten different concentration of the formerly mentioned compounds W/WO addition of surface active materials.

2. Materials and Methods

2.1. Materials

N-vinylpyrrolidone (NVPy) monomer, 2, 2' Azobisiso-butyronitrile (AIBN) as free radical initiator and silver nitrate $AgNO_3$ were all supplied from Aldrich. Sodium dodecyl sulphat (SDS) as a surfactant was provided by Al-Gomhoria Company. Pomegranate fruits were collected from the local market.

2.2. Equipment

2.2.1. Fourier Transform Infrared Spectroscopy (FTIR)

IR measurement was carried out with KBr methods for polyN-vinylpyrrolidone (PNVPy) nanocomposite samples (2 mg), which was dried overnight at 60°C under reduced pressure, were mechanically well-blended with 100 mg of KBr. The thickness of the KBr disk was 0.5 mm. The KBr disk of the mixed powder was desiccated for 24 h at 110°C under reduced pressure and then its IR spectrum was recorded with a Shimadzu FTIR-4200 spectrometer using a disk of 100 mg KBr as a reference. The maximum intensity of the IR absorption band was determined by the baseline method.

2.2.2. X-ray Diffraction (XRD)

X-Ray Diffraction patterns of the sheet samples were recorded on a X-ray diffractometer (D/Max2500VB2+/Pc, Rigaku Company, Tokyo, Japan) with area detector operating at a voltage of 40 kV and a current of 50 mA using Cu Kα radiation (λ = 0.154 nm). The scanning rate was 1°/min and the scanning scope of 2θ was from 5° to 50° at room temperature.

2.2.3. Transmission Electron Microscopy (TEM)

The crystal size samples have been investigated by (TEM, Model JEM-200CX, JEOL, and Japan). A few quantities of nanocomposite and copolymer were dispersed in 10 ml ethylene glycol and sonicated for 30 min. A few drops of the suspension were placed on a covered copper grid.

2.2.4. Tested Organisms

The tested compounds were evaluated against four of microbiofouling causing bacteria, Gram negative bacteria (*Pseudomonas aeruginosa ATCC 10145, Escherichia coli ATCC 23282)* and Gram positive bacteria (*Bacillus subtilis NCTC- 10400 and Staphylococcus aureus ATCC 29737*).

2.3. Preparation

2.3.1. Preparation of Silver Nanoparticles [36]

20g of pomegranate fruit peel was weighted and added in 100 ml of distilled water in 250 ml Erlenmeyer flask and boiled for 20 minutes. The boiled materials were filtered to

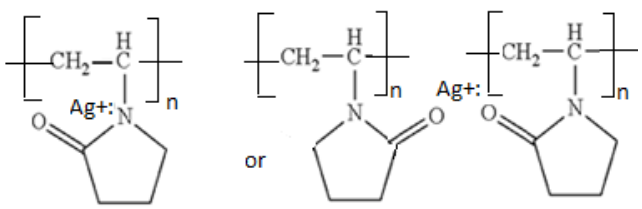
get aqueous fruit peel extract then kept in refrigerator 24 hours which was used as such for metal nanoparticles synthesis. 1mM aqueous solution of silver nitrate was prepared for 100 ml then 5 ml of filtrate was added and kept for 24 hours incubation with intermittent shaking. After 24 hours the brown colour development indicated the formation of silver ion nanoparticles Ag^{+}. The bio reduction of Ag+ ion in aqueous solution was centrifuging 5000 rpm at 5 min. The resulting suspension was redispersed in 100 ml of sterile distilled water. Preparation of silver ion nanoparticles Ag+ was repeated in presence of surfactant sodium dodecyl sulphate (SDS). The purified suspension was then analyzed by IR Tracer-100 Shimadzu for FTIR. The purified silver nanoparticles after centrifugation were dried to powder form and analyzed by Fb quanta 200 Tranmission Electron Microscopy (TEM) for the structure, composition and average size identification. The silver nanoparticles synthesized using Pomegranate Peel extract was tested for antimicrobial activity by disc diffusion method against Staphylococcus aureus ATCC 29737, Pseudomonas aeruginosa ATCC 10145 and Escherichia coli (ATCC 23282).

2.3.2. Preparation of Poly N-vinylpyrrolidone PNVPy [37]

11.4g of monomers of (NVPy) with 11.4 g water, 0.035 gm free radical initiator, 2,2' Azobisiso-butyronitrile (AIBN) were added in a beaker and stirred for about 20 min at room temperature to dissolve all the AIBN into water. The reaction was carried out inside a commercial microwave for 5 min with power 100 W. The resulting polymer was precipitated by pouring the reaction mixture in large excess of water. The precipitated polymer was vacuum filtered and washed several times with methanol and water then dried at 65°C for 10 h till constant weight. The molecular weight of the polymer was determined by gel permeation chromatography (GPC); by using CRYETTEA instrument- Automatic cryoscope.

2.3.3. Preparation of Poly N-vinylpyrrolidone PNVP / Silver Nanoparticles AgNPs [37]

PNVPy / AgNPs with and without SDS was prepared in situ by adding 11.4g of monomers of (N-VPy) with 11.4 g water, 0.035 gm free radical initiator 2, 2' Azobisiso-butyronitrile AIBN in a beaker, different concentration (0.1, 0.2, 0.4 and 0.8ml) of silver nanoparticles and stirred for about 20 min at room temperature to dissolve all the AIBN into water. The reaction was carried out inside a commercial microwave for 5 min with power 100 W. The resulting polymer was precipitated by pouring the reaction mixture in large excess of water. The precipitated polymer was vacuum filtered and washed several times with methanol and water then dried at 65°C for 10 h till constant weight Figure 1.



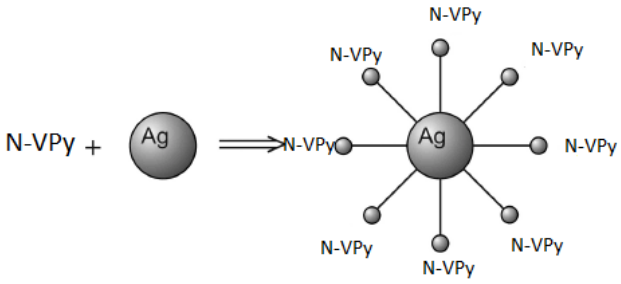


Figure 1. *PNVPy / silver nanoparticles.*

2.4. Tested Organisms

2.4.1. Minimum Inhibitory Concentrations (MICs) Test

The MIC is the lowest concentration of an antimicrobial compounds that inhibits the visible growth of a microorganism after overnight incubation [38]. Serial dilutions of the four tested compounds were prepared in macro dilution tubes with concentrations in the range between ranging between 1/2 and 1/64. Bacterial suspensions were adjusted to the logarithmic-phase growth to match the turbidity of a 0.5 McFarland standard, yielding approximately 108 CFU/mL. The same amounts of bacteria were added to all tubes and the tubes were incubated at 37°C for 24 h. Each tube was examined for growth and compared to the control. Positive control tubes are referred to bacterial suspension added to tubes filled with nutrient broth with each of the tested organisms. Tubes containing nutrient broth and each of the tested compounds are considered as the negative control. The absence of growth in the macro dilution tubes was defined as antibacterial activity.

2.4.2. Minimum Bactericidal Concentrations (MBCs) Test

The MBC is the lowest concentration of antibiotic required to kill a particular bacterium [38, 39]. The dilutions were run in duplicate for the MBC test. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC was determined by sampling all the macroscopically clear tubes (1dilution below the MIC was used for the levels to be assessed in the MBC assay) and the first turbid tube in the series. Running dilutions above the known MIC permits detection of tolerance to normally bactericidal [40]. The suspension was inoculated onto plates of nutrient agar. The plates were incubated for 24 h at 30°C. Each experiment was carried out 3 times and was correlated against the controls.

3. Results and Discussion

3.1. Molecular Weight Mw

The method is based on Avogadro-Gerhadt law chromatography (GPC) in THF. It was found that the weight average molecular weight Mw. of polyN-vinylpyrrolidone PNVPy is 10000.

3.2. Fourier Transform Infrared (FTIR)

The structures of the N-vinylpyrrolidone monomer and poly N-vinylpyrrolidone were confirmed by FTIR spectra as

shown in Figure 2. A strong absorption band observed at 1700 cm^{-1} is due to the –C = O of NVPy. It is observed that the peak at 1645 cm^{-1} (C=C vinyl mono substituted) in NVP was disappeared due to formation of the PNVPy. The peaks at 1373 and 1427 cm^{-1} are due to C-N-C imide and C-N stretching [37]. Increasing in the intensity and shifting towards higher wave number of the absorption peak assigned to Ag-PNVPy stretching, corresponded to strong interaction between polymer matrix and dopant. The PNVPy is hydrophilic and thus some evidence of water can be present in the spectrum. In fact, the peak at 3445 cm^{-1} due to the presence of polyphenols in the edible part of the fruit [41].

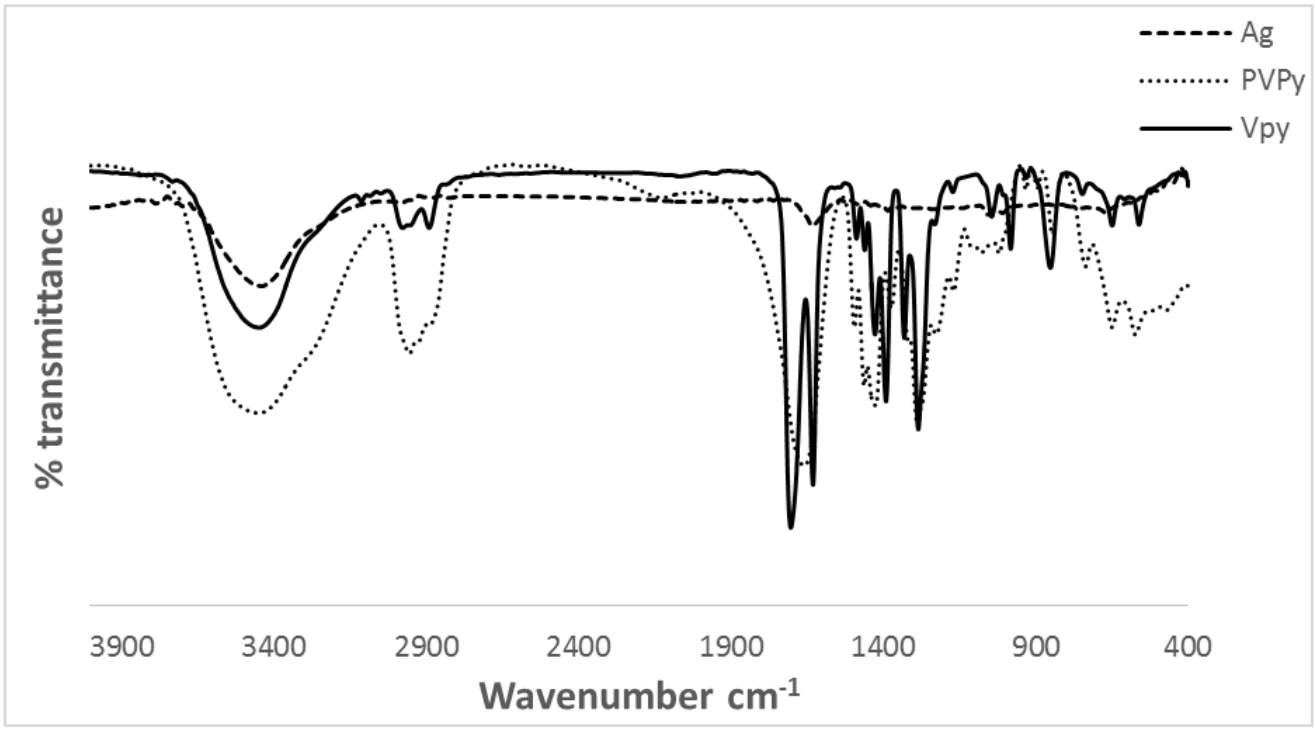


Figure 2. *FTIR of Ag nanoparticles, VPy and PVPy.*

3.3. X-ray Diffraction of Silver Nanoparticles

The crystalinity phase and purity of the silver nanoparticle synthesized using pomegranate peel extract was analyzed by using X-Ray diffraction as shown in the Figure 3.a.

The diffraction pattern shows four sharp and well defined diffraction lines at 2θ = 38°, 44°, 64° and 77° with d-spacing of 2.3, 2.0, 1.44, and 1.23 A° which can be assigned to the (111), (200), (220) and (311) reflections of the face centered cubic (fcc) structure of metallic silver, respectively. The lattice parameter calculated from XRD pattern is a = b = c = 4.082975 A° in agreement with the literature value a = 4.086 A°. The diffraction pattern is in well agreement with the literature report JCPDS File No. 04-0783. The well-defined intense peaks in diffraction pattern confirm excellent crystallinity of silver nanoparticles (42). The calculated particle size was approximately 25 nm, similar to the results obtained by scanning electron microscopy. Figure 3.b shows the X-ray diffraction pattern of PNVPy/AgNPs, it is amorphous because the silver nanoparticle was disappeared in the polymer when prepared insitu during the polymerization (41).

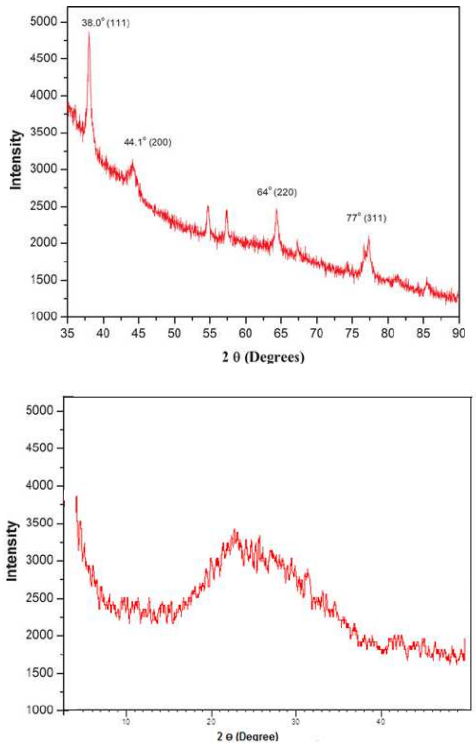


Figure 3. *XRD pattern of a. silver nanoparticles synthesized and b. poly N-vinylpyrrolidone/silver nanoparticles.*

3.4. Transmission Electron Microscopy (TEM)

It can see from Figure 4 that the morphology of the silver nanoparticles synthesized using pomegranate peel extract is mainly represented by clusters of 20–50 nm nanoparticles. With adding silver nanoparticles in PNVPy, the particle was dispersed well. Silver nanoparticles would coordinate with N or O in PNVPy as shown in Figure 1 and a covered layer was generating on the surface of the particle so AgNPs neither growth nor agglomerate [43].

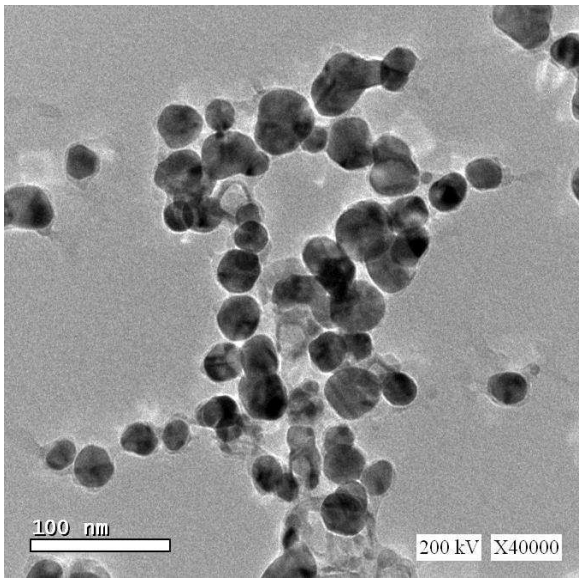


Figure 4. *TEM image of AgNPs at different magnification levels.*

3.5. Antimicrobial Activity

The synthesized compounds were evaluated for their microfouling action against *E. coli and P. aeruginosa* as Gram-negative and *B. subtilis and S. aureus* as Gram-positive bacteria. Table 1 showed the results of anti-micro fouling activity of the prepared compounds which revealed that addition of the surfactant (SDS) enhanced the antimicrobial activity of AgNPs increasingly as the silver nanoparticles concentrations increased. The obtained optimum mixture was the tested polymer with 0.4 Ag NPs with the addition of the tested surfactant. Regarding the suggested mechanism, Samiey et al. (2014) declaimed` that the structure of self-assembled surface active compounds and their electric charge can interact differently with other compounds which may catalyze or inhibit the reaction rates of the substrates [44]. Commonly, Gram-negative bacteria are resistant towards antibacterial substances due to the presence of lipo polysaccharide molecules in their outer membrane so the anti-micro fouling activity of the prepared samples in case of Gram-positive is more than Gram-negative [45–47].

While on 2008, Kvítek et al.[48] had proved that aggregation stability of some surfactants such as sodium dodecyl sulphate (SDS), polyoxyethylene sorbitane monooleate (Tween 80), polymers as polyvinylpyrrolidone (PVP 360) and polyoxyethylene sorbitane were significantly enhanced the antibacterial activity of silver NPs causing a decreased minimum inhibition concentration (MIC).

Table 1. *Anti-microfouling activity of the prepared compounds.*

Compound	Tested Microfouling-Causing Organisms							
	(Gm-ve 1) *E. coli*		(Gm-ve 2) *P. aureginosa*		(Gm+ve 1) *B. subtilis*		(Gm+ve 2) *S. aureus*	
	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL
Pure Polymer	12.0	16.0	10.0	18.0	10.0	10.0	10.0	10.0
Polymer+0.1 Ag	8.0	16.0	8.0	16.0	6.0	12.0	6.0	12.0
Polymer+0.1 Ag+Surf	6.0	12.0	8.0	16.0	5.0	10.0	5.0	10.0
Polymer+0.2 Ag	12.0	16.0	10.0	18.0	10.0	10.0	10.0	10.0
Polymer+0.2Ag+ Surf.	5.0	5.0	5.0	5.0	3.0	6.0	3.0	6.0
Polymer+0.4 Ag	5.0	10.0	5.0	10.0	5.0	5.0	5.0	5.0
Polymer+0.4 Ag+Surf.	5.0	5.0	5.0	5.0	2.5	2.5	2.5	2.5

4. Conclusion

Silver nanoparticles were synthesized by chemical reduction method from pomegranate Peel with and without of surfactant sodium dodecylsulphat (SDS). The reduction of the metal ions through pomegranate Peel extracts leading to the formation of silver nanoparticles of fairly well defined dimensions 20-50 nm. PolyN-vinylpyrrolidone (PNVPy) nanocomposite was polymerized in situ in presence of various compositions of AgNP with different concentration (0.1, 0.2, 0.4 and 0.8ml). All prepared samples were characteristics with FTIR, XRD and TEM. The synthesized compounds were evaluated for their microfouling action against *E. coli* and *P. aeruginosa* as Gram-negative and *B. subtilis* and *S. aureus* as Gram-positive bacteria. It was found that addition of the surfactant (SDS) enhanced the antimicrobial activity of AgNPs increasingly as the silver concentrations increased. The obtained optimum mixture was polymer with 0.4 Ag NPs in presence of surfactant.

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