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Preparation of Antibacterial Cotton Wound Dressing By Green Synthesis Silver Nanoparticles Using Mullein Leaves Extract

S. Najmeh Aboutorabi[1], Majid Nasiriboroumand[2,*], Pourya Mohammadi[1], Hassan Sheibani[1,*] and Hossein Barani[3]

[1]Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran.

[2]Department of Carpet, Shahid Bahonar University of Kerman, Kerman, Iran.

[3]Department of Carpet, University of Birjand, Birjand, Iran.

[*]Corresponding Authors: Majid Nasiriboroumand. Email: Nasiri5757@yahoo.com; Hassan Sheibani. Email: hsheibani@uk.ac.ir.

Abstract: Silver nanoparticles (AgNPs) were synthesized by a bio-reduction method using an aqueous extract of mullein leaves (*Verbascum thapsus* L.) functioning as reducing as well as a stabilizing agent. Various synthesis parameters such as reaction time, temperature and concentration of the extract were also studied for the synthesis of AgNPs. The so prepared AgNPs were characterized by various techniques including UV-Vis spectroscopy, X-ray diffraction, scanning electron microscopy (equipped with energy dispersive analysis of X-rays), and transmission electron microscopy. The electron microscopy images suggest the formation of polydispersed spherical AgNPs with average particle size of about 20 nm. The kinetic analysis revealed that the rate of bio-reduction of silver ions was very slow for initial 1h; however, later the reduction was fast as the development of characteristic color of AgNPs was completed within 5 hrs. This observation was concomitant with the appearance of the surface plasmon absorbance peak at ~ 430 nm. Further, these nanoparticles were used for the treatment of wound dressings by the exhaustion method. The so developed wound dressings showed good antibacterial activity against a gram positive bacterial strain *Staphylococcus aureus*.

Keywords: Silver nanoparticles; mullein; antibacterial; biochemical; medical application

1 Introduction

Silver nanoparticle is a good suggestion for applications in medical, pharmaceutical and textile industry [1,2] due to its good antibacterial properties. Among the different methods to synthesize silver nanoparticles, the green methods have been extensively investigated. The non-toxic materials are used in the green synthesis procedures [3], and therefore the method is an environmentally friendly [4]. In this process two components are required to concern in the synthesis of silver nanoparticles; silver salt and reducing agent like plant extract, besides it has a stabilizing agent role [5].

Several plant extracts have been used to synthesize AgNPs [6-9]. Natural compounds in various plants can act as a reducing and stabilization in synthesis of AgNPs. Shankar et al reported that terpenoid, protein, and other organic compounds in Pelargonium leaves are responsible for the bio-reduction of Ag ions to AgNPs [10]. The extracted plant of *Capsicum annuum* L. comprises of biomolecules, such as proteins, polysaccharides, and vitamins contain which could be used as a reducing and stabilizing agents in the synthesis of AgNPs [11]. Leaves of Camphor have a several polyols components such as terpenoids, polysacharides, and flovonoids which can reduce silver ions to silver atoms [12]. The Rumex hymenosepalus plant is a natural antioxidant rich and its phenolic compound such as flavan-3-ols is a good reducing agent for the synthesis of the silver nanoparticle [5]. Polyphenols and flavonoids in *Reseda Luteola* L., are able act as reducing agent in the synthesis of AgNPs from silver salt [13].

Verbascum thapsus L. (Common mullein), a member of the family Scrophulariaceae, is a famous herb that is found all over Europe, in temperate Asia, in North America and is well-reputed due to its medicinal properties. This medicinal herb contains various chemical constituents like flavonoids, vitamin C and minerals. It is famous in various communities worldwide for the treatment of various disorders of both humans and animals aliments [14]. A number of pharmacological activities such as anti-inflammatory, antioxidant, anticancer, antimicrobial, antiviral, antihepatotoxic and anti-hyperlipidemic activity have been ascribed to this plant [15].

Traditional wound dressing products including gauze, lint, plasters, bandages (natural or synthetic) which are used as primary or secondary dressings for protecting the wound from contaminations [16]. A wound dressing is a sterile pad or compress applied to promote a wound to promote healing and protect the wound from further harm and contaminations. Modern wound dressing have been developed to facilitate the function of the wound rather than just to cover it [17]. Nanotechnology offers a superlative approach to hasten the healing of acute and chronic wounds, by stimulating the proper movement through the different healing phases. A considerable percentage of nanomaterials are used in various biomedical applications for wound dressings, drug delivery and other medical purposes [18].

In the current study, the cotton wound dressing was treated with the green synthesized AgNps to obtain antibacterial property. AgNPs were synthesized using mullein leaf extract as a reducing and stabilizing agent. Effects of process parameters such as reaction time, temperature and extract concentration were studied on the synthesized AgNPs.

2 Experimental Section

2.1 Mullein Leaf Extract

The Mullein leaf plant was purchased from an Iranian traditional shop and washed with distilled water and dried at room temperature. Then aqueous-ethanolic extracts were taken by soxhlet for 8 h. Air-dried extract was dissolved in ultrapure water to obtain an aqueous extract solution (2.5%w/w) and used for further experiments.

2.2 Bio-Reduction of AgNPs

As a precursor, the extra pure silver nitrate was purchased from Merck (Germany) and dissolved in ultrapure water. 1 ml of mullein extract was added into the 7 ml of silver salt solution (1.5 mM) at 50°C and stirred for 5 h. The effect of synthesis parameters such reaction time (1, 3, 5 h), reaction temperature (25, 50 and 75°C) and extract concentration (1, 2, 3, 4 ml of extract solution) was studied on the characteristics of the synthesized nanoparticles.

2.3 Characterization of Synthesized AgNPs

The synthesized AgNPs were characterized using a UV-Vis spectrophotometer, Scanning Electron Microscopy (SEM) which was equipped with Energy Dispersive X-ray spectroscopy (EDX), Transmission Electron Microscopy (TEM), and X-Ray Diffraction (XRD).

2.4 Coating of Wound Dressing with Mullein Leaf Extract

1 gram of the cotton gauze was immersed in a solution containing 43 ml of distilled water and 7 ml mullein extract for 2 h at 50°C. Finally, the treated wound dressing was rinsed with cold distilled water and dried at room temperature.

2.5 Coating of Wound Dressing Whit Synthesized AgNPs

In the reaction flask, a piece of the wound dressing (4 × 4 cm) was immersed in 50 ml of AgNPs with a material to liquor ratio of 1:50 for 2 h at 50°C, rinsed with distilled water, and dried at room temperature.

2.6 Antibacterial Test

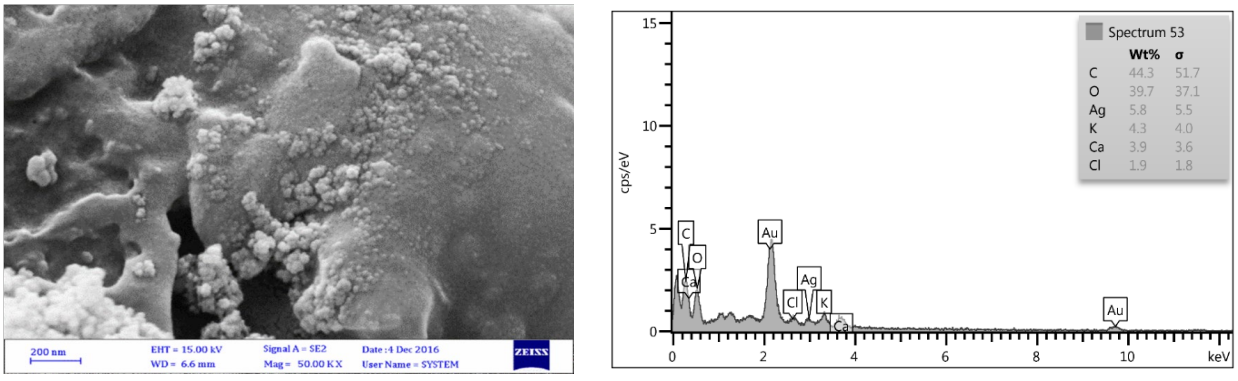
In order to specify the antibacterial efficiency of synthesized silver nanoparticle coated wound dressings against *Staphylococcus aureus*, the bacteria with a final concentration of 10^7-10^8 CFU/ml was prepared. To confirm the bacteria concentration, absorbance of bacteria solution at 600 nm was set at 0.11-0.15 (OD). The circular wound dressing with 4.8 cm in diameter was inoculated with 0.5 ml of bacteria solution and incubated at 37°C for 24 h. After incubation, each sample was immersed in 40 ml of neutralizing solution and shacked vigorously for a minute to separate any bacteria. Antibacterial test continued with serial dilution and pour plate 0.5 ml of each solution in nutrient agar. The total bacterial count was determined after 24 h incubation at 37°C. The percentage of bacteria reduction (E) was calculated according Eq. (1), where A is the number of bacteria recovered from the inoculated treated test immediately after inoculation and B is the number of bacteria recovered from the inoculated treated test after 24 h.

$$E = \frac{A-B}{A} \times 100 \quad (1)$$

3 Results and Discussion

3.1 Characterization of Biosynthesized AgNPs

The SEM image and respective EDX analysis of biosynthesized AgNPs are presented in Fig. 1. The SEM image (Fig. 1(a)) shows that the synthesized particles are fairly polydispersed and the EDX analysis (Fig. 1(b)) of the region shown in the SEM image suggests the presence of silver element in our sample. The signals for other elements such as C, Cl, and O are due to the organic components of the mullein leaf extract. It is also expected that these organic components act as capping molecule which imparts colloidal stability to the biosynthesized AgNPs. Further, the synthesized AgNPs were also characterized for the size and surface morphology by TEM imaging (Fig. 2), which showed polydispersed nanoparticles with average particle size distribution of 20 nm (Fig. 2(a), inset). The enlarged TEM image of the synthesized AgNPs clearly revealed that majority of nanoparticles are spherical in shape and of ~20 nm in diameter with few smaller particles of ~2-5 nm (Fig. 2(b)).



a b

Figure 1: SEM image micrograph (a) and EDX profile of synthesized AgNPs (b)

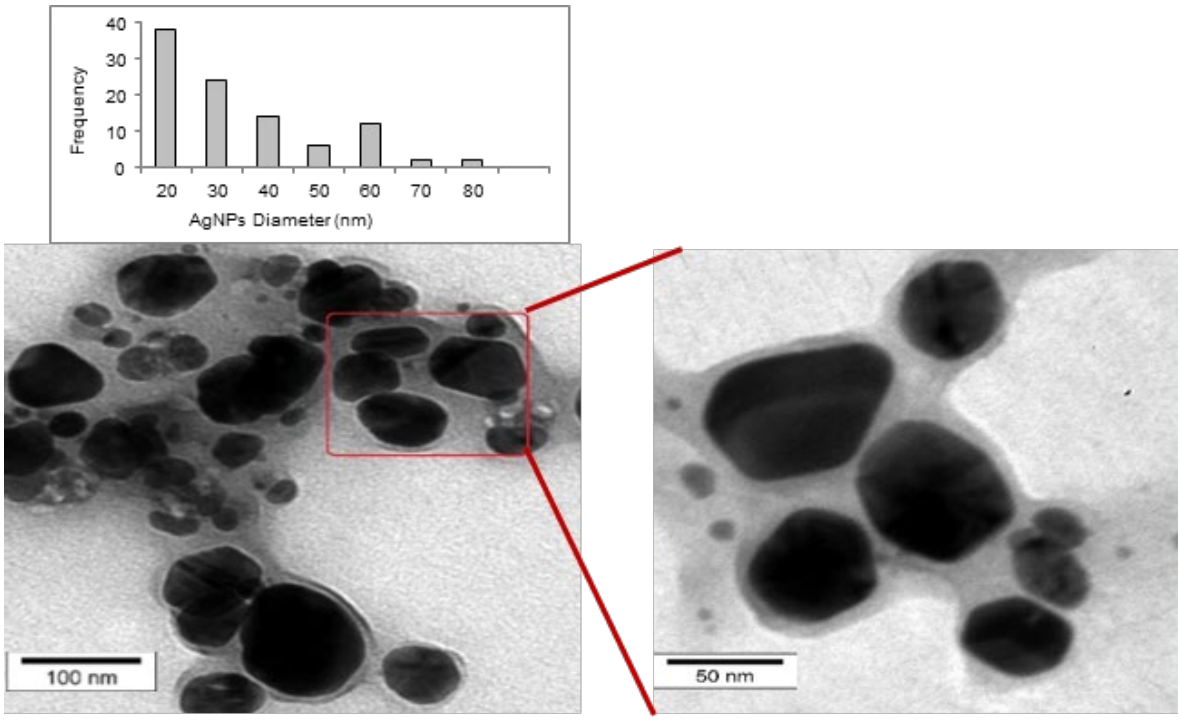


Figure 2: TEM image of green synthesized AgNPs and their size distribution

The XRD pattern of synthesized AgNPs (Fig. 3) suggests that the particles are crystalline in nature. The obtained Bragg's diffraction patterns were index based on the characteristic diffraction pattern of silver. The diffraction patterns obtained at 2θ value of 38.21, 44.35, 64.54 and 77.53 were found to correspond with (111), (200), (220) and (311), crystal planes for cubic face-centered AgNPs, respectively [19]. The unindexed diffraction peaks are expected due to the organic compounds of mullein extract [20,21]. The average size of synthesized AgNPs was calculated using the Scherrs formula, which is 38.54 nm.

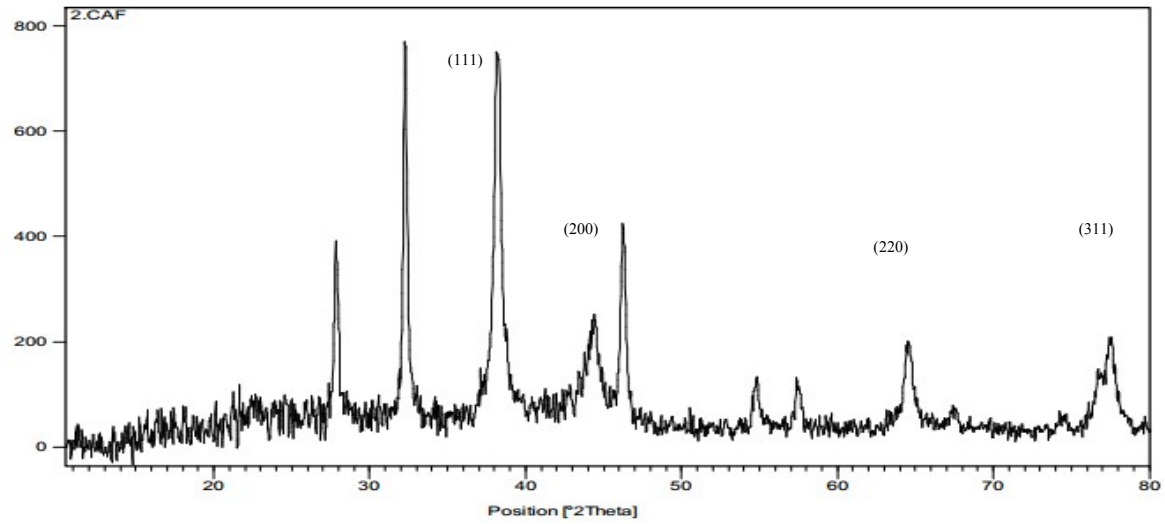


Figure 3: XRD pattern of synthesized AgNPs with mullein extract

3.2 UV-Vis Spectroscopy

It is well known that formation of AgNPs can be confirmed by change in the solution color from colorless to brownish-yellow due to the Surface Plasmon Resonance (SPR). The SPR property is depend on the size and shape of AgNPs. This characteristic SPR peak for AgNPs appears in the range of 400-500 nm in UV-Vis spectroscopy [22] and in our case the characteristic SPR of synthesized AgNPs using mullein was centered at 430 nm (Fig. 4(a)).

3.2.1 Effect of Reaction Time

We studied that effect of reaction time between silver salt and mullein extract over AgNPs synthesis by following the SPR by UV-vis spectra. Plant extract contains compounds which are weak reducing agents and therefore result slow reduction of silver ions [23]. We observed that up to 1hr incubation time there was no reduction of silver ions into AgNPs as there was no SPR observed (Fig. 4(b)). However, as time progressed, we found that after 3 h of incubation the formation of AgNPs was started as there was some signal of SPR. After 5 h of reaction time, we observed a complete SPR formation form the mixture of silver ions and mullein extract. This observation suggests that a minimum of 5 h is required for the synthesis of AgNPs by the use of mullein extract (Fig. 4(b)).

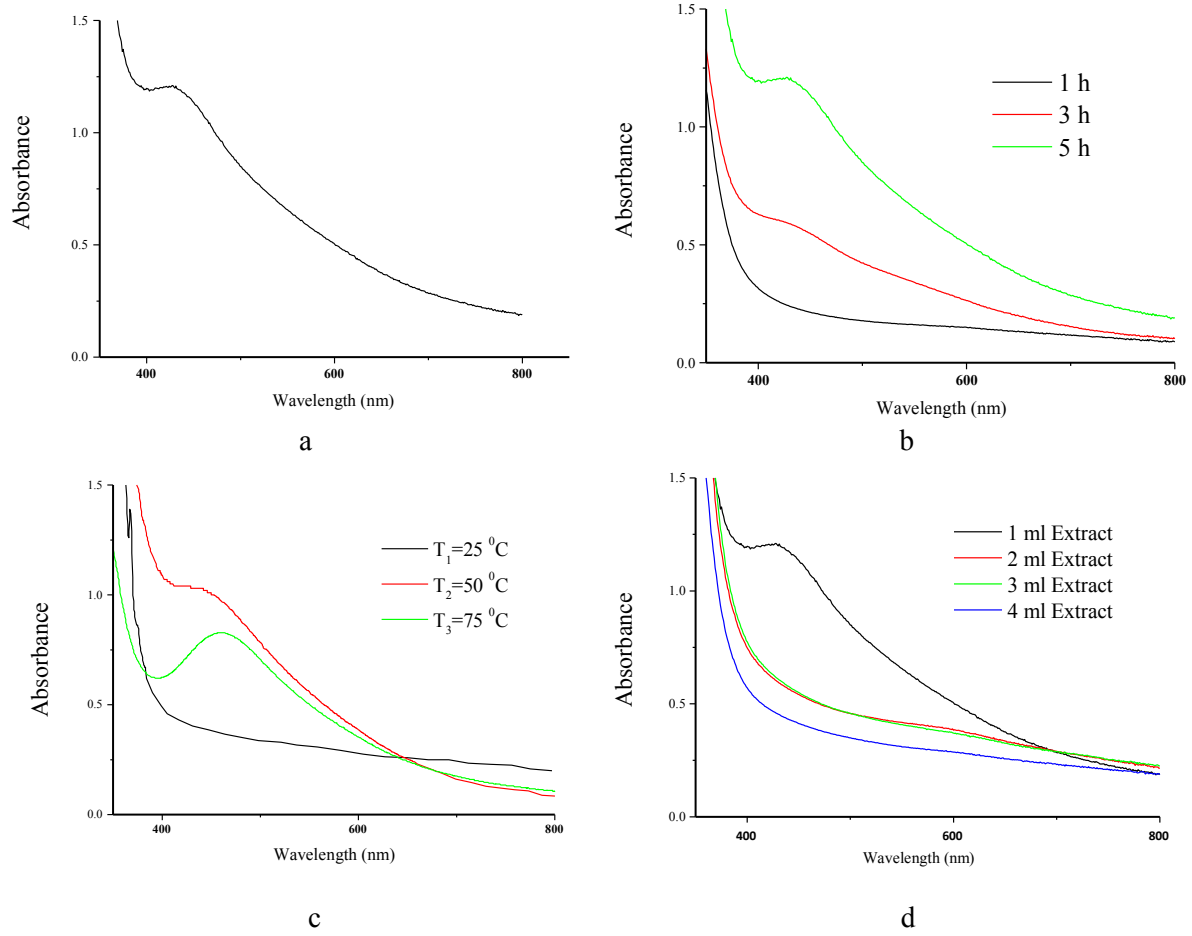


Figure 4: UV-Visible spectra of synthesized AgNPs at 437 (a), different procedure time (t_1 = 1, t_2 = 3, t_3 = 5 hrs) (b), different reaction temperature (T_1 = 25ºC, T_2 = 50ºC, T_3 = 75ºC) (c), and different concentrations of mullein extract (1, 2, 3, and 4 ml of 2.5 %w/w solution) (d)

3.2.2 Effect of Reaction Temperature

Among the other AgNPs synthesis parameters, the temperature had a significant effect over the AgNPs formation as well as their shape, size, and distribution. We considered three temperatures, 25, 50, and 75ºC, and studied the formation of AgNPs with the reaction between silver ions and mullein extract (Fig. 4(c)). The characteristic peak was not observed when the reduction reaction was performed at 25ºC. Subsequently, increase of the reaction temperature to 50ºC resulted in the increase in absorbance (SPR) intensity at 450 nm suggesting the formation of AgNPs. In contrast, increase of the reaction temperature to 75ºC resulted in reduced absorbance intensity, which might be due to the degradation of certain essential biomolecules of mullein extract required for nanoparticle formation and capping. Moreover, at 50ºC, the UV-Vis spectra shows the SPR peak at the lower wavelength (450 nm) compared to 75ºC (466 nm), which indicate the formation of smaller nanoparticles at lower temperature [24].

3.2.3 Effect of Mullein Leaf Extract Concentration

We also varied the mullein leaf extract concentration during the synthesis of AgNPs (Fig. 4(d)). It is reported that a variation in the amount of the reducing agents and metal salt concentration can influence the nanoparticle formation procedure [25]. As shown in Fig. 4(d), the SPR band of synthesized AgNPs was observed only at concentrations of 1 ml of extract, however other concentrations (2, 3, and 4 mL) did not show any SPR peaks. With increasing mullein leaf extract concentration, the synthesized AgNPs might be completely were coated by extract components and resulting to hide SPR band.

3.3 Antibacterial Efficiency

The synthesized AgNPs were tested for their potential as antibacterial agent after incorporating them into wound dressing material. As clearly evident from Tab. 1 the pristine (uncoated) wound dressing did not show any reduction in bacterial (*Staphylococcus aureus)* population. The mullein plant extract have been traditionally used as a natural medicine for wound healing. Antibacterial properties of aqueous mullein extract against a broad range of bacteria has also been reported [15]. Therefore, when wound dressing was treated with only mullein extract (heated at 50 and 75ºC) showed significant decrease in the growth of bacterial population (42, and 53%, respectively). Interestingly, when AgNPs (synthesized at 50 and 75ºC) were coated on the wound dressing showed almost complete inhibition in bacterial growth.

Table 1: Antibacterial results

Sample	Bacteria Reduction
Untreated wound dressing	0%
Treated wound dressing with extract at 50ºC	42%
Treated wound dressing with extract at 75ºC	53%
Treated wound dressing with synthesized AgNPs at 50ºC	99%
Treated wound dressing with synthesized AgNPs at 75ºC	99%

4 Conclusion

Based on the above discussed results, it can be concluded that the extract of mullein leaves can be used for the synthesis of AgNPs. The shape and size of nanoparticles were analyzed by SEM and TEM imaging. The process of synthesis of AgNPs was optimized by various reaction conditions using UV-Visible spectroscopy. It was observed that the maximum bio-reduction of silver ions can be seen after 5 h of reaction with leaf extracts. The effect of temparature on nanoparticle synthesis suggest that maximum silver ion reduction can be obtain when reduction reaction was performed at 50ºC. The antibacterial test of synthesized silver nanoparticle coated wound dressings showed significant inhibiton to bacterial growth.

Althouh the obtained results are motivationg, however, more in-depth result is required to establish this method of silver nanoparticle synthesis and their coating on wound healing dressings in future.

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