

Synthesis of silver nanoparticles using herbal plants and its application to nano-gel systems for treatment of wounds

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ABSTRACT

The present study aims that a simple, rapid and economical method to synthesize silver nanoparticles (AgNPs) from herbal extract of coriander sativum linn. The optimized AgNPs were characterized using Particle size analysis, Zeta potential, FE-SEM and UV-vis analysis. It was observed that the average particle size of AgNP was 73.67 nm which confirmed that the silver ions were reduced into nanoparticles. The polydispersity index (PDI) of the silver nanoparticle was found to be 0.327; which indicates the broad distribution of globules and its homogeneity. The zeta potential was found to be -23.9 mv. The topical formulation was prepared by incorporation of optimized AgNPs into the carbopol gel base. Further, the gel was evaluated in vitro antibacterial assay by agar well diffusion method. The antibacterial inhibitory efficiency of prepared AgNPs incorporated nano gel was found similar to the commercial product against the Staphylococcus aureus, E. coli and Pseudomonas aeruginosa. From the results, it can be concluded that the formulation has potential to use for the treatment of wounds.

Keywords: Silver Nanoparticles, Wound healing, Hydrogel

INTRODUCTION

Nanotechnology offers fields with effective applications, ranging from traditional chemical techniques to medicinal and environmental technologies. AgNPs have emerged with leading contributions in diverse applications, such as drug delivery,¹ ointments, nanomedicine,² chemical sensing,³ data storage,⁴ cell biology,⁵ agriculture, cosmetics,⁶ textiles,⁷ the food industry, photocatalytic organic dye-degradation activity,⁸ antioxidants,⁹ and antimicrobial agents.¹⁰ Despite the contradictions reported on the toxicity of AgNPs,¹¹ its role as a disinfectant and antimicrobial agent has been given considerable appreciation. The available documented data^{12,13} and the interest of the community in this field prompted us to work on plant-mediated green synthesis and biological activities of AgNPs.

Green silver nanoparticle synthesis has been achieved using environmentally acceptable plant extract and ecofriendly reducing and capping agents. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-

friendly, and a single-step method for biosynthesis process. Silver nanoparticles incorporated into nanogel have shown a broad-spectrum microbicidal effect and have potential to use treatment of wounds. The goal of the research was to prepare silver nanoparticles by using plant extract then formulate and evaluate various polymers with varying concentrations for the preparation of a safe, effective and stable gel containing silver nanoparticles and to evaluate the in vitro evaluation and the antibacterial activity for prepared formulations.

EXPERIMENTAL METHODS

Preparation of Extract

The leaves of coriander sativum linn were washed in water to remove the dust and foreign material from the surface then air dried under shade at room temperature. The air dried plant material was coarse powdered and subjected to methanol extraction using soxhlet apparatus by reflux for

24 h at 60°C. A grey colored semisolid mass was obtained, dried under vacuum and kept in desiccators until use.

Synthesis of Nanoparticles

In the single step green synthesis, 5 ml of leaf extract was added to 95 ml of 1 mM aqueous silver nitrate solution and kept in the dark place at room temperature for 24 h. Silver nanoparticles are formed by reduction of pure silver ions and it was monitored by measuring absorption of the reaction medium in the wavelength range of 300-700 nm using UV spectrophotometry. The synthesized silver nanoparticle (AgNP) was purified by centrifugation at 1000 rpm for 15 min. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried using cooling microfuge to get dried, purified and characterized the AgNPs.

UV-Visible Spectral Analysis

Silver nanoparticles were formed by reduction of silver ion; it was monitored by measuring the absorption spectra in the wavelength range of 300-700 nm using Shimadzu UV-1800 Spectrophotometer. The spectrum was recorded and the maximum absorption wavelength was determined.

Preparation of Topical Formulation

Topical gel formulations were prepared by cold mechanical method with defined quantity of carbopol-934 and HPMC polymer. The specified quantity (1g) of polymers such as Carbopol 934 and HPMC were weighed separately and sprinkled slowly on surface of purified water. To this defined quality of double distilled water was added with vigorous stirring and left overnight for dissolving the polymer. To the polymer solution, drug silver nanoparticles were added to the gel with continuous stirring. Required quantity of glycerol was added and mixed well by using magnetic stirrer. After complete dispersion, the pH of the gel was adjusted to neutral pH 7 by using sodium hydroxide. Distilled water was added and made up to 100g.

Physicochemical Evaluation of Formulations

Spreadability

Glass slides with standard dimension (length of 6.0 cm) were taken. Topical gel formulation was placed on the one

side of the glass slide and sandwiched with the help of another slide. Remove the adhering gel on the outer surface of the glass slides by wiping. Slides are fixed in a stand that only upper slide to slip off freely without any disturbance by force of weight (20g) tied to it. Time taken for the movement of upper slide to the distance of 6.0 cm was measured. Measurement of spreadability was done in triplicate and calculated by using the following formula:

$$\text{Spreadability} = (\text{Weight} \times \text{Length}) / \text{Time}$$

Where, S=Spreadability; m=Weight tied to the upper slide (20 g); l=Length of the glass (6.0 cm); t=Time taken in seconds.

Determination of zone of inhibition

Antibacterial activity was checked by agar well diffusion method. In this method a previously liquefied medium. In each of these plate extract, silver nanoparticle medium was inoculated with 0.1 mL Bacterial suspension having a uniform turbidity at temperature of 40°C. In a sterile petri dish having an internal diameter of 8.5 cm was taken, 20 mL of culture medium was poured into it. Care was taken to form a uniform thickness of the medium in different plates. Wells were made aseptically with cork borer having 6 mm diameter after complete solidification of liquefied inoculated and topical gel formulation were placed carefully. Plates were kept for pre diffusion for 30 min at room temperature; then the plates were incubated at 37°C for 24 h and the zones of inhibition were measured.

RESULTS AND DISCUSSION

Green Synthesis of AgNPs

Green synthesis of AgNPs was prepared from plant extract and shown in Figure 01. On mixing plant extract with the silver nitrate solution, a change in the solution color from pale yellow to dark brown was observed which indicates the reduction of silver ions and formation of silver nanoparticle



Figure 01: Image of silver nanoparticles preparation

Characterization of Silver Nanoparticles

UV-Visible Spectral Analysis

The UV absorption spectrum of coriander sativum linn has shown a peak specific in the range between 400 and 450 nm. Peak specific in this region might be due to Mie scattering effect²⁴. The UV spectrum of silver nanoparticle is shown in Figure 02.

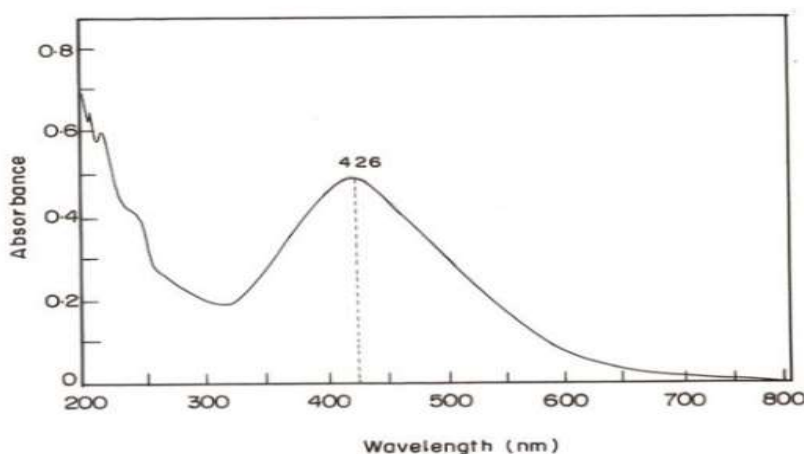


Figure 02: UV-Visible spectra of Silver Nitrate Nanoparticles

The particle size analysis was done by measuring the average particle size in the aqueous reaction mixture after the completion of reaction using the zeta sizer in dynamic light scattering mode. It was observed that the average particle size was 74.37 nm which confirmed that the silver ions were reduced into nanoparticles. The polydispersity

index (PDI) of the silver nanoparticle was found to be 0.327; which indicates the broad distribution of globules and its homogeneity. The particle size and polydispersity index is shown in Figure 3.

Results

| | Size (d.nm): | % Intensity: | St Dev (d.nm): |
|--------------------------------|----------------------|--------------|----------------|
| Z-Average (d.nm): 74.37 | Peak 1: 73.67 | 84.5 | 32.93 |
| Pdi: 0.327 | Peak 2: 1051 | 10.2 | 487.4 |
| Intercept: 0.917 | Peak 3: 4107 | 5.3 | 1069 |
| Result quality : Good | | | |

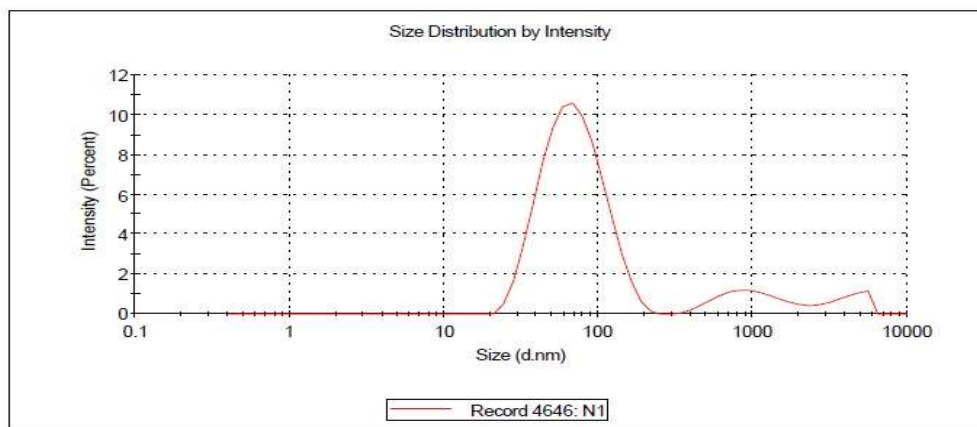


Figure.03: Particle size and poly dispersity index of silver nitrate nanoparticles

Spreadability

Bioavailability and therapeutic property of the topical formulation depends upon the spreadability. The spreadability is expressed of time in seconds based on the slip off from the gel by upper slide under certain load. Time taken for the separation of the two slides is less which indicates the topical formulation has better spreadability. The spreadability value was found to be 6.9 ± 0.1 (g.cm/sec) for gel prepared by Carbopol.

Antibacterial Activity of the Formulation

The antibacterial activity study results of the formulated herbal gel showed antibacterial activity against *Staphylococcus aureus* (S. aureus) bacteria was depicted in Figure 04. The antibacterial study reveals that the silver nanoparticle of coriander sativum linn showed higher activity than the extract against all the pathogens.



Figure No 04: In vitro antimicrobial activity of silver nitrate nanoparticles incorporated hydrogel, Plain Silver Nanoparticles, Marketed silver nitrate gel

CONCLUSION

Concerning the environmental protection, green synthesis of nanoparticle has gained friendly and growing demand. Among the different metal nanoparticle, AgNPs has an excellent antibacterial agent due to its non-toxic effect on the human cells. Medicinal plants have been used as a home remedy from ancient time due to its variety of metabolites and its phytoconstituents. These phytoconstituents and metabolites can reduce the silver ions and assist synthesis of AgNPs from plant extracts. These AgNPs are having

strong binding affinity with many functional groups of the plant extracts. The present study reveals a simple, rapid and economical method to synthesize AgNP silver nanoparticle from coriander sativum linn. The antibacterial activity is well demonstrated by agar well diffusion method. The synthesized AgNP silver nanoparticle using coriander sativum linn leaves extract showed higher activity than the extract. AgNP of coriander sativum linn in an aqueous gel base can be used as an appropriate formulation for the treatment of wound healing.

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