Silver nanoparticles: preparation, characterization, cytotoxicity, and its impact on cotton fabrics

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Abstract:

In this study, silver nanoparticles (AgNPs) were synthesized from silver nitrate by using glucose and Poly vinyl alcohol (PVA) as reducing and capping agent at the same time. Their particle size ranges from 15-35 nm. Ultraviolet –visible spectrophotometry (UV-vis.) and transmission electron microscope (TEM) analysis were used to characterize the synthesized AgNPs. Cotton Fabrics was treated with silver nanoparticles to be used for medical purposes. The fabrics were treated with AgNPs with three concentrations 10, 20 and 50 ug/ml to determine the best effective treated fabric structure from Atomic absorption and ultra violet protection factor (UBF) to be applied to cytotoxicity test in vitro. Cytotoxicity of the prepared silver nanoparticles were evaluated using cell viability assay from MMT and IC_{50} values and these results confirm that we can use AgNPs safely in contact medical treatment with skin.

Keywords: silver nanoparticles, cytotoxicity, antibacterial, green chemistry, medical textiles.

1. Introduction:

Metal nanoparticles represents main area in nanotechnology to give new physical and chemical properties such as catalytic, electronic and magnetic properties [1, 2]. Chemical reduction of metal ions is the most methods used to prepare metal nanoparticles by using reducing agents such as sodium borohydrides or other chemical materials[1, 3-5]. The main problem that these chemicals are cytotoxic so that the researchers start to apply some green chemistry principles to prepare these nanoparticles specially silver and gold nanoparticles [6].

Silver nanoparticles were first time prepared via green concept by Raveendran et al [7] by using a glucose as reducing agent and starch as capping agent. Solvents, reducing and capping agents represents the main three aspects for nanoparticles green synthesis[8].

Silver metal and its ions exhibit antibacterial activity towards both Gram-positive and Gram negative bacteria. Their antibacterial mechanism is through passing via some types of interactions of these ions with thiol groups of enzymes and proteins. These bacterial organelles are vital for their respiration and materials transport through cell membrane in addition to that silver ions bounded to bacterial cell membrane cause bacterial death [9].

Human physiological system can change silver metals into silver ions to interact with bacterial cells. High amount of silver ions can cause damage of the normal human cells

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causing cytotoxicity of these cells. High surface area of silver nanoparticles gaining it higher antibacterial activity than normal silver metal [10]. Nanocomposite system consists of polymer and metal nanoparticles accepted it as a capping agent because its unique properties such as optical, catalytic, biomedical and electrical properties [9].

Silver nitrate can be reduced to silver nanoparticles by using glucose (soft reducing agent) in the presence of polyvinyl alcohol (as good hosting material). This reducing system was widely used in biomedical process and semiconductor due to its high thermal stability and chemical resistance [11-14].

Several in-vitro studies were performed to illustrate the cytotoxicity of silver nanoparticles to its oxidative stress. So that recent trends used some natural polymers, such as cellulose, chitin and chitosan, to cape silver nanoparticles and reduce its cytotoxicity [15].

The main aim of this study is preparation of silver nanoparticles using green system from glucose and polyvinyl alcohol to minimize their cytotoxicity comparing to chemical reduction method to be used in medical textiles on large scale. Polyvinyl alcohol was used as capping and reducing agent. The prepared silver nanoparticles were characterized by using transmission electron microscope (TEM) and UV-Vis spectroscopy. These silver nanoparticles then used to impart gauze antibacterial activity with minimum cytotoxicity. Antibacterial activity and cytotoxicity of gauze treated with silver nanoparticles were evaluated.

2. Materials and Methods

Materials:

Mill desized, scoured, and bleached 100% cotton fabric were supplied by the Misr Company for Spinning and Weaving, Mehala El-Kura, Egypt. The fabric has the following specifications: plane weave, warp 36 yarn/cm, weft 30 yarn/cm, fabric weight, 150 g/m^2.

Silver nitrate was purchased from Fisher Scientific. Polyvinyl alcohol (M.W. 115,000, Polymerization 1700-1800, viscosity 25-32 and hydrolysis (mole %): 98-99) was purchased from Alpha achemika, India. Glucose was purchased from Aldrich Chemical. Other chemicals were used at analytical grade without further purification.

Preparation silver nanoparticles:

Different concentrations of $AgNO_3$ (0.001M, 0.01M, and 0.1M) were prepared into an aqueous solution of PVA (3 g/100 ml) in the presence of glucose (0.15 g/100 ml) for every experiment to overcome the oxidation of silver. The reduction time was 72 h. at room temperature [16].

Silver nanoparticles prepared by chemical reduction as described in previous study [17]

Finishing of Fabrics with Silver nanoparticles: -

The prepared silver nanoparticles (AgNPs) were applied on washed and dried fabrics using pad-dry-cure method. 30x30 cm of fabrics were immersed in the silver nanoparticles (AgNPs) (0.005 -0.5 g/100 ml) solution containing acrylate binder (1%) for 30 min., and then it was passed through a padding mangle with 100% wet pick-up for all of the treatments. Then the fabrics were dried at 80°C for 5 min., followed by thermo-fixation at 140°C for 3 min. Finally, samples washed and dried to be ready for characterization and antibacterial evaluation.

Characterizations of Silver Nanoparticles (AgNPs):-

- UV-Vis spectra has been proved to be quite sensitive to the formation of silver colloids because AgNPs exhibit an intense absorption peak due to the surface Plasmon excitation which describes the collective excitation of conductive electrons in a metal.
- Shape and size of AgNPs were practically obtained using TEM; JEOL-JEM-1200. Specimens for TEM measurements.
- The metal content of the nanometal oxides-loaded fabric samples was assessed by flame atomic absorption Spectrophotometer GBC-Avanta-Australia.
- Microscopic investigations on fabric samples were carried out using a Philips XL30 scanning electron microscope (SEM) equipped with a LaB6 electron gun and a Philips-EDAX/DX4 energy-dispersive spectroscope (EDS). Images were taken at different magnifications (from 1509 to 30009), using secondary electrons (SE) in accordance with the clarity of the images. Fabric samples were fixed with carbon glue and metalized by gold vapor deposition to record images.

The tests was carried out at the Central Unit for analysis and scientific services at National Research Center.

Evaluation of AgNPs cytotoxicity:

Cytotoxicity of the prepared AgNPs on A-549 cells were evaluated via cell viability test using MMT method (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and determination of IC_{50} values [15, 18, 19].

3. Results and Discussion

Reducing agents can reduce silver ion to silver metal. Herein we use mild and safe reducing system composed of glucose in the presence of polyvinyl alcohol. In addition, it's reducing power PVA used as capping agent and allow colour change from coluorless for silver nitrate to brownish yellow passing through faint yellow and yellow colour.

Reductions of silver nitrate into silver nanoparticles without glucose accompanied with silver oxide formation instead of silver nanoparticles; these reactions confirm glucose role through silver nanoparticles formation [14].

Polyvinyl alcohol-Ag^+ redox system occurs via oxidation of hydroxyl groups of PVA into carbonyl groups and reduction of Ag^+ into Ag^0 at the same time. Glucose support the PVA reduction power by introducing more oxidation active sites via its free aldehyde groups [20, 21].

There are main two factors affecting the color of colloidal silver nanoparticles from yellow to brownish yellow; these are silver nitrate and poly vinyl alcohol concentrations. $AgNO_3$ concentration changes from 0.001M to 0.1M and PVA concentration changes from 3-9% (Wt./V).

Absorption in UV region were used to study the formation of AgNPs via green system from glucose and PVA composite at various concentrations from $AgNO_3$ and PVA to optimized the reaction for obtaining AgNPs with lower cytotoxicity towards human beings.

Figure 1 illustrate the UV-Visible absorption spectra of AgNPs at variable parameters from $AgNO_3$ and PVA concentrations. In figure 1a, AgNO3 varied from 0.001 M to 0.1 M at

constant concentration of PVA; 6 g/100 ml where as in figure 1b, PVA varied from 3 % to 9 % wt/wt at constant concentration of AgNO3; 0.01 M. Where we obtain homogeneous narrow band at 440nm at the concentrations of 0.01M silver nitrate and 6% (Wt./V) PVA. Lower concentrations cannot be able to form nanoparticles and higher concentrations accompanied with red shift and particle size increase. [22].

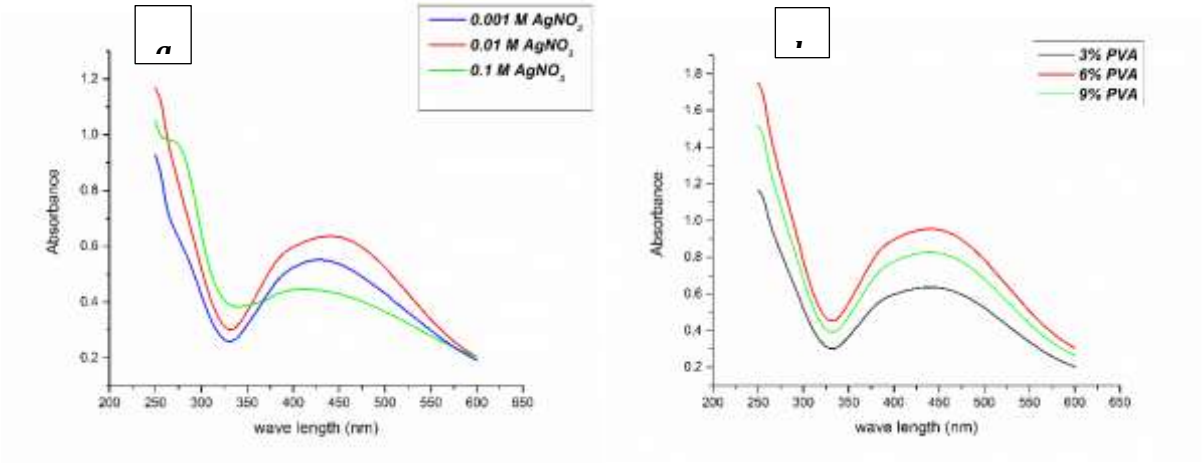

Figure1 shows the UV-Vis spectra of the silver nanoparticles with different concentrations from PVA and $AgNO_3$. ()

The Particles size of silver nanoparticles was observed by using transmission electron microscope (TEM) as shown in Figure 2. From Figure 2. AgNPs were normally distributed with spherical shape at 0.01M $AgNO_3$ and 6gm/100ml water PVA. So that we can concluded that the particles size of AgNPs and its distribution can be controlled by controlling silver nitrate and PVA concentrations.

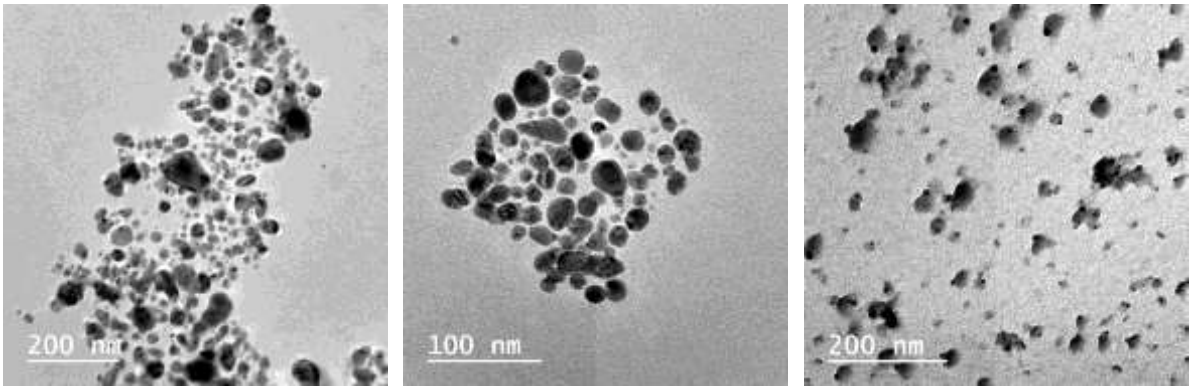

Figure 2 TEM images of silver nanoparticle synthesized using $AgNO_3$ (0.01M) and PVA (6 g/100 ml)

Finishing of Fabrics with Silver Nanoparticles:-

We used two fabrics named Sample 1, 100% cotton and Sample 2, 50:50% cotton: polyester blend. These fabrics were treated with the prepared AgNPs to impart new desirable properties and antibacterial activity. Atomic absorption was used to confirm the presence of AgNPs on fabrics as shown in Table 1. There are some differences in atomic absorption at the same treated AgNPs concentration. These differences are attributed to their differences in fabric components and construction [23].

Table 1:- Atomic absorption values of the fabrics treated with AgNPs

Description	AgNPs (mg/L)
(Sample 1) untreated	0.000
(Sample 2) untreated	0.000
(Sample 1) treated with 0.015 M	97
(Sample 2) treated with 0.015 M AgNPs	123
(Sample 1) treated with 0.02 M AgNPs	219
(Sample 2) treated with 0.02 M AgNPs	149
(Sample 1) treated with 0.025 M AgNPs	384
(Sample 2) treated with 0.025 M AgNPs	234

- *All these data were measured by using 6 g/100 ml PVA*

Scanning electron microscope (SEM) and EDX analysis of untreated and treated cotton samples were shown in Figure 3. EDX confirm the presence of AgNPs in these samples. As shown in the SEM images the prepared AgNPs are homogeneous, smooth and regular distributed on the fabric surface.

SEM images of AgNPs shows that they were dense with low porosity for blended samples; and uniform distributed in case of 100% cotton samples. In addition from SEM images, it is evident that AgNPs were strong attracted to the fabrics due to chemical interaction between them [24].

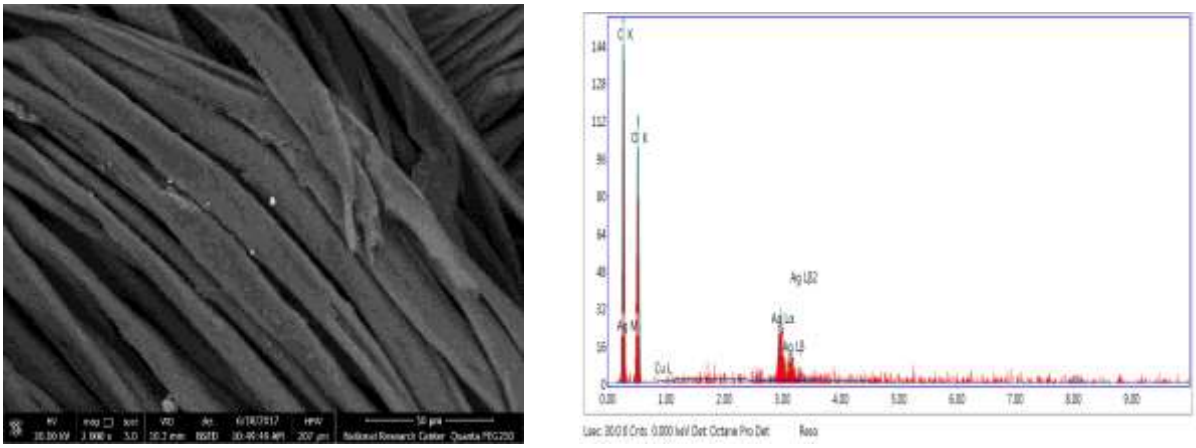

Figure 3:- SEM and EDX spectra cotton treated with AgNPs

Cytotoxicity of AgNPs evaluated by two protocols: IC_{50} and MTT viable cells:

Colloidal suspensions of silver nanoparticles are toxic to human cells [25]. In our study A549 cell line exposed to different concentrations of AgNPs (0.5-2.0 μg/ml) prepared via PVA/glucose composite compared with that prepared by chemical reduction for 24 h at the same concentration. It is known that AgNPs prepared via PVA/glucose were synthesized through chemical reduction. Herein we used two types of silver nanoparticles for cytotoxicity.

Type I for chemical reduction prepared AgNPs and type II for AgNPs prepared from PVA/glucose composite as shown in Table 2. .

MTT protocol assay used to evaluate cell viability. As expected MTT viable cells show that AgNPs prepared by chemical reduction has higher toxicity than that prepared by PVA/glucose green composite. These results prove that the presence of PVA and glucose as green reduction system can reduce the toxicity of AgNPs towards human cell. So that the toxicity of AgNPs depends on the exposed amount of particles. This green composite is safe and has minimum toxic effects on these cells [26-28]

Table 2 MTT Test (metabolic Activity of the Mitochondria) of silver nitrate, silver nanoparticles type I and II

Material	MTT expressed in viable cells	
	After 3hrs.	After 24 hrs.
$AgNO_3$ (1 mM)	22.1	5.3
Type I AgNPs	12.3	3.2
Type II AgNPs	74.2	49.6

***Type I for chemical reduction prepared AgNPs and type II for AgNPs prepared from PVA/glucose composite**

Table 3 shows that the IC_{50} of silver nanoparticles prepared by chemical methods were less toxic than silver nitrate itself but more toxic than that prepared by green composite. These results data were agreed with MTT data. In this study $AgNO_3$ decreased mitochondrial activity more than AgNPs as shown in Table 3 which agreed with several studies of many researchers [24, 27, 29, 30] .

Table 2 IC50 A549 cell line after exposing to AgNO3, AgNPs types I and II (for 24 h)

Material	EC50,
$AgNO_3$ (1 mM)	0.51 µg/ml
Type I AgNPs	0.23 µg/ml
Type II AgNPs	4.50 µg/ml

Type I for chemical reduction prepared AgNPs and type II for AgNPs prepared from PVA/glucose composite

So that results in both Tables 2 and 3 show that AgNPs prepared by chemical reduction was more toxic than $AgNO_3$ whereas that prepared through green method by using PVA/glucose was less toxic than $AgNO_3$.

4. Conclusion

We succeeded to prepare silver nanoparticles by two different methods: chemical reduction and PVA/glucose reduction. The size and size distribution are controllable by adjusting the molar ratio of PVA: $AgNO_3$. At the optimum conditions, well dispersed and regular spherical silver nanoparticles were obtained with size ranges within 15-35nm particle size. The AgNPs were characterized by using several devices e.g. X-ray diffraction, UV absorption, FT-IR and TEM imaging. The cytotoxicity of these AgNPs were evaluated by using MTT assay and IC_{50} values to show the effect of AgNPs preparation method on the cytotoxicity of these nanoparticles on A549 viable cells. Ag+ decreased mitochondrial activity more than AgNPs

prepared by chemical reduction but it is less than that prepared by PVA/glucose. The free Ag^{+} in AgNPs suspensions play an important role in its cytotoxicity towards a viable A549 cells. The toxic effects of AgNPs related to the release of Ag^{+}. These properties are essential for manufacturing of silver nanocomposites, with minimal toxicity compared with these prepared by chemical reduction by using PVA/glucose composite.

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