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Green Synthesis of Silver Nanoparticles: Estimation of their Antimicrobial Activity and their Electrochemical Fabrication on FTOs

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Abstract

The research project focused on fabricating the conducting Fluorinated Tin Oxide (FTO) glass with green synthesized metallic nanoparticles (NPs) of silver. Stable and size-controlled metallic nanoparticles of silver were synthesized by using lemon leaf extracts. One-pot, clean, and eco-friendly synthesizing strategy led to spherical-shaped metallic nanoparticles having a particle size range between 5–50 nm. Characterization techniques like SEM, XRD, and Particle size analyzer revealed the synthesis of well-formed spherical nanoparticles. In UV-Visible spectra, the plasmonic peaks of silver NPs in regions between 400-450 nm also confirmed the formation of NPs. FTO plates were fabricated by depositing fine and uniform layers of nanoparticles, using the electrochemical method. These silver NPs were successfully used for electro-catalytic water splitting and showed a low onset potential ~1.5V at room temperature. Silver NPs showed moderate bactericidal activity against Gram-positive and Gram-negative bacteria.

Keywords: FTO, Electrode Fabrication, Silver nanoparticles, Electrochemical deposition, Green Synthesis.

Introduction

Global energy demand has grown inexorably in the last decades due to enormous industrial development and population growth. Abundant usage of natural energy resources like coal, natural gas, and petroleum products not only led to a depletion of these energy resources but also contributed a huge part to environmental pollution and global warming. Environmental pollution is touching alarming levels so now there is a need to switch to alternate energy resources, definitely to water fuels. Water comprises 70% of our earth's parts and is one of the best options for energy purposes. Water splitting produces H_2 gas which can be used for fuel purposes. It contains three times more energy as compared to natural gas combustion along with no by-products hence no more pollution.

Abundant availability and no by-product formation during the combustion of water fuels make it one of the best choices as an energy resource. There are many ways to split water into H_2 and O_2 like electrolysis, thermal decomposition, chemical production, etc. [1-9]. However electrochemical and photoelectrochemical (PEC) methods are better methods and are mostly used nowadays [10-12]. Nanotechnology is one of the enchanting technologies and has attracted a number of scientist's research interest due to versatile material development of desired

properties at the nano level [13]. Nanoparticles (NPs) of different metals are successfully being used for water splitting nowadays [14-17].

There are many approaches to synthesize NPs but the biological method is one of the environment-friendly and non-toxic methods of nanoparticle formation [18]. In chemical methods, many toxic chemicals are used so these methods are expensive and also impart toxic environmental effects. Among all biological methods, NPs formation using plant leaf extract is an easier, controllable, and cheaper method as compared to other chemical methods [19]. Different parts of plants like leaves, roots, and stems can be used for NPs synthesis as they contain different phytochemicals that can be used as reducing and capping agents [20]. NPs of different metals are synthesized by green methods and fabricated on different substrates for water splitting purposes [21].

Transition metals dichalcogenides are being used for active oxygen production at a low over-potential of 232 mV [22]. Manganese based mesoporous NPs and Microwave assisted Ag/AgO NPs are also tested for oxygen production at over-potentials of 480 mV and 450-600 mV, respectively, by electrocatalytic method [23-24]. Magnetic nanocomposites of transition metals (size 50-60 nm) prepared by ion doping and fabricated on fluorinated tin oxide (FTO) showed enhanced water catalytic activity [25]. Recently, magnetically recyclable nanocomposites of manganese, silicone, and silver have been used for the reduction of dye pollutants, which has a very sustainable effect on the environment [26]. Greener methods are used to synthesize nickel NPs with plant leaf extracts and different metal alloys showed outclass applications as electrochemical sensors, catalysts, antibacterial agents, and catalytic clean energy conversion [27-29].

Materials and Methods

Preparation of Plant Leaves Extracts

Leaves of lemon *(Citrus limon)* were taken from Lahore, Pakistan, and verified by the Botany Department, University of the Punjab, Lahore, Pakistan. These leaves were washed with distilled water and shade-dried. 20 grams of plant leaves were cut into small pieces and soaked in 20% ethanol solution for 10 days. Then leaf extract was double filtered and stored in air tight bottles at 0 °C.

Metal Solutions and NPs synthesis

1 mM solution of silver chloride (AgCl) (Sigma Aldrich 99.8% purity) was prepared in double distilled water. Plant extract was first centrifuged at 5000 rpm for 30 minutes and then 10 mL extract was pipetted out in a conical flask. 180 mL of metal solution was added dropwise and the solution was stirred for two hours at room temperature. A clear change in the color of the plant extract solution was observed after adding AgCl solution thus exhibiting AgNPs formation.

Deposition of NPs on FTO

FTOs were purchased from Dawn Scientific Company Lahore (having surface resistivity~10 Ω/sq). Metallic NPs were deposited from the solution by cyclic voltametric method using Potentiostat (PGSTAT-30 from Metrohm Auto Lab). 100 scans of each voltametric cycle were done with a scan rate of 0.02, voltage between +0.5V to -0.5V, and NPs solution was taken as electrolyte. A very fine and even layer of AgNPs was coated on FTO plates which were further subjected to water splitting investigations. AgNPs gave a dark brown color layer on FTO after fabrication (Fig. 1).

Figure 1. **FTO fabricated with AgNPs**

Results and Discussion

Hydrodynamic Volume Analysis

Hydrodynamic volume analysis of NPs was carried out by using a Particle size analyzer (Anton Paar Litesizer 500, using DLS Technology, λ=658 nm). AgNPs with 47 nm hydrodynamic volume were obtained on reduction with lemon leaf extracts (Fig. 2).

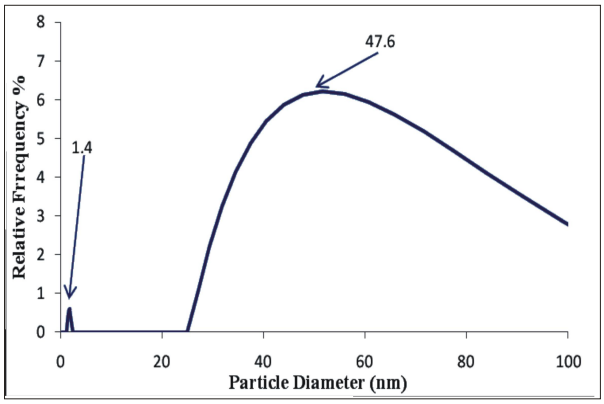

Figure 2. **Hydrodynamic volume of AgNPs different plant leaf extracts**

Singh et al., and Torabfam and Yuce [30,31] synthesized AgNPs using *Symphytum officinale* leaf extract and microwave reduction of silver nitrate solution using an algal source, *Chlorella vulgaris,* respectively. The zeta potential showed the negative surface charge (-25.5 mV) and (-17 mV) of AgNPs, respectively. AgNPs exhibited good photoaging and antibacterial properties.

Kaur and Joya studied the interaction and binding of plasma proteins with AgNPs. DLS measurements revealed that the average particle hydrodynamic diameter (z-average) increased after the incubation of AgNPs with plasma [32].

SEM Analysis

The particle size and shape were determined by Scanning Electron Microscope (SEM) (TESCAN VEGA3). SEM images of AgNPs with lemon leaf extracts (Fig. 3), show the formation of nano-sized spherical cluster-shaped AgNPs sized between 5-50 nm.

Previously many authors have synthesized AgNPs by a green method using Royal Jelly extract, Grape Pomace extract, and Alovera extract. They reported that spherical cluster-shaped AgNPs having size 20-100 nm were obtained and showed promising antibacterial properties [33-36].

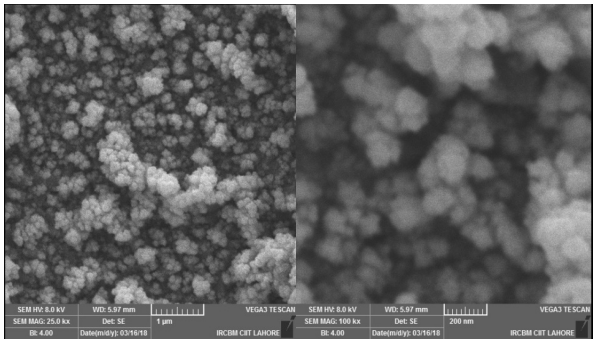

Figure 3. **SEM images of AgNPs with lemon leaves extracts**

UV-Vis Analysis

Optical absorbance spectra of NPs were measured using an Agilent UV-Visible Spectrophotometer (Carry 60 UV-Vis). NPs of silver showed higher absorption peaks in the 400-450 nm region as compared to pure plant leaf extracts which clearly indicates the reduction and formation of NPs after mixing metal solution and leaves extract. The UV-Visible spectra of AgNPs with lemon leaf

extract clearly shows a strong absorption peak between 400-450 nm which is a characteristic region for AgNPs as reported in literature [36] (Fig. 4).

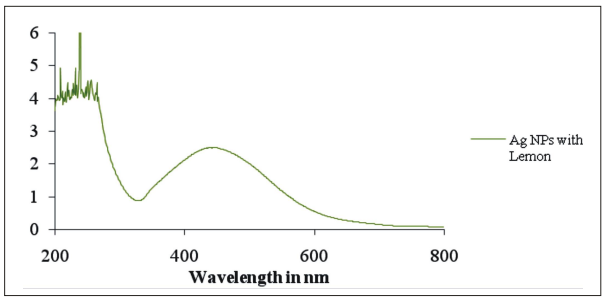

Figure 4. **UV-Visible spectra of AgNPS with lemon leaf extracts**

FT-IR Investigations

Functional group studies were carried out using FTIR Agilent Carry 630. The peak at 3450 cm^{-1} and 1650 cm^{-1} corresponds to N–H stretching and bending vibrations, respectively in amines from proteins of plants. While stretching vibrations of the O–H bonds at 3250 cm^{-1} (alcohols and phenols) and C–H bonds at 2900 cm^{-1} arise from plant metabolites and C=O at 1700 cm^{-1} peak, arise from carboxylic acid. Two peaks at 1650 cm^{-1} and 1500 cm^{-1} correspond to C=C stretching vibrations from aromatic rings, present in plant metabolites. Three peaks, viz. 1200 cm^{-1}, 1090 cm^{-1}, and 1020 cm^{-1} correspond to carboxylic acid, ester, and C–O stretching from alcohol; allowing functional groups of metabolites and proteins covering the AgNPs [37] (Fig. 5).

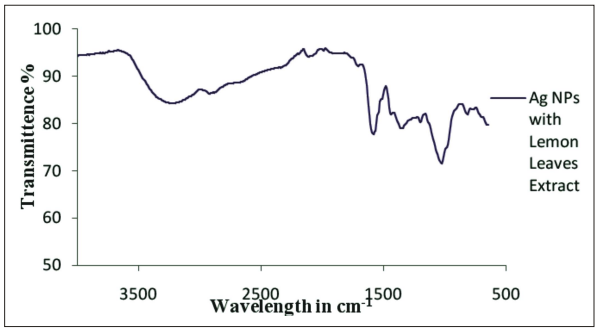

Figure 5. **FT-IR spectra of AgNPs with lemon leaf extracts**

Electrochemical Studies

Electrochemical and cyclic voltammetric measurements were investigated in a three-electrode configuration cell using Potentiostat (PGSTAT-30 from Metrohm AutoLab). AgNPs thin films were used as working electrodes, Ag/AgCl (3 M KCl) was used as the reference electrode, and a Platinum plate of 1 cm^2 was used as a counter electrode. Cyclic voltametric analysis of fabricated FTOs was done under a basic environment 0.1 M KOH (pH=11) using Ag/AgCl and Platinum electrodes as a reference and counter electrode, respectively. FTOs fabricated with NPs of silver showed higher O_2 generation at the anode. Graphs clearly show the low onset potential for water splitting AgNPs (Fig. 6) hence more water reducing properties as compared to the blank FTO. The FTO without AgNPs showed no water splitting activity at potential 0-1.6 V. AgNPs with lemon leaf extract showed low onset oxidation potential (~1.5 V) as compared to literature [38].

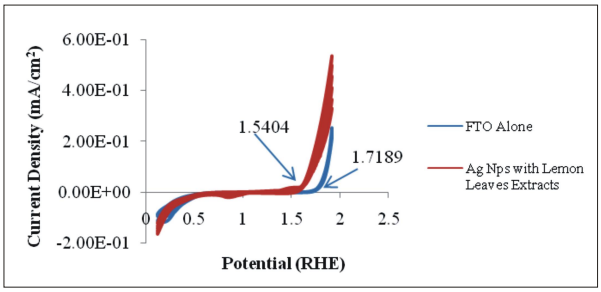

Figure 6. **Cyclic voltametric analysis of AgNPs with lemon Leaves extract**

X-Ray Diffraction

In x-ray diffraction spectra, four peaks were identified at 37.76°, 44.36°, 65.55°, and 77.38° related to planes (111), (200), (220), and (311), respectively. The unassigned five peaks are due to some bio-organic impurities present in the sample. The mean grain size of the synthesized silver nanoparticles measured from XRD data using the Debye-Scherrer

equation was 32.15 nm. The morphology and crystallite nature of AgNPs were found to be cubic close packed (ccp), also recognized as face centered cubic (fcc) lattice. This confirms the crystalline nature of AgNPs. The principle surface morphology was silver, and the other phases evident as contaminants were also recorded in the XRD analysis (Ag XRD Ref. No. 02-088-0125). It could be concluded from the XRD pattern that the green synthesized AgNPs contain both phases crystalline and amorphous.

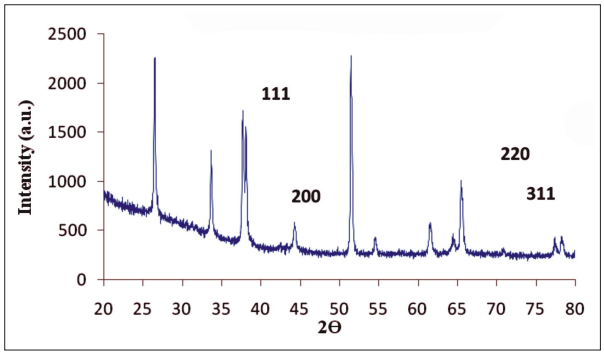

Figure 7. **XRD graph of AgNPs with lemon leaf extracts**

Antibacterial Activity

In literature, Silver nanoparticles are reported using various reducing agents like; *Symphytum officinale* leaf extract, algal source; *Chlorella vulgaris*, Royal Jelly extract, Grape Pomace extract and Alovera extracts. AgNPs synthesiszed from all above said materials showed very prominent antibacterial activity [30-36].

In this research, antimicrobial activity of AgNPs was estimated using Agar Disc Diffusion Method using bacteria *Pseudomonas aeruginosa* (Gram-) and *Bacillus subtilis* (Gram+) under ambient conditions (72 hours incubation). Antimicrobial results revealed that pure extracts of all plant leaf extracts showed no antibacterial activity but the AgNPs with Sapodilla, Banyan, and Jujube leaf extracts showed maximum resistance against gram-positive bacteria (Table 1) and gram-negative bacteria (Fig. 8).

Table 1. **Comparison of antibacterial activity of AgNPs against Gram- and Gram+ bacteria.**

NPs with different Plant Leaves Extract	Gram Positive (*Bacillus subtilis*) ZOI (mm)	Gram Negative (*P. aeruginosa*) ZOI (mm)
Sapodilla Leaves Extract	12	13
Banyan Leaves Extracts	11	10
Lemon Leaves Extracts	10	13
P. Lime Leaves Extracts	10	12
Jujube Leaves Extracts	11	11

*ZOI (zone of inhibition)

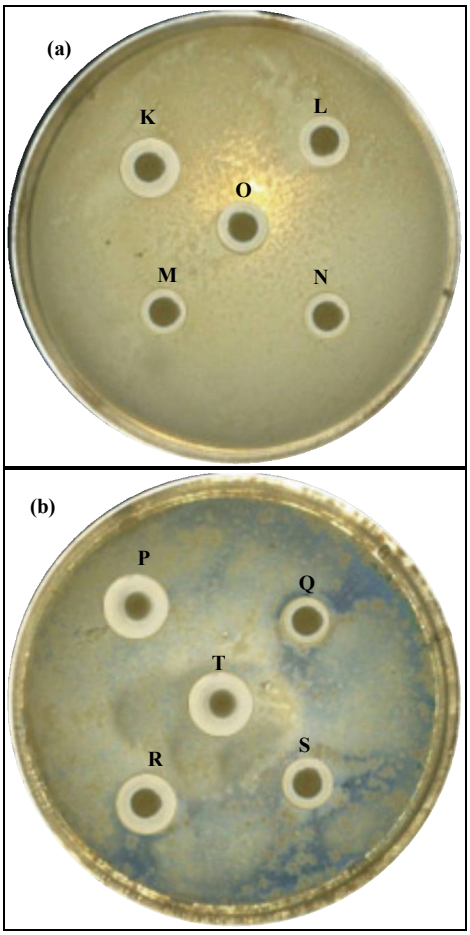

Figure 8 (a). **(K, L, M, N, O); AgNPs with plant leaves extracts of Sapodilla, Banyan, P. Lime, Lemon, and Jujube, respectively on gram positive bacterial medium (*Bacillus subtilis*) *(b).* (P, Q, R, S, T); Ag NPs with plant leaves extracts of Sapodilla, Banyan, P. Lime, Lemon and Jujube, respectively on gram negative bacterial medium (*Pseudomonas aeruginosa*)**

Conclusion

Silver nanoparticles were successfully synthesized by using leaf extracts of different plants. The electrochemical method of deposition gave fine layered fabrication of NPs on FTOs. NPs prepared by lemon (*Citrus limon)* leaf extracts gave optimal nano-sized particles that can be efficiently used for water splitting. AgNPs with lemon leaf extract reported a low onset potential of ~1.5V. AgNPs also showed little bactericidal activity against Gram+ and Gram- Bacteria and hence can be used in medicinal areas. The method and process for the synthesis of these NPs are very simple, one-pot, and convenient.

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Conflict of Interest

The authors declare no conflict of interest.

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