Research Article

Synthesis, Characterization and Size Evaluation of Biosynthesized Silver Nanoparticles by UV–Vis Spectroscopy

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Abstract

Mie-Gans (MG) fitting model theoretical model utilizing the phenomena of scattering light to determine the morphologies, shape, and size of metallic nanoparticles in solution. In the present work, the average radius of biosynthesized silver nanoparticles (AgNPs) was evaluated based on the fitting of their Ultraviolet-visible (UV-Vis) spectra by the MG fitting model for spherical and non-spherical particles. Biosynthesis of AgNPs using Lemon (Citrus Limon) leaves extract as a reducing agent and Gum Acacia as a capping and stabilizing agent was studied for various concentrations of Citrus Limon leaves extract. The investigation of structural and optical properties was carried out for the synthesized samples using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and UV-Vis spectroscopy. XRD confirmed the structure of AgNPs and revealed that the structure of these nanoparticles was face-centered cubic (fcc). FTIR measurements indicate the presence of citric acid in Citrus Limon leaf extract which is responsible for reducing bio-reduced AgNPs. UV-Vis spectroscopy determined the surface plasmon resonance (SPR) for AgNPs; the peaks of resonances of samples appear at 436-461nm range. MG fitting evaluations show that most of AgNPs were spherical in shape with an average radius in the range of 39-47nm. Moreover, this model allows the estimation of the fraction of nonspherical and aggregated AgNPs. These unique characteristics of AgNPs have made them applicable in a large number of fields like water treatment, biomedical, energy science, catalysis, etc.

Keywords: silver nanoparticles, biosynthesis, characterization, Mie and Mie-Gans models, fittings

1. Introduction

In recent years metal nanoparticles (MNPs) have attracted immense attention of the scientific community for their novel properties and technological fields, from biology to materials science,

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electronics, or mechanics and they are still charming the scientists to explore new dimensions for their respective worth which is generally attributed to their corresponding small sizes [1]. Among noble MNPs, silver has attained a special focus due to its uncommon optical, mechanical, electronic, and chemical properties, depending on their size and shape, thus opening many possibilities for technological applications [2, 3]. It is swiftly gaining renovation in a large number of fields such as biomedical, drug-gene delivery, food and feed, water treatment, mechanics, chemical industries, cosmetics, space industries, energy science, catalysis, electronics, light emitters, single-electron transistors, nonlinear optical devices, and photo-electrochemical applications. The tremendous growth in these expanding technologies had opened applied frontiers and novel fundamentals [4].

Several methods have been employed for the synthesis of AgNPs involving physical, chemical, photochemical, and biological synthesis. The biological synthesis of MNPs is an emerging branch of nanotechnology in which environmentally benign materials [5] like plant leaf extract, bacteria, and fungi were used for the synthesis. It has numerous advantages such as simplicity, cost-effectiveness, compatibility for antibacterial, antioxidant, and antitumor activity of natural products [6, 7]. The use of plants extract as reducing and stabilizing agents for the synthesis of AgNPs holds unique attention among researchers. *Solanum xanthocarpum* berry, *Apiin*, *Azadirachta indica*, *Musa paradisiaca*, *Garcinia mangostana*, and other plant extracts have been found suitable for the green synthesis of AgNPs and highly effective against different multi-drug resistant human pathogens [8]. G. Suresh et al (2014) investigated the green synthesis of AgNPs using aqueous root extract of *Delphinium denudatum* (Dd) by reduction of Ag^+ ions from silver nitrate (AgNO_3) solution, and they demonstrated the possible application of DdAgNPs in the medical field as it shows antibacterial activity against human pathogenic bacteria as well as its anti-mosquito activity against dengue vector *A. aegypti* [9]. The biomedical applications of the synthesized AgNPs using aqueous *Piper longum* fruit extract (PLFE) was substantiated by their potent free radical quenching effect, antibacterial activity and cytotoxic effect against MCF-7 breast cancer cell lines. Reddy et al study 2014 [10]. A lot of works have been done but, none of these studies have studied the effect of the Citrus limon leave extract concentrations on AgNPs. Citrus Limon is a commonly available fruit and a principal source of such important nutrients. It contains vitamin C also provides organic acids such as citric acids and ascorbic acids, which; were suggested to be bioreductant in the formation of AgNPs, dietary fibre, and bioactive components such as carotenoids [11]. Moreover, Citrus Limon is well known to be rich in certain phytonutrients especially flavonoids that protect humans against cancer and cardiovascular diseases, also rich in minerals such as Calcium, Iron, Magnesium, Manganese, Phosphorus, Potassium, and Zinc that help in lowering blood pressure levels and substantially reduce the risk of stroke [12].

Since the Mie model for spherical particles together with the Gans model for spheroids allows an easy characterization of the structure of the nanoparticles, also a fast and reliable interpretation of experimental UV-Vis spectra based on Mie theory. Moreover, the MG fitting model allows estimation of the fraction of nonspherical and aggregated MNPs, which is useful for the quantitative detection of aggregation processes even in their early stages [13,14,15]. S. Baset et al., 2011 presented a technique for size measurement of metal and semiconductor nanoparticles synthesized by laser ablation method. The technique includes a comparison between UV-Vis spectra fitting of the colloidal nanoparticles and theoretical calculation of absorption spectra. Mie theory was used and found this theoretical method was

in excellent agreement with size measurement taken from TEM observation by experimental method [16]. Also, V. Amendola and M. Meneghetti have investigated a method for the evaluation of the average size of gold nanoparticles (AuNPs) based on the fitting of their UV-Vis spectra by the Mie model for spheres. As result, the method gives good results using a calibration of the dumping frequency of the SPR and accounting for the presence of nonspherical AuNP in solution by the Gans model for spheroids. And they found an accuracy of about 6% on the average size of nanoparticles to size measured by TEM. Furthermore, the fitting model has provided other information not available from TEM like the concentration of AuNP in the sample and the fraction of nonspherical nanoparticles [13].

Therefore, there is a need to develop novel and simple theoretical methods for the characterization of nanoparticles; hence, the purpose of the present study was to use Mie-Gans fitting for calculating the average radius, particle size distribution, and the fraction of spherical to nonspherical of the green synthesized AgNPs.

In this present study, AgNPs were synthesized biologically. It demonstrated the use of a natural, renewable and low-cost biological reducing agent, such as Citrus Limon leaves can produce metal nanostructures in an aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents. The plant leaves have a high level of citric acid and ascorbic acid as well as lignin which can act as reducing agents, and gum Acacia extracts as capping. The effect of extract concentrations was also evaluated to optimize the structure and optical properties of producing nanoparticles.

2. Experimental

2.1 Materials

AgNO₃ (99%) was obtained from CDH laboratory reagent, and distilled water was used throughout the experiments from the chemical laboratory of Al- Neelain University, Fresh leaves of Citrus Limon were collected from Toti Island in Khartoum, Sudan, Gum Acacia was purchased from the local market.

2.2 Preparation of Citrus Limon extract

Twenty grams of fresh leaves of Citrus Limon were washed several times with tap water and then washed with distilled water to remove the dust. They were then cut and soaked in 100ml of distilled water then boiled for 30min and filtered through quantitative filter paper, and finally, the yellow extract was collected and stored to be used for biosynthesis of AgNPs from silver nitrate [17].

2.3 Preparation of Gum Acacia extract

Dried gum acacia was bought from the local market. The gum was dried by the oven for 1h to completely remove the moisture. A fine powder was obtained from the dried gum using a mortar. A homogeneous 0.01g/ml gum stock solution was prepared by adding the fine powder gum to distilled water with constant stirring on a magnetic stirrer at room temperature for 30minutes [18].

2.4 Preparation of silver nanoparticles

For the green synthesis of AgNPs, 1ml of Citrus Limon leaves extract was mixed to 100ml aqueous solution of AgNO₃ (2mM) and stirred continuously for 5min at room temperature and the reduction of

AgNO₃ to silver ions was completed slowly take more than 12hours and confirmed by the changing of the colour which gives silver colloid (L1). Similarly by adding 3 and 5ml of extract two more sets of samples, (L3 and L5 respectively) were prepared.

After three weeks of preparation of silver colloids, 5ml of Gum Acacia extracts was added to the samples (L1, L3, and L5) and stirred continuously for 5min at room temperature, which henceforth called LG1, LG3, and LG5 respectively. UV–Vis spectra were taking when the samples were in solution form. Then the solutions were dried at 200°C for 2 hours. The dried powders were characterized by XRD and FTIR [19].

2.5 Characterizations of the synthesized nanoparticles

2.5.1 Experimental Techniques

Synthesis of AgNPs by reducing silver ion solution with Citrus Limon leaves extract is easily observed by UV-Vis spectroscopy. The absorption spectra of the prepared nanoparticles were measured using a Shimadzu spectrophotometer (UV mini 1240) in 190–800nm range. The formation and quality of the samples were checked by the XRD technique using a Shimadzu MAXima_X XRD_7000. FTIR measurements of the samples were obtained on Mattson, model 960m0016 spectra. With transmission from 4000 to 400cm⁻¹, by using KBr pellets.

The studies of size, morphology, and composition of the nanoparticles were performed by MG fitting model and log-normal fitting analysis and were done using MATLAB R2106a program.

2.5.2 Mie-Gans Fitting Model

The extinction cross-section of spherical AgNPs can be successfully calculated using the Mie model for compact spheres. The Mie model is based on the resolution of the Maxwell equations in spherical coordinates using the multipoles expansion of the electric and magnetic fields and accounting for the discontinuity of the dielectric constant between the sphere and the surrounding medium. The Mie model expressions of extinction cross-section σ_{ext} for a single sphere of radius R [13]:

$$\sigma_{ext} = \frac{2\pi}{|k^2|} \sum_{L=0}^{\infty} (2L + 1) \text{Re}(a_L + b_L) \quad 1$$

The Mie model accounts only for spherical particles, while AgNPs in the solution can have different structures. Since aggregation can involve two or more particles and most isolated particles with nonspherical shape are low aspect ratio spheroids, one can account for the contribution of small aggregates and nonspherical particles to the overall extinction spectra using the Gans model that is the Mie theory extension to particles with the spheroidal shape. For a prolate spheroid of aspect ratio a/b, where b is the smaller axis (a > b = c), the σ_{ext} averaged over all possible orientation in space is

$$\sigma_{ext} = \frac{2\pi V \epsilon_m^{3/2}}{3\lambda} \sum_j \frac{\frac{1}{p_j^2} \epsilon_2(\omega, R)}{(\epsilon_1(\omega, R) + \frac{1-p_j}{p_j} \epsilon_m)^2} \quad 2$$

Where λ is the wavelength of incoming photons, m is the matrix real dielectric constant, $V = (4\pi/3) ab^2$ is the volume [13].

We accounted for particles with different shapes as spheroidal particles with a distribution of aspect ratios, and we assumed a Gaussian probability G (a/b) centered at a/b=1 for such a distribution:

$$G(a/b) = \frac{1}{S_c \sqrt{2\pi}} \exp \left[\frac{-(a/b-1)^2}{2S_c^2} \right] \quad 3$$

2.5.3 Log-normal Mie Gans (LNMG) fitting model

The MG model provides information about AgNPs samples, but it does not provide data about the size distribution. In principle, estimation of the size distribution of a given monodispersed AgNPs solution from its UV-Vis spectrum was possible.

In this LNMG fitting model $\sigma_{ext}(\omega, R)$ is weighted by the probability given by the log-normal distribution $LN(R)$ according to the following expressions [13]:

$$LN(R) = \frac{1}{\sqrt{2\pi}(wR)} \exp \left[\frac{-1}{2w^2} \left(\log \frac{R}{R_c} \right)^2 \right] \quad 4$$

$$\sigma_{ext}(\omega) = \frac{\int_{R=0}^{R=\infty} LN(R) \sigma_{ext}(\omega, R) dR}{\int_0^{\infty} LN(R) dR} \quad 5$$

3. Results and Discussion

3.1 XRD analysis

The XRD patterns of the dried AgNPs synthesized using Citrus Limon leaves extract with various concentrations (LG1, LG3, and LG5) were shown in **Figure 1**. Four intense peaks in the whole spectrum of 2θ values ranging from 20° to 80° appeared, XRD spectra of pure crystalline silver structures have been published by the Joint Committee on Powder Diffraction Standards.

A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as verified by the peaks at 2θ values of 38.1° , 44.3° , 64.4° , and 77.5° corresponding to (111), (200), (220), and (311) reflections of (fcc) structure of silver phases. These peaks appeared with a noise background that indicates the AgNPs need more temperature for annealing, or more grind to be smooth.

Two small peaks were observed at 32.3° and 54.5° . These peaks were attributed to the presence of other organic substances in Citrus Limon leaves extract. These peaks were much weaker than those of silver, which indicates that silver was the main material in the composite compared with those of the bulk counterpart [20]. Based on our XRD data, as the concentration of Citrus Limon leaves extract increases the diffraction peaks corresponding to silver have become sharpened. The sharper peaks indicating the crystallite size were large whereas broader peaks indicative of small crystallite materials [21].

This was also confirmed by the estimation of crystalline size using the Debye–Scherrer formula by using the width of the (111) Bragg's reflection and tabulated in **Table 1**. It was found to be increasing particle size with increasing extract concentration. The lattice parameter and cell volume of LG1, LG3, and LG5 were evaluated and tabulated in **Table 1**, which is in good agreement with the standard data file JCPDS No. 04-0783.

Figure 1 XRD pattern of silver nanoparticles LG1, LG3, and LG5 (* due to Citrus Limon extract)

Table 1 crystalline size, lattice constant and cell volume of the prepared silver nanoparticles (LG1, LG3 and LG5)

Sample	Crystalline size (nm)	d_{hkl} (Å)	Lattice constant (a) (Å)	Cell volume (V) (Å ³)
LG1	3.027	2.359	4.0874	68.3
LG3	3.178	2.359	4.0874	68.3
LG5	3.572	2.358	4.0856	68.2

3.2 UV-Vis spectral analysis

The extract concentration variation study was carried out by measuring its optical spectra of L1, L3, and L5 recorded at various concentrations. Since concentration is an important parameter in the synthesis of nanoparticles.

The optical spectrum of aqueous Citrus Limon leaves extracts exhibits, one band, at 665nm shown in **Figure 2**. The absorption peak indicated the presence of flavonoids, alkaloids, and antioxidant vitamins in Citrus Limon leave extract [17].

Figure 2 Optical absorption spectra of Citrus Limon leaves extract (inset: Citrus Limon leaves and its extract)

It is well known that silver nanoparticles exhibit yellowish-brown colour in an aqueous solution due to the excitation of surface plasmon vibrations in silver nanoparticles [19]. Reduction of the silver ion to silver nanoparticles during exposure to the plant leaf extracts was followed by colour change (see **Figure 3**) and as well as by UV–vis spectroscopy. A concentration variation study of AgNO_3 using Citrus Limon leaves extract was carried out with various volumes of lemon leaves extract.

Figure 3 Concentration dependent colour changes of the solution in reaction system:

(a) 1ml, (b) 3ml, and (c) 5ml of Citrus Limon leaves extract in 0.02 M/L AgNO_3)

Figure 4 shows that the concentration of Citrus Limon leaves extract plays an important role in the formation of AgNPs, revealed by the intensity of SPR band around 420nm, which increases with increasing concentration of Citrus Limon leaves extract. Indeed, when a large volume of Citrus Limon leaves extract (L3 and L5) were added to silver ions solution, the SPR band around 420nm increases in intensity, which can be correlated with an enhancement in the number of nanoparticles in the reaction medium [22].

Figure 4 Optical absorption spectra of silver nanoparticles at different concentration of Citrus Limon leaves extract

An enhancement in the SPR bands of the samples was observed when the gum acacia extract was added as capping and stabilizing to the silver samples (L1, L3, and L5) as can be seen in **Figure 5**. The position of the plasmon absorption peak depends on the particle size, shape and the adsorption of nucleophile or electrophile to the particle surface [21]. As the concentration of leaves extract increases the SPR bands of the prepared samples (LG1, LG3, and LG5) were red-shifted: from 436nm to 461nm for the LG1 and LG5, respectively. This result represents that the diameter of the prepared silver nanoparticles increases with increasing concentration of the leaves extract, such a red-shift is associated with the withdrawal of electron density from the surface and is a characteristic of an increased nanoparticle size [20, 22]. It is well known that adsorption of the nucleophile to the particle surface increases the Fermi level of the silver particle owing to its donation of electron density to the particles [20]. In this case, observed redshift indicates that the silver nanoparticles are larger in size, which is confirmed by the experiment.

Figure 5 Optical absorption spectra of prepared LG1, LG3, and LG5 after stabilized by gum acacia

3.3 FTIR analysis

FTIR measurements were carried to identify the possible biomolecules in Citrus Limon leaves extract, which is responsible for the reduction and capping of the prepared silver nanoparticles. **Figure 6** shows the FTIR spectra of AgNPs (LG1, LG3, and LG5) and dried Citrus Limon leaves extract powder. It shows interesting peaks at 1107, 1384, 1634, and 3425 cm^{-1} .

The band at 1107 cm^{-1} corresponded to CN stretching vibrations of amine [17]. The band at 1384 cm^{-1} is due to the COH deformation and CH_3 wagging mode of citric acid. The band at 1634 cm^{-1} corresponded to amide I, arisen due to CO stretching of proteins. Where the CH stretching of vitamin C is responsible for the weak peak observed at 2925 cm^{-1} , and the band observed at 3425 cm^{-1} is due to the OH stretching of citric acid [22]. The more prominent vibrational mode observed at 1384 and 3425 cm^{-1} in AgNPs, indicates that citric acid present in Citrus Limon leaves extract is responsible for the reduction of AgNPs (see **Table 2**).

Table 2 FTIR analysis of AgNPs samples

Vibration mode	Wavenumber (cm^{-1})
CN stretching of amine	1107-1117
COH deformation and CH_3 wagging mode of citric acid	1384-1386
CO stretching of protein	1634-1638
CH stretching of vitamin C (ascorbic acid)	2925-2928
OH stretching of citric acid	3425-3430

Figure 6 FTIR spectra of AgNPs (LG1, LG3, and LG5) and Citrus Limon leaves extract

3.4 Calculated Results

The fitting of AgNPs surface plasmon absorption (SPA) at about 400nm with the MG fitting model allows an easy characterization of the structure of the nanoparticles [24]. MG fitting model has been employed to characterize the size, shape and morphologies of formed AgNPs.

Colloids of spherical AgNPs have a characteristic yellow colour due to the SPA located near 400nm, but in **Figure 5**, can also observe an asymmetric broadening of the SPA toward longer wavelengths, which are due to a fraction of particles with spheroidal shape. The origin of these spheroids can be attributed to aggregation processes that are very probable in a colloidal solution of free metal nanoparticles. The SPA of spheroidal metal particles can be well reproduced by the Gans model, an extension of the Mie model for nonspherical particles [24].

Figure 7(a) shows the UV-Vis experimental spectrum of AgNPs LG1, where the SPA maximum was observed at 435nm. It also shows the separate contributions of spheres and spheroids to the final fitting. All silver nanoparticles were found to spherical (100%) with a Gaussian distribution of aspect ratio with a standard deviation $S_G = 0.1$. The MG fitting of the UV-Vis spectrum was found an average radius of $R = 39\text{nm}$. As can be seen in **Figure 7(b)**, the size distribution was fitted by a log-normal curve with a peak at 39.05nm and a width of 0.1.

Figure 7 (a) UV-Vis spectrum of LG1 (line), the MG fitting (circles), the spherical particles contribution to the fitting (dashed line) and the spheroids contribution (dashed dot line). (b) The log-normal measured size distribution of LG1

Figure 8 (a) displays the UV-Vis spectrum of LG3, where the SPA centered at 460nm. The spheroid's contribution was found to be 5% with $S_G=0.5$. And it is illustrated in figure together with the sphere's contribution. The MG fitting of the UV-Vis spectra indicates an average radius of $R = 40.60\text{nm}$, the log-normal curve in **Figure 8 (b)** shows a peak at 41.05nm and a width of 0.1.

Figure 8 (a) UV-Vis spectrum of LG3 (line), the MG fitting (circles), the spherical particles contribution to the fitting (dashed line) and the spheroids contribution (dashed dot line). (b) The log-normal measured size distribution of LG3

Figure 9 (a) shows the UV-Vis spectrum of LG5, in which the SPA located at 460nm. The MG fitting resulted that an average radius $R = 47.7\text{nm}$ with a fraction of 5% of spheroids with $S_G = 0.33$. **Figure 9 (b)** shows the log-normal curve of the distribution of particles has a peak at 49nm and a width of 0.1.

Figure 9 (a) UV-Vis spectrum of LG5 (line), the MG fitting (circles), the spherical particles contribution to the fitting (dashed line) and the spheroids contribution (dashed dot line). (b) The log-normal measured size distribution of LG5

These calculated results were confirmed the experimental results, in which the larger particle size found at the higher concentration of Citrus Limon leaves extract is due to the aggregation of the AgNPs (as can be observed in **Figure 10**).

Figure 10 plot of the AgNPs (LG1, LG3, and LG5) average radius versus the concentration of Citrus Limon leaves extract

4. Conclusion

The present study was aimed to rapid synthesise of AgNPs by a natural, low-cost biological reducing agent. AgNPs were synthesized using Citrus Limon leaves extract by varying the concentration of the extract. This natural method for the synthesis of AgNPs offers a valuable contribution in the area of green synthesis and nanotechnology avoiding the presence of hazardous and toxic solvents and waste.

The SPR of prepared AgNPs were confirmed by UV–Vis spectral analysis. As the concentration of leaves extract increases, absorption spectra shows red shift with increasing particle size. XRD indicated that these nanoparticles had been synthesized as the best possible. The XRD revealed that the AgNPs had a structure of (fcc) with average crystalline size of 3nm. FTIR analysis shows that the presence of amine, ascorbic acid, and citric acid in Citrus Limon leaves extract with related bonds results in the perfect condition for reducing silver in AgNPs compounds. And indicates that the proteins present in gum acacia extracts were used as capping agent. MG fitting model of UV-Vis spectroscopy measurements shows that the average radius of the prepared AgNPs was in the range of 39-47nm and it was spherical in shape.

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