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ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2019;8(3):541-4

Green synthesized silver nanoparticles: Morphology and antibacterial contact effects

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Received 06 December 2018; Accepted 25 February 2019
Available online 22.02.2019 with doi:10.5455/medscience.2018.07.8996

Abstract

In the study in which the green synthesis method was preferred, silver nanoparticles (Ag NP's), which preferred fig (Ficus carica) leaf extracts as a reducing agent, were obtained from their saline solutions. The morphological characteristics of the obtained Ag NPs were studied and antibacterial effects on 8 different bacteria (K. pneumonia, E. coli, P. mirabilis, Shigella, A. baumannii, S. aureus, S. aureus, S. epidermidis) were investigated. Transmission Electron Microscopy (TEM) and UV-Vis spectrophotometer determined morphology and formation of nanoparticles, respectively. The UV-vis absorbance spectra of AgNP exhibit increasing surface plasmon peaks with time of 446 nm. Nanoparticle sizes are at an average value of 13 nm in the 7-33 nm range. The antibacterial effects of Ag NPs vary from bacterial to bacterial and depending on the applied dose and show a contact effect. Positive results were obtained against both gram-positive and negative bacteria and the results were shared.

Keywords: Fig, Ficus carica, UV-Vis spectrophotometer, bacteria, silver nanoparticles

Introduction

The bioactivities and uses of nano-sized metal particles are the research areas for increased interest [1]. Nano size metal particles exhibit extraordinary physical, chemical and biological properties due to their wide surface area and high reactivity comparison. The optical properties of Ag NP's according to their size, structure and chemical properties have been studied in detail [2,3]. In addition, Ag NP's have great interest in the use of silver as a special class of biocidal agents due to the extraordinary antibacterial properties [4,5]. For instance, sore wraps including Silver materials that are sprayed monocrystalline are used in clinical practice to prevent bacterial infection of burn wounds today [4]. Similarly, there are numerous Silver composite materials containing Ag NP's as an active antibacterial component [6-8].

In recent years, researchers in the field of nanotechnology have discovered that metal nanoparticles have all the undisclosed benefits. They are typically prepared from noble metals, that is, silver (Ag), gold (Au), Platinum (Pt), Cupper (Cu) and İron (Fe). Ag NP's being most exploited [9]. They obtain applications in various fields such as medicine, electronics, energy saving, environment, textile, cosmetics, and so forth. Due to their applicability in such wide sectors, their demand is increasing at an overwhelming rate. The increased request has consequently resulted in increased production. Researchers are continuously developing newer methods for synthesis of highly monodisperse Ag NP's which are efficient in terms of synthesis rate as well as energy usage [10].

Traditionally, nanomaterials were synthesized using chemical or physical methods including left process, sol-gel, chemical sedimentation, hydrothermal method, pyrolysis and chemical vapour deposition [11]. Some of these methods provide easy control over the size of the crystal by restoring the reaction environment. The problem still occurs with the overall stability of the product, but it uses these methods in single-dispersion nanositm [12]. Moreover, most traditional techniques were found to be capital intensive and inefficient in the use of materials and energy [13].

Biological methods have emerged as an alternative to traditional methods in order to synthesize nanoparticles (NP's). Synthesis of inorganic particles by biological systems ensures that nanoparticles are environmentally friendly and biologically more compatible

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[14]. Additionally, the procedure is cost effective too [10]. A lot of bacterial as well as fungal species were used for Ag NP's synthesis [15]. But most of them was reported to amass Ag NP's intracellularly. Intracellular synthesis always takes longer reaction times and moreover needs subsequent extraction and recovery steps. In contrast, plant extract mediated synthesis always takes place extracellularly, and the reaction times was stated to be very short compared to that of bacterial synthesis. Most importantly, the method be able to suitably scaled up for large scale synthesis of NP's.

A lot of plants such as Turmeric [16], [16], Madhuca longifolia [17], Lemongrass [18], Aloe vera [19], Emblica officinalis [20], Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium [21] and many weeds [10] were shown the potential of lessening silver nitrate(AgNO3) to give formation of silver nanoparticles.

In this study, green synthesis method was preferred and Ag NP's were obtained from AgNO3 solutions using fig leaf extracts. Morphological characteristics of the obtained nanoparticles and their antibacterial effects on eight different bacterial cultures were investigated.

Material and Methods

AgNPs synthesis

1.00 gr fresh shaded dried figs (fucis Carica) leaves were milled and mixed on a magnetic stirrer in 100 ml distilled water for 30 minutes. The resulting solution was passed through filter paper and then the fig extracts were obtained. The fig extracts were mixed at a ratio of 1:1 with 2mM AgNO3 dissolved in distilled water. The mixtures were allowed to stand at room conditions for the synthesis of colloidal Ag NP's.

Characterization of Ag NPs

UV-vis absorbance spectrums of the synthesis colloidal Ag NP's were recorded in the wavelength range of 200-800 nm using Hitachi U–3900H double-beam spectrophotometer operating at a resolution of 1 nm. On the spectra, the fig extract diluted at a ratio of 1-1 with distilled water was taken as the reference solution, and the extract-AgNO3 mixtures were examined in the presence of this reference solution. Quartz cuvettes were used in the spectrophotometer. The formation kinetics of Ag NP's were determined by absorbance spectra taken at periodic intervals of 20 minutes. Morphological characteristics of the Ag NP's were determined by ZEIS-LEO 906E brand-model Transmission Electron Microscopy (TEM).

Antibacterial activity

The antibacterial activity of the synthesized Ag NP's was assayed in Gram (-) bacteria (Klebsiella pneumonia ATCC 700603, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 27853, Shigella, Acinetobacter baumannii ATCC BAA 747) and Gram (+) bacteria (Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 95923, Staphylococcus epidermidis ATCC 12228) by disc diffusion method on solid media. All the strains were obtained from Acıbadem University Medical Microbiology Laboratory. The bacterial strains were grown for 16 hours at 37 °C in Nutrient Agar (NA). The densities of bacterial suspensions were adjusted to 0,5 McFarland turbidity standard (1,5 x 108 CFU/mL) by diluted 1:100 with Nutrient Broth. Subsequently, 100 µl of bacteria cells were spread onto the NA Petri dishes using sterile spreader. Afterwards, sterile Whatman filter papers (6-mm-diameters) were placed over the medium using sterile forceps and were impregnated with 10, 30, 50, 70, 100 µl of Ag NP's. Ficus carica leaf extract was used as a negative control and Cefoperazone 75 mcg/disc and Doksisiklin 30 mcg/disc were used as positive control. The plates were incubated for 24 h at 37 °C. The growth inhibition zones of each disc were measured in millimeters. All experiments were duplicated.

Results

As soon as, Fig extract was mixed in aqueous solution of silver ion complex, the reduction of pure Ag+ ions to Ag0 was monitored by measuring UV–vis spectrum of the reaction media at regular intervals. UV–vis spectra were recorded as function of reaction time. The increase in peak intensities is related to the size of the nanoparticles as they are related to nucleation. Figure 1, nanoparticle sizes formed at the end of 40 minutes and nanoparticle sizes formed at the end of 100 minutes will be different from each other as can be seen from the peak intensities. As is known, nanoparticle sizes directly affect the antibacterial effects of Ag NP's.

Table 1 shows the particle sizes and shapes of Ag NP's obtained from different plant extracts. According to this table, which contains literature data, almost all of the nanoparticles have spherical shape and their dimensions vary from 4 to 150 nm. This change in particle size can be seen to be influenced by plants used as reducing agents. In this study, it was observed that the size distributions of the Ag NP's synthesized from the fig plant varied between 7 and 33 nm and the average 13 nm shapes were circular in accordance with the literature in Figure 2.

Table1. Dimensions and shapes of Ag NP's obtained by green synthesis method.

Reducing agent	Particles size (nm)	Particles Shape	SPR peak	Ref.
Turmeric	18 ±0.5	Spherical	432	16
Madhuca longifolia	30-50	Spherical. oval	436	17
Grass waste	15	spherical-oblate	430-450	18
Carica papaya	7-25	Spherical	429	19
Green Tea	3.9 ±1.6	Spherical	410	26
Abutilon indicum	50-100	Spherical	420	27
Rheum turkestanicum	26	Spherical	441	28
Bauhinia purpurea	12-50	Spherical	435	29

UV-vis absorbance spectra of Ag NP's obtained by green synthesis are given the table 1. According to these spectra, the values of the SPR peaks related to the formation of Ag nanoparticles range from 408 to 450 nm. In this study, it was observed that the UV-vis absorbance spectra of mixtures exhibited increasing SPR peaks at 446 nm, similar to the literature.

The antibacterial activity of mixtures that contain Ag NP's and Ficus carica leaf extract tested against the human pathogen Gram (-) bacteria (Klebsiella pneumonia ATCC 700603, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 27853, Shigella,

Acinetobacter baumannii ATCC BAA 747) and Gram (+) bacteria (Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 95923, Staphylococcus epidermidis ATCC 12228) at various concentration. The results were given in Table 2.

According to results, the synthesized Ag NP's and Ficus carica leaf extract mixture inhibited the bacterial growth both Gram (+) and Gram (-) bacteria strains, especially in Klebsiella pneumonia ATCC 700603. Inhibition zone changes according to the bacterial strain, for example antibacterial activity starts at 70 µL for Proteus mirabilis.

The morphology of silver nanoparticles synthesized using fig leaves and the antibacterial effect on G (+) and G (-) bacterial strains were investigated. As a result, the minimum dose amounts that the antibacterial effect changed with respect to the bacteria and which should be used were determined. Dose data of the study with a concentration of 1 mm was obtained. It is suggested to obtain new bacteria in different bacterial strains at different concentrations.

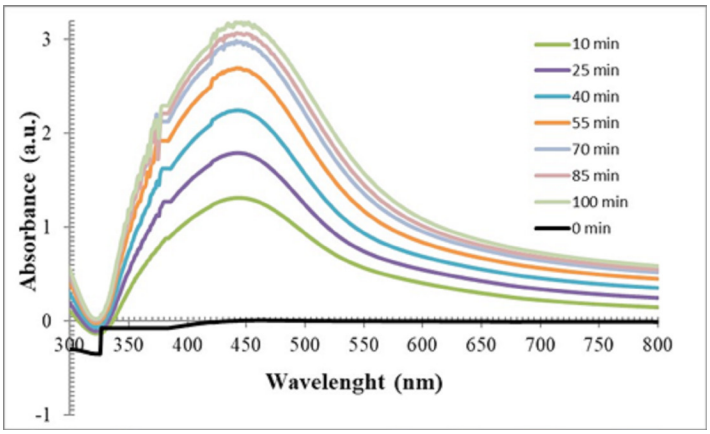

Figure 1. The UV-vis absorbance spectra of the fig extract-AgNO3 mixture as a function of time.

Table 2. The inhibition zone in diameter (mm) formed around the discs impregnated with mixtures in various volume

Bacteria Species	Plant extract	10 µl/ disc	30 µl/ disc	50 µl/	70 µl/ disc	100 µl/ disc	Doxycycline 30 mcg/disc	Cefoperazone 75 mcg/disc
K. pneumonia ATCC 700603	-	-	11	disc	18	22.5	20	
E. coli ATCC 25922	-	9	11.5	16.5	13.5	15.5	23	
P. mirabilis ATCC 27853	-	-	-	12.5	12	15	24	
Shigella	-	-	-	-	12.5	14.5	17	28
A. baumannii ATCC BAA	-	0	10	9	14	18	23	
S. aureus ATCC 29213	-	7.5	9.5	13	13	13.5	29	25
S. aureus ATCC 95923	-	-	11	11	14	14.5	30	24
S. epidermidis ATCC 12228		-	7	12	13	16.5	28	23

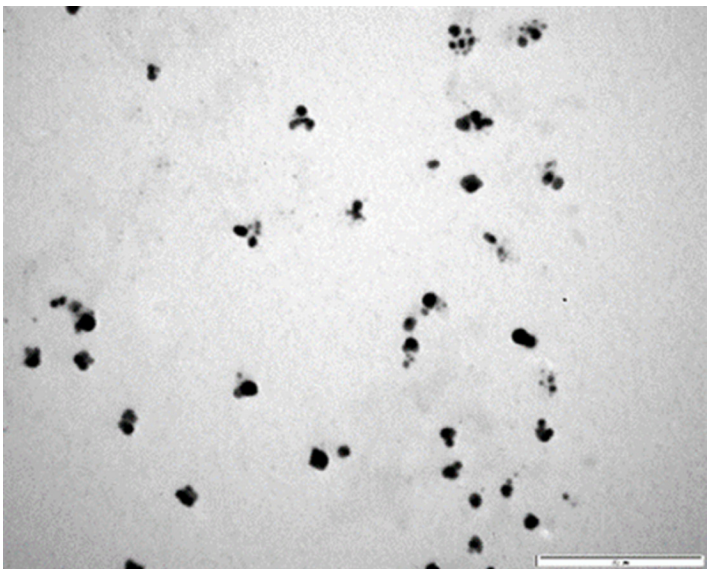

Figure 2. Transmision Electron Microscopy (TEM) imaging of Ag NP's.

Discussion

In many studies, the antibacterial effects of nanoparticles were emphasized, and the effects of nanoparticle solutions on different bacteria were investigated (Table 1). In these studies, it is emphasized that the inhibition area is expanding. In some of these studies, it was suggested that the interaction of the bacteria with the nanoparticle solution increased due to the increase of the volume. The Ag-NPs are capable of inhibiting the bacterial growth due to their small size and large surface area which provide sufficient contact with the bacteria [30]. Since this volumetric increase will not directly increase the nanoparticle concentration on the surface of the unit, it can be said that the antibacterial effect will not increase due to the solution volume and the expansion of the field may cause antibacterial effect due to the increase in contact with bacteria due to the fluidity of the solution. As the volume of the nanoparticles increases, the area it covers on the petri increases and this expands the inhibition area. Ag NP's are positively charged and can easily bind and interact with the negatively charged bacterial cell wall. Nanoparticles that move to the cytoplasm prevent expression of essential proteins for cell and cause cell death [22-25]. As a result, the contact effect of Ag NP's dependent on the plant which is used can be mentioned.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Consent of ethics was approved by the local ethics committee.

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