Chemistry Research Journal, 2016, 1(6):17-21

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Green Synthesis of Silver Nanoparticles from the Lemon Leaves flower extract and their Antibacterial Activity

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Abstract Silver nanoparticles were synthesized according to chemical reduction method but by using citrus limonum leaves (lemon leaves) extract. Silver nanoparticles are characterized by UV-Visible spectrometer, particle size analyser (PSA), and Scanning electron microscope (SEM). Further, the AgNPs showed an effective antibacterial activity toward pseudomonas and proteus by disk diffusion method.

Keywords Biosynthesis, Silver nanoparticles, lemon leaves extract, Characterization, Antibacterial activity

Introduction

Silver nanoparticles were synthesized according to chemical reduction method but by using citrus limonum leaves (lemon leaves) extract. Silver nanoparticles are characterized by UV-Visible spectrometer, particle size analyser (PSA), and Scanning electron microscope (SEM).

Figure 1: (a) Citrus limonum (lemon leaves), (b) Citrus limonum leaf extract

Figure 2: (a) 0.001 N Silver nitrate solution, (b) silver nanoparticles after addition of extract

Preparation of Extract of *Citrus limonum* leaves

Fresh leaves of *Citrus limonum* (lemon leaves) were collected from A. M. Jain College campus. The extract was prepared by taking 10g of fresh citrus limonum leaves. The leaves were washed several times with distilled water. Then, the clean and fresh *Citrus limonum* leaves were cut into small pieces with the help of sterilized knife. The chopped leaves were boiled in 100 ml of distilled water taken in a 250 ml borosil beaker at 80-90 °C for 20 min. The boiled leaves were filtered using whatmann 40 filter paper and the extract was collected in a clean container. The extract is used as a reducing and stabilising agent for the preparation of silver nanoparticles [1, 3-4].

Synthesis of Silver Nanoparticles

0.001 N aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. The equal proportions of 25 ml of 0.001 N Silver nitrate solution is mixed with 25 ml of *Citrus limonum* (Lemon leaves) extract after the 24 hours observation the mixture turned into a reddish brown colour which confirms the presence of silver nanoparticles [2,7,9].

Result and Discussion

UV–Visible absorbance studies

The Ultra-Violet Visible absorption spectroscopy (UV-Vis) indicating the formation of silver nanoparticles is shown in the Fig.3. for the sample prepared as mentioned before.

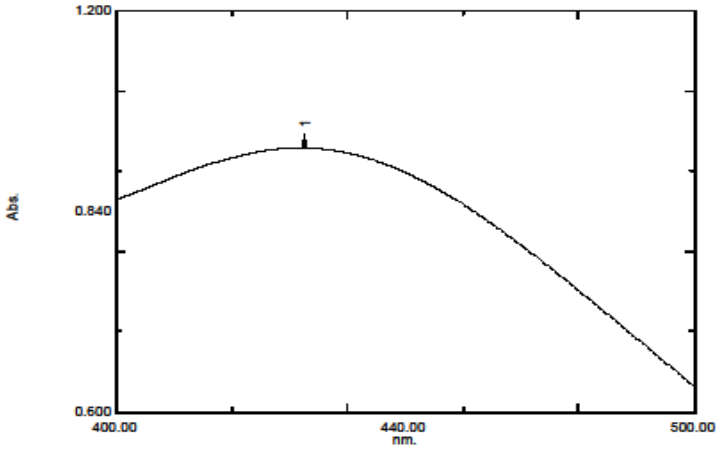

Figure 3: UV-Vis absorption spectrum of silver nanoparticle

The addition of lemon leaf extract to silver nitrate ($AgNO_3$) solution resulted in color change of the solution from transparent to brown due to the production of silver nanoparticles. The colour changes arise from the excitation of surface plasmon vibrations with the silver nanoparticles. The SPR of silver nanoparticles produced a peak centered near 432.50 nm. UV–Vis absorbance of the reaction mixture was taken from (Fig. 4). It was observed that the absorbance peak was centered near 0.994 nm, indicating the reduction of silver nitrate into silver nanoparticles. It was also observed that the reduction of silver ions into silver nanoparticles started at the start of reaction and reduction was completed at almost 24 hours at room temperature, indicating rapid biosynthesis of silver nanoparticles [10].

Size and Morphology of Silver Nanoparticles

SEM analysis finally confirmed the synthesis of spherical and polydispersive Ag NP_S in the reaction mixture.

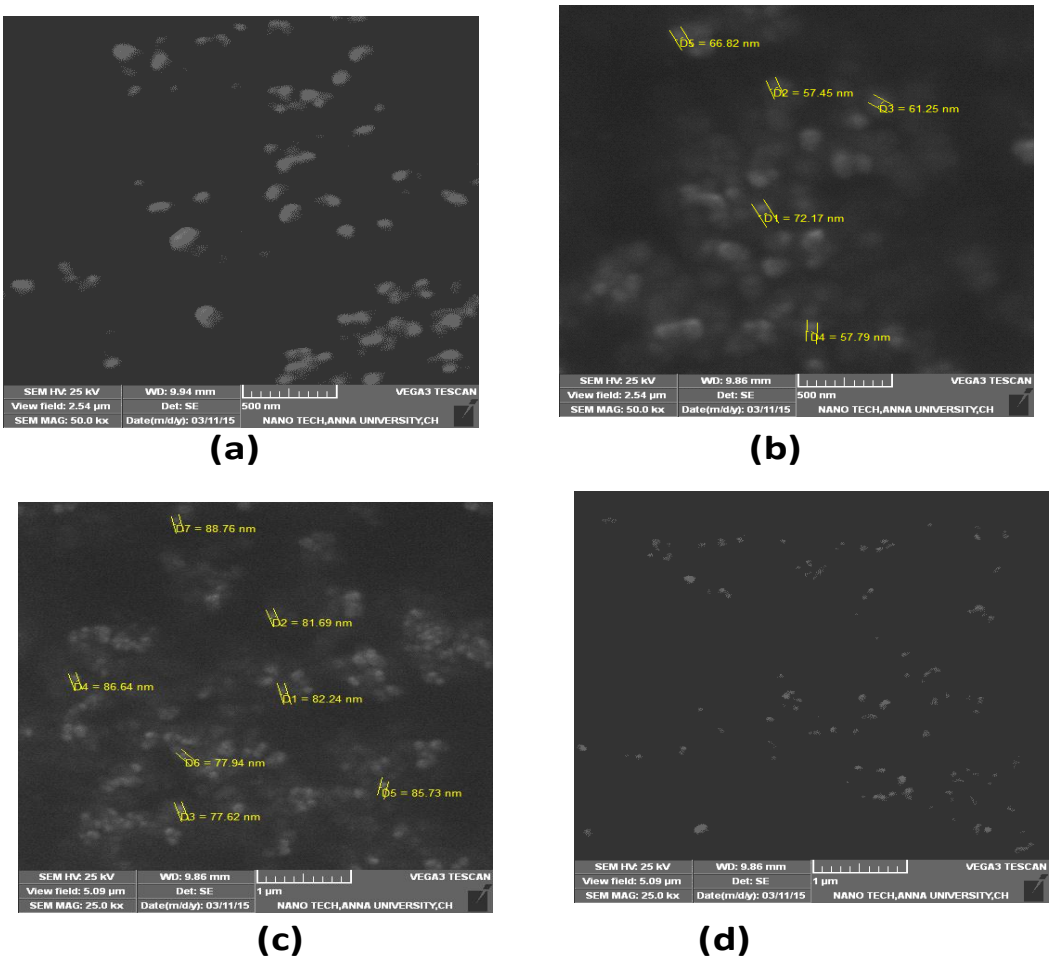

Figure 4: SEM Image of silver nanoparticle

The SEM micrographs of the sample is shown in the Fig.4. The SEM pictures of silver nanoparticles are shown in resolution with scale bar of 500 nm. The particle size distribution of silver nanoparticle of sample is shown in the Fig.4. (b) and (d). This shows the size of the spherical silver nanoparticles is around 60-80 nm in diameter. The silver nanoparticles are associated with one another and forms clusters. It leads to agglomeration of silver nanoparticles after 24 hrs.

Particle Size Analysis

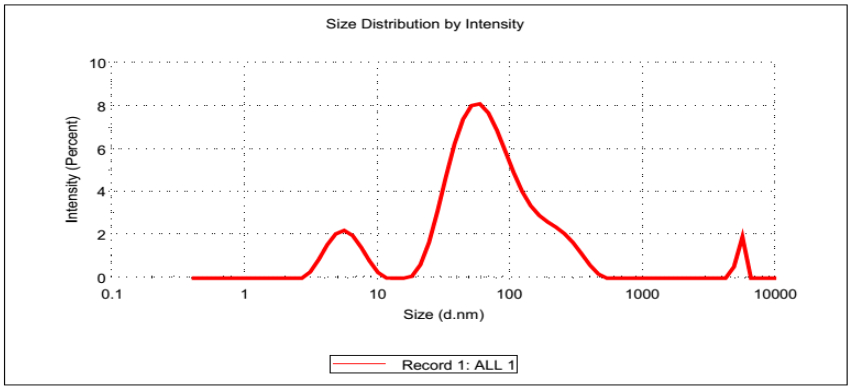

Figure 5: Particle size analysis of silver nanoparticles

The particle size analysis shows that the average size silver nanoparticles is 100 nm as shown in Fig.6. This may be because of translational diffusion coefficient, which will not depend only on the size of the particle 'core', but also on any surface structure, as well as the concentration and type of ions in the medium. This means that the size will be larger than measured by electron microscopy, for example, where the particle is removed from its native environment. It is important to note that dynamic light scattering produces an intensity weighted particle size distribution, which means that the presence of oversized particles can dominate the particle size result.

Anti-bacterial studies

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against pseudomonas and proteus by disk diffusion method (Fig.6). It was observed that microbial growth of pseudomonas and proteus was independent on AgNPs concentration.

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of silver nanoparticles that inhibited the visible growth of *E. coli*. It was found that the MIC (µg/l) for silver nanoparticles is 0.5, for silver nitrate 1.8 mg, and for standard antibiotic 0.6 mg. The zone of inhibition of pseudomonas ranged from 20-24 mm and proteus ranged from 4-6mm [5,6,9].

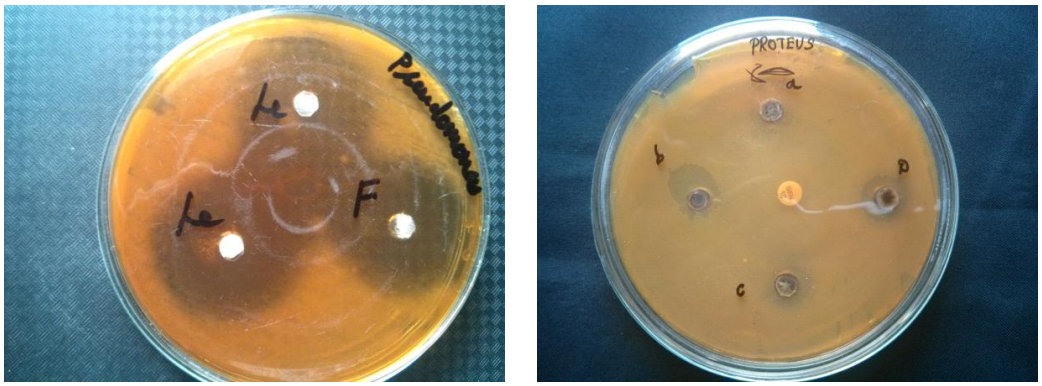

Figure 6: (a) Antibacterial activity of pseudomonas (b) Anti bacterial activity of proteus

Conclusion

We have developed a fast, eco-friendly, and convenient green method for the synthesis of silver nanoparticles from silver nitrate using Lemon leaf extract at ambient temperature. Lemon leaf extract is found suitable for the green synthesis of silver nanoparticles within 24 hours at ambient conditions. Spherical, polydisperse AgNPs of particle sizes ranging from 10 to 100 nm with an average size of 50 nm are obtained. Color changes occur due to surface plasmon resonance for the silver nanoparticle which is confirmed by UV–Vis spectroscopy. Particle size analyzer and SEM image confirmed the size of the silver nanoparticle. Biomolecule responsible for the bioreduction process. The antibacterial activity of biologically synthesized silver nanoparticles was evaluated against Pseudomonas and proteus.

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