GREEN SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL OF SILVER NANOPARTICLES

Claudia Ionela DRAGAN[1], Cristian PANTILIMON[2], Elisabeta Elena POPA[3], George COMAN[4], Catalin GRADINARU[5]

The aim of this research was to present an eco-friendly method to prepare silver nanoparticles (AgNPs). AgNP was confirmed and characterized by ultraviolet-visible spectroscopy, scanning electron microscopy, fourier transforming infrared, x-ray diffraction and atomic force microscopy. More their antibacterial activity was examined against three bacterial, including the Aspergillus niger, Penicilium corylohilum and Aspergillus ochraceus. The antibacterial activity showed that the inhibition zone increased in proportion to the increase in the amount of solution added to the paper disks. The purpose of the study was to synthesis the AgNPs in order to obtain the best conditions necessary for high antibacterial effectiveness and potential application in water treatment.

Keywords: green synthesis, silver nanoparticles, antimicrobial,

1. Introduction

The wastewater treatment has become a major problem for the worldwide. The mainly responsible for contamination of water are non-biodegradable effluents such as heavy metals and organic materials which are generated from industries. Nanomaterials based on CoFe2O4, TiO2, Fe3O4, TiO2-Fe3O4, Pd/Ag have been developed and play an important role in applications including wastewater purification, catalysis and magnetic devices.[1-4] Several reports present oxide-based nanocomposite/hybrid materials composed of two or more components used for the removal of toxic metals ions such as Cr(VI), Zn(II), Pb(II), and Ca(II)[1, 3-7].

[1] Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: draganclaudiaa@yahoo.com
[2] Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: Cristi_pantilimon@yahoo.com
[3] University of Agricultural Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnology, e-mail: Elenush_68@yahoo.com
[4] Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: George.coman@ecomet.pub.ro
[5] Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: Catalin.gradinaru@ecomet.pub.ro

It is well known that to achieve higher potential to remove contaminants, several wastewater treatment processes have been combined (sedimentation, precipitation, coagulation, filtration, ion exchange, advance oxidation, adsorption and biological) [8]. However, sometimes resistant pathogens fail to be eliminated by wastewater treatment processes. Even if, there are chemical treatment such as chlorination, iodination, ozonation, UV and reverse osmosis which are applied to remove microbes, these present several disadvantages [9]. Consequently, due to their unique physical and chemical proprieties, a new generation of materials has attracted intensive interest in wastewater treatment [10-12]. It was attributed that silver nanoparticles (AgNPs) have a toxic effect on microbes. Several methods have been adopted for synthesis and different advantages and disadvantages presented each of them such as cost, stability, size distribution and application. Among several synthetic methods for AgNPs, a green chemistry shows much promise, which seem to be simple, rapid, eco-friendly and non-toxic. [13]

The aim of the research is the synthesis, characterization and tested of AgNPs using extract plant. The synthesis of AgNPs was confirmed by UV-VIS, ATR-FTIR, XRD, SEM and AFM. Furthermore, antibacterial efficacy against three bacterial, including the Aspergillus niger, Penicilium corylohilum and Aspergillus ochraceus was investigated. These results are encouraging for using AgNPs in a treatment process.

2. Experiment

2.1. Materials

All chemicals used in this experiment were analytical grade and used without further purification. To obtain silver nanoparticles, the silver nitrate solution interacts with extract plant. The leaves were collected and washed several times with distilled water and dried at 100°C. The extract plant solutions used for synthesis silver nanoparticles were prepared by using 2 g of dried leaves and 100 ml of distilled water were boiled 30 minutes until the color of the solution has changed. After the color of the solution was changed the extract was cooled and filtered through 0.45 µm filter. The collected extract was brought into contact with 0.1M AgNO3 and distilled water at a molar ratio of 1:1:8 under magnetic stirring and heating. After 60 min, the color of the solution was change from colorless to dark brown, and the formation of AgNPs was also confirmed by UV-VIS.

In order to observe details regarding morphology, distribution, purity, sizes of particles and topography and characteristic peaks, the nanoparticles have been characterized using XRD (X-ray diffraction), AFM (Atomic force microscopy), SEM (Scanning electron microscopy), FTIR (Fourier Transforming Infrared) and UV-VIS spectrometry.

2.2. Characterization methods

To observe purity and size of crystallites of the studied material, a Panalytical X'Pert PRO MPD X-ray diffractometer with intensity Cu–Kα radiation (λ = 1.54065 A) and 2θ range from 10 to 90° was used for obtaining XRD patterns. The morphology and structure of the materials were evaluated by SEM using a Quanta 450 FEG scanning electron microscope equipped with a field emission gun and a 1.2 nm resolution X-ray energy dispersive spectrometer with a resolution of 133 eV. The topography of the surface and the particles dimensions were determined by atomic force microscopy (AFM) (MultiView 4000SPM/NSOM). The functional groups of nanoparticles were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) and scanned in the range of 700–4500 cm-1 at a spectral resolution of 4 cm^{-1}. The obtained data were processed using specific data analysis software (Origin Pro 8.0). For the UV-VIS determinations, a Cintra 220 UV spectrophotometer from 300 to 900 nm was successfully used in order to identify the characteristic peaks for analyses solutions and materials suspensions.

2.3. Antimicrobial activity of silver nanoparticles

Antimicrobial activity was assessed by diffusion method against Penicillium corylophilum, Aspergillus ochraceu, Aspergillus niger. For further experimentation these fungi were grown on potato dextrose agar (PDA – dissolving peptone (4g/L, glucose (20g/L) and agar (15g/L) during 7-9 days at 25°C. Then, disc of 6 mm was made on agar medium and different concentrations such as 10, 20, 30, 40 µl of AgNPs were checked for antifungal activity. Incubation was done at 27°C for 7 days. The evaluation of the samples was performed by daily visual monitoring of mycelium growth and measuring the area of the inhibited region. Each experiment was repeated at least four times.

3. Results and discussion

In this research was reported the detailed study on synthesis of silver nanoparticles by extract plant. The reduction of the Ag^+ ions was visually highlighted by the colloidal solution brown color and monitored using the UV-VIS spectrophotometer (Shankar et al., 2004). Figure 1 shows the adsorption spectra of the AgNPs obtained from the reduction of Ag^+ ions using extract plant recorded in the range of 350 – 1000 nm at different reaction time. It can be observed that by fixing the amount of extract plant at 2 mL and increasing the reaction time from 5 to 60 min the spectra comprises a fast-increasing absorption at about 450 nm, which is characteristic for silver nanoparticles SPR band. Further increasing in the intensity of SPR showed broad band indicating agglomeration of

AgNPs or increase in particle size. The effect of the concentrations has investigated by fixing amount of extract plant as 2 mL and reaction time 1 hr. With increasing the concentrations from 1 to 100 mg/L, there is variation in λ_{max} values signifying the changes in particle size, due to change in concentration of ion metal. On increasing the amount of extract plant to 8 mL the intensity of SPR band has increased indicating the instability of the nanoparticles. From the above optimized conditions, the AgNPs have been synthesized, characterized and tested for antimicrobial activity.

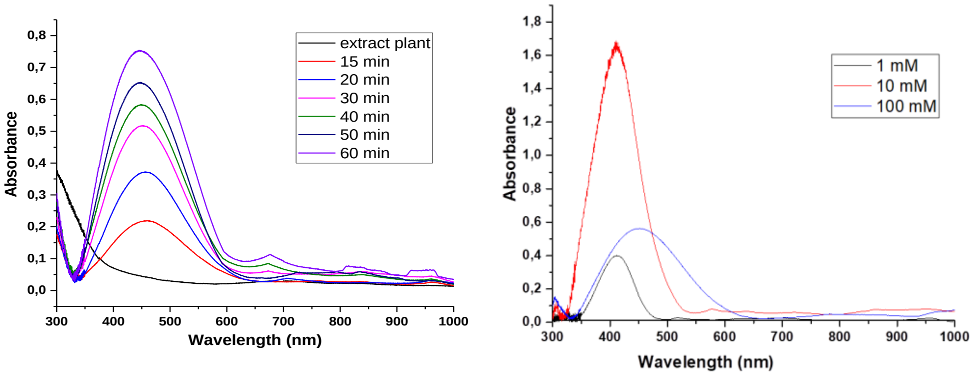

Fig.1 a) UV-VIS absorption spectra of AgNPs as a function of reaction time 100mM, b) UV-VIS absorption spectra of AgNPs as a function of concentrations from 1 to 100 mM with fixed amount of extract plant (2mL) after 30 min.

Fourier transform infrared spectroscopy (FTIR) was carried out to identify possible biomolecules responsible for the reduction of the silver ions. The functional groups were identified from ATR-FTIR spectrum recorded from extract plant in the 4000 – 700 cm^{-1} (Figure 4). Responsible for the reduction of metals ions using vegetable materials are the functional groups located at 1638 and 2101 cm^{-1}, which have been attributed to amine groups of protein. The peaks at 1638 cm^{-1} and 1344 cm^{-1} indicating the strong band of carboxylic acids and the medium bands of aromatic amines and alkyl [14]. ATR-FTIR results demonstrated that the biomolecules in the extract plant were responsible for the bioreduction and stabilization of silver nanoparticles

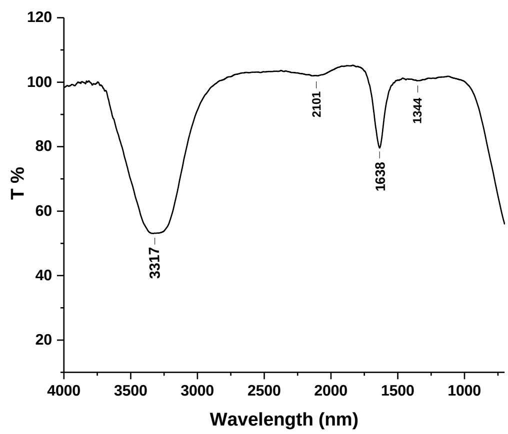

Fig. 4 ATR-FTIR spectra of AgNPs

Fourier transformation infrared (FTIR) and UV-Vis spectroscopy have been used to study the relationship between absorbance spectra and particle size distributions. The functional groups present over the silver nanoparticle surface may acts as agent for stabilization.

The morphology and crystallite structure of the silver nanoparticles using extract plant have been characterized by SEM and XRD analysis. Crystalline nature of AgNPs was identified using XRD analysis at 20-90°C. The diffraction peaks observed in Figure 2 represented the characteristic diffraction peaks at 38.16, 44.35, 64.51 and 77.42 corresponding to (111), (200), (220), (311) planes of face-center cubic. Furthermore, additional peaks were also observed at 32.27, 46.24, 81.52. These peaks are due to the organic compounds which are present the extract and responsible for silver ions reduction and stabilization of resultant nanoparticle [15]. The strong intensity diffraction peaks indicate that the AgNPs formed by reduction of Ag^+ ions by the extract plant are highly crystalline. The crystallite size of silver nanoparticles was estimated using the Debye-Scherrer's equation [16],[17]:

$$D \ ¼ \ K\lambda=\beta \cos\theta$$

Where λ is the wavelength of X - ray radiation, β is the full width at half maximum of the diffraction peak, θ is the Bragg angle of diffraction and K is Scherrer constant. The particles size correlates with the results obtained by SEM analyses.

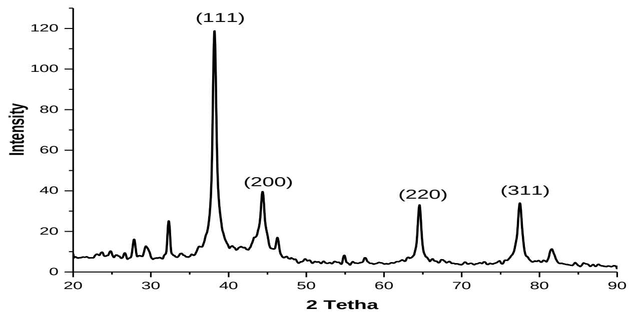

Fig. 2 XRD pattern of AgNPs

From XRD data using Debye-Scherrer's equation was approximately 85 nm, which indicated that the AgNPS synthesized by green method were nanocrystalline.

Morphology and size of synthesized AgNPs was analyzed through SEM imagine. Figure 3 showed relatively spherical shape with a little bit agglomeration and an average grain size distribution in the range of 70 – 110 nm. It can be observed that the agglomeration was formed from small nanozied crystals. These nanosized crystals were also evidenced by the crystallite size calculation from XRD.

The purity of material was examined by energy dispersive X-ray spectroscopy (EDS) (figure 3 which confirms the presence of AgNPs. The typical optical absorption peak of silver nanocrystals generally was shown at approximately 3 keV due to the surface plasmon resonance, which confirms giving that silver has been correctly identified [18].

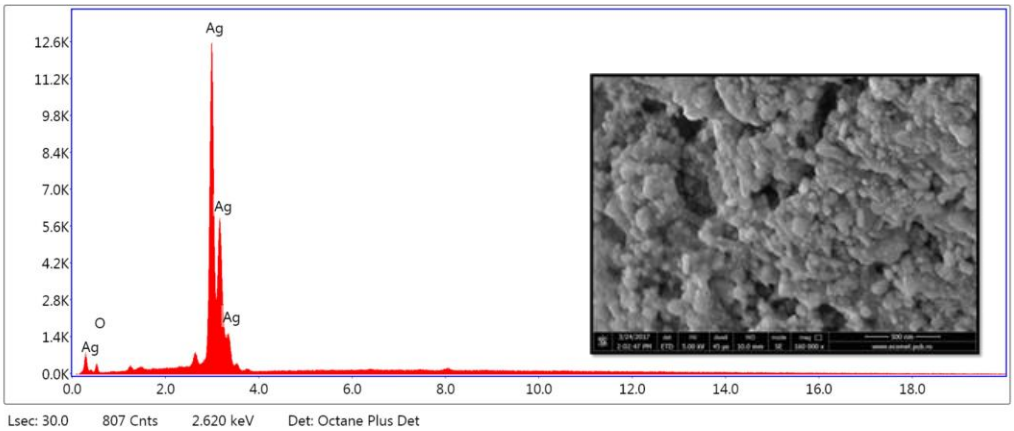

Fig. 3 EDS spectrum of the silver nanoparticles.The inset shows the SEM micrograph of silver nanoparticles

Surface topology of the synthesized AgNPs was studied by 3.8 x 3.8 μm atomic force microscopy (AFM) analysis figure 5. The topography of AFM micrographs indicates that the AgNPs possess spherical shape, the average particle size of 80 nm and agglomerations.

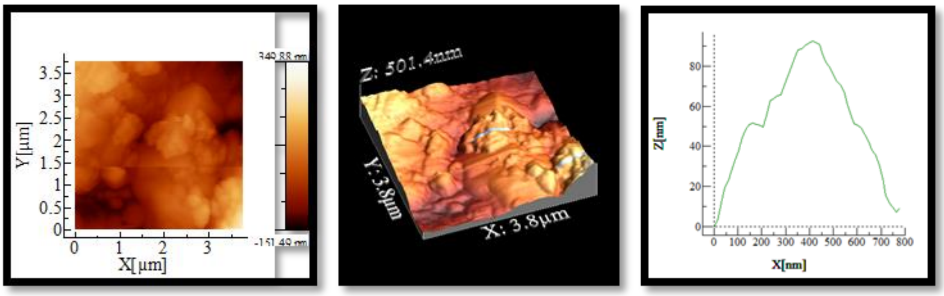

Fig. 5 AFM images and size distributions of AgNPs synthesized

Silver nanoparticles were analyzed for their antimicrobial activity against Aspergillus ochraceu followed by Penicillium corylophilum and Aspergillus niger. The higher levels of inhibition were obtained for Aspergillus ochraceus and Penicillium corylophilum compared to the results obtained for Aspergillus niger. It can be noticed that increasing the concentration of silver nanoparticles the inhibition zone is increasing also. The results showed that all samples have a value of inhibition zone above 10 mm which present a very good activity. Figure 6 shows the Zone of Inhibition of AgNPs with different concentrations against Aspergillus ochraceu, Penicillium corylophilum and Aspergillus niger.

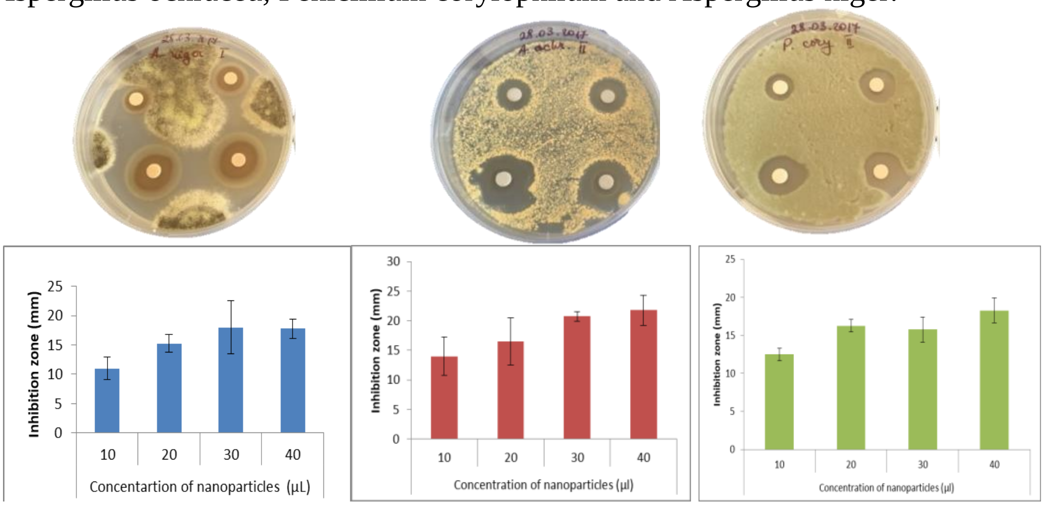

Fig. 6 Zone of Inhibition of AgNPs with different concentrations against Aspergillus ochraceu, Penicillium corylophilum and Aspergillus niger.

6. Conclusions

The aim of this study was to develop a fast and eco-friendly method for the synthesis of silver nanoparticles from extract plant. The morphology, distribution, purity, sizes of particles and topography and characteristic peaks of the silver nanoparticles have been characterized using XRD (X-ray diffraction), AFM (Atomic force microscopy), SEM (Scanning electron microscopy), and UV-VIS spectrometry. The results for the XRD pattern are correlated with the results obtained by SEM analyses. The antimicrobial activity of silver nanoparticles was demonstrated by the zone inhibition against Aspergillus ochraceu, Penicillium corylophilum and Aspergillus niger. According to these results, the green method of silver nanoparticles has high potential application in water treatment.

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