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# Assessment of biopolymer stabilized silver nanoparticle for their ecotoxicity on *Ceriodaphnia cornuta* and antibiofilm activity

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ABSTRACT

Sodium alginate (biopolymer) stabilized silver nanoparticles (SA-AgNPs) were synthesized and characterized by UV–vis spectroscopy, XRD, FTIR, SEM, EDAX and TEM. UV–vis spectra recorded the absorbance peak at 407 nm due to its surface plasmon resonance. XRD measurements indicated the crystalline nature of particle with various Bragg's reflection peaks at (111), (200) (220), (311) and (222) planes. FTIR spectra showed the possible functional groups at 3443, 1616, 1384, 1092, 1209, 835 and 774 $cm^{-1}$. SEM and TEM showed that the particles were spherical in shape and the size was in the range of $23 \pm 2$ nm. EDAX analysis showed that the weight percentage and elemental silver in the composition was 57.80. The ecotoxicity of SA-AgNPs was assessed on the freshwater crustacean, *Ceriodaphnia cornuta*. SA-AgNPs caused 100% mortality of *C. cornuta* at 40 $\mu g\,L^{-1}$, whereas $AgNO_3$ caused100% mortality of *C. cornuta* at 10 $\mu g\,L^{-1}$. The accumulation of $Ag^+$ by *C. cornuta* was increased at increasing concentration and reached 3.4 $\mu g/g$ dry weight at 50 $\mu g\,L^{-1}$. *C. cornuta* exposed to $AgNO_3$ showed blackening of intestine at 5 and 10 $\mu g\,L^{-1}$. On the otherhand, the visible uptake, ingestion and accumulation of SA-AgNPs in the intestine of *C. cornuta* were clearly visualized under CLSM after treated with 10, 20 40 and 50 $\mu g\,L^{-1}$. The abnormal swimming, reduced heart rate and thoracic limb movement of *C. cornuta* were also observed after exposure to 50 $\mu g\,L^{-1}$of SA-AgNPS. The synthesized SA-AgNPs were tested for its antibiofilm activity against Gram positive (*Listeria monocytogenes*) and Gram negative (*Vibrio parahaemolyticus*) bacteria. SA-AgNPs effectively inhibited the biofilm growth of *L. monocytogenes* and *V. parahaemolyticus* at 75 $\mu g\,mL^{-1}$.



## 1. Introduction

Nanotechnology is the most recent and an emerging field that deals with eco-friendly synthesis of nanomaterials by using biological materials for an application in the medical field to control various human diseases [1]. Among different methods used for the synthesis of nanoparticles, biological methods using plant extracts, enzymes and microorganisms is most promising and eco-friendly [2]. Recently, researchers have begun the green synthesis of AgNPs using polymer matrices because they can be easily designed into almost any shape required for a particular application [9,10]. This includes the use of different biopolymers such as starch [3], chitosan, [4], cyclodextrins [5] and bacterial biomass [6] that acts as stabilizing and reducing agent.

It was proposed that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell [7]. Smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs [7]. It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [8]. However, in order to fully enhance the antibacterial properties of silver nanoparticles, they are usually dispersed on the surface of the polymer matrix without the formation of large aggregates, which otherwise dramatically reduce the antimicrobial effect of silver [9]. Most of the synthetic polymers are nondegradable and the monomers of the polymers are toxic, which restricts the utilization of nano-silver/polymer composites. With increasing concern over microbial infections, there is a growing demand for effective and safe antimicrobial agents [10,11]. Sodium alginate

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(SA) is a linear anionic natural polysaccharide composing of 1,4-linked residues of β-D-mannuronic (M) acid and a-L-guluronic acid (G) [12]. The sodium alginate stabilized AgNPs offer numerous benefits of eco-friendliness and biocompatibility for pharmaceutical and biomedical applications.

*Listeria* monocytogenes is food borne pathogen and is able to form biofilms on surfaces of food industry premises and the ability of *L. monocytogenes* to form biofilm has been reported earlier [13,14]. *Vibrio* parahaemolyticus is responsible for the most common *Vibrio*-associated, seafood-borne gastroenteritis. Recent studies reveal that the ability of vibrios to form biofilms [15].

Invertebrates have a key position as consumers in aquatic ecosystems and certain invertebrate species have been used as ecotoxicity test organisms. The freshwater crustaceans like *Daphnia* and *Ceriodaphnia* are bioindicators and an extremely important member in aquatic ecosystems and has been used to determine the toxicity of chemicals or nanoparticles. They interact with large portions of the environment and therefore have a greater potential to be affected by ingestion of pollutant particulates compared to that of other aquatic organisms [16]. Hence, in the present study, an ecofriendly, novel and biocompatible approach was employed to synthesize silver nanoparticle using sodium alginate biopolymer (SA-AgNPs) as the stabilizing material. The synthesized SA-AgNPs was tested for their ecotoxicity against the freshwater crustacean, *Ceriodaphnia cornuta*. In addition, the antibiofilm efficacy of SA-AgNPs was determined against Gram positive and Gram negative bacteria.

## 2. Materials and methods

### 2.1. Chemicals used

Silver nitrate ($AgNO_3$) (MW 169.87 g/mol), sodium alginate (MW 500,000, low viscosity) and sodium borohydride ($NaBH_4$) (MW 37.83 g/mol) was procured from Sigma Aldrich, USA. All the chemicals used were of analytical grade.

### 2.2. Synthesis of sodium alginate stabilized AgNPs (SA-AgNPs)

1 g of sodium alginate was dissolved completely in 100 mL of deionized water for an hour. Subsequently, 2 mL of 1 M aqueous silver nitrate ($AgNO_3$) (Sigma Aldrich, USA) solution was dropped into the sodium alginate (Na-Alg) solution under magnetic stirrer. After 1 h, freshly prepared 5 mM sodium borohydride ($NaBH_4$) (Sigma Aldrich, USA) solution (2 mL) was added to the mixture. A rapid color change to dark brown indicated the formation of AgNPs. The mixture was stirred for 3 h at room temperature to ensure that complete reduction had occurred. The mixture was then dialyzed using a dialysis tube with a molecular weight cut-off of 1000 Da (Membrane Filtration Products Inc., USA) with repeated water changes for more than 2 days to eliminate any unreacted chemical remains of $AgNO_3$, $NaBH_4$, and any salts formed during synthesis. The sodium alginate–silver nanocolloid was freeze-dried for 3 days and used for analysis [17].

### 2.3. Physico-chemical characterization of SA-AgNPs

The physico-chemical characterization of SA-AgNPs such as shape, size, crystalline nature, elemental composition and functional groups of the capping agent were analyzed through UV–vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and Energy dispersive X-ray analysis (EDX).

### 2.4. UV–visible spectroscopic analysis

The bioreduction of $Ag^+$ ions was monitored by periodic sampling of aliquots (1 mL) of the aqueous solution and measuring the UV–vis spectra of the solution. UV–vis spectra of these aliquots were monitored as a function of time of reaction on a spectrophotometer in 200–800 nm range operated at a resolution of 1 nm [18].

### 2.5. X-ray diffraction (XRD) analysis

The synthesized SA-AgNPs were dispersed in distilled water via 30 min of sonication, and subsequently centrifuged at speed of 9000*g* for 30 min to isolate the pure SA-AgNPs and remove any unbound ligand. They were then freeze dried and lyophilized to obtain a powdered sample. The particle size and crystalline nature of the powdered SA-AgNPs were determined using XRD. XRD measurements were carried out using a powder diffractometer (PANalytical X'per PRO model X-Ray diffractometer), operating at a voltage of 50 kV and a current of 30 mA. The particle size of silver nanoparticles was determined using Debye Scherrer's equation.

$$D = 0.9\lambda/B\cos\theta$$

where λ is the wavelength (Cu Kα), β is the full width half-maximum (FWHM) of the Ag (111) line and θ is the diffraction angle [19].

### 2.6. Fourier transform infrared (FTIR) analysis

FTIR was performed following the method of Chandran et al. [20]. Two milligram of SA-AgNPs was mixed with 200 mg of potassium bromide (FTIR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FTIR spectra were recorded in FTIR spectroscopy at a resolution of 4 $cm^{-1}$.

### 2.7. Transmission electron microscopy (TEM) analysis

The size and surface morphology of SA-AgNPs was determined following the method of Deepak et al. [21]. TEM analysis was performed by placing a small volume of SA-AgNPs on carbon-coated copper grids and the solvent was allowed to evaporate for 30 min. TEM measurements were performed on JOEL model instrument 1200 EX instrument on carbon coated copper grids with an accelerating voltage of 80 kV.

### 2.8. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis

SEM analysis of SA-AgNPs was done using Hitachi S-4500 SEM machine. Thin films of SA-AgNPs were prepared on carbon coated copper grid by just dropping very small amount of sample on the grid. The extra solution was removed using a blotting paper and then the film on SEM grid was allowed to dry, by placing it under a mercury lamp for 5 min. The presence of elemental silver was determined using EDX Zeiss Evo 50 equipped with SEM [21].

### 2.9. Antibiofilm assay

Bacterial colonies such as Gram positive *L. monocytogenes* (HQ 693279) and Gram negative *V. parahaemolyticus* (HQ 62565) ($1 \times 10^6$ cfu $mL^{-1}$) were allowed to grow on glass slides (dimension $1 \times 1$ cm) and placed in 24-well polystyrene plates with 1 mL of nutrient broth along with different concentrations of SA-AgNPs (25–75 μg $mL^{-1}$) and incubated at 37 °C for 24 h. Glass slides were stained with 0.04% crystal violet and examined under a Nikon