

Full Paper

Synthesis of Silver Nanoparticles and their Applications

Shashanka Rajendrachari^{1,2}, Bahaddureghatta E. Kumara Swamy^{2,*}, Sathish Reddy² and Debasis Chaira¹

¹*Department of Metallurgical and Materials Engineering, National Institute of Technology Rourkela-769008, India*

²*Department of P.G .Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta 577451, Shimoga, Karnataka, India*

*Corresponding Author, Tel.:+91 8282 256225; Fax: +91 8282 256255

E-Mail: kumaraswamy21@yahoo.com

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Abstract- Silver nanoparticle was synthesized by using acacia melanoxyton fresh leaves extract. The obtained silver nanoparticle was characterized by using X-ray diffraction (XRD), infrared absorption spectroscopy (IR), UV-Vis absorption spectroscopy (UV-Vis) and Zeta-potential analysis. The prepared silver nanoparticle/carbon paste electrode (AgNps/CPE) exhibits diffusion-controlled process and enhanced electrochemical sensing property for the detection of uric acid in presence of dopamine at physiological pH. The antibacterial property was checked the prepared silver nanoparticle is also shows good antibacterial property.

Keywords- Silver Nanoparticle, Dopamine, Uric Acid, Acacia Melanoxyton, Cyclic Voltammetry

1. INTRODUCTION

Silver nanoparticles (AgNPs) egressed as a boon product from the field of nanotechnology due to its good conductivity, chemical stability, catalytic and antibacterial activity [1-3]. In recent years, biological synthesis of nanoparticles especially from plant extract is gaining importance due to its simplicity, non-toxic, cost effective and eco-friendly in nature. There were many gold and silver nanoparticles from different plants has been

reported they are Alfalfa [4,5], Aloe vera [6], Cinnamomum camphora [7], Emblica officianalis [8], Carica papaya [9], Eucalyptus hybrid [10], Capsicum annum [11] and tamarind [12]. Acacia melanoxylon is one among the above plants whose leaves are used to synthesis silver nanoparticles.

Uric acid (2,6,8-trihydroxypurine, UA), a major nitrogenous compound in urine, is a product of purine metabolism in human body and its higher levels leads to many clinical disorders [13]. High levels of UA in the blood (hyper uricemia or Lesch-Nyhan syndrome) are linked with the body disorders like gout, kidney and cardiac problems. Many epidemiological studies have suggested that elevated serum UA is also a risk factor for cardiovascular disease [14-19] and dopamine (DA) is one of the very important neurotransmitter [20]. In brain fluids UA is present in relatively high concentration (1-50 μM), while dopamine level are of the order of 100 nM [21-23]. Hence, to explore a simple, accurate and reliable method for the determination of uric acid in presence of interference became an urgent need. Electrochemical methods were commonly accepted as the fastest analytical assay compared with traditional spectroscopic [24], chemiluminescence [25,26], chromatographic [27], spectrofluorometric [28], colorimetric [29,30], electrophoretic [31,32] methods.

In order to increase the sensitivity and selectivity, various oxides such as CuO, ZnO, $\text{Mg}_2\text{Fe}_2\text{O}_4$, CdO, NiO/ZnO hybrid nanoparticles modified carbon paste electrodes are reported [33-37]. In the work, Silver nanoparticle was synthesized by using acacia melanoxylon fresh leaves extract and characterized using XRD, IR, UV-Vis and Zeta-potential analysis. The obtained results shows that silver nanoparticle/carbon paste electrode act as good electrochemical sensor for detection of UA in presence DA. In addition, there is a great need of finding new antibacterial agents against various pathogenic bacteria which causes some infectious diseases. Here prepared silver nanoparticles accomplish the need and acts as a promising antibacterial activity.

2. EXPERIMENTAL

2.1. Materials and Stock solution

Analytical grade dopamine hydrochloride (DA), uric acid (UA), sodium dihydrogen ortho phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), silver nitrate (AgNO_3) and silicon oil were procured from Himedia chemicals and Graphite powder (particle size μm) (Merck chemicals). All chemicals are used without any further purification. Dopamine stock solution (25 mM) was prepared by taking a known quantity of dopamine in 0.1 M perchloric acid, 25 mM uric acid solution was freshly prepared using 0.1 M NaOH solution. Phosphate

buffer (pH 7.4) was prepared as per the literature with 0.2 M NaH_2PO_4 and Na_2HPO_4 solution in double distilled water.

2.2. Characterization

The silver nanoparticle was characterized by powder XRD using $\text{Cu-K}\alpha$ radiation ($\lambda=1.5438 \text{ \AA}$) in a Philips XRD 'X' PERT PRO diffractometer. IR absorption spectra was recorded in a FT-IR SPECTRUM 1000 PERKIN ELMER spectrometer on thoroughly dried samples using KBr as dilutant, UV-Vis spectra was obtained using Perkin Elmer UV-Vis Spectrophotometer and Zeta potential was performed in Malvrin Zeta sizer. All the electrochemical experiments were carried out in a three-electrode cell with the bare CPE, silver nanoparticle/CPE as a working electrode an aqueous saturated calomel electrode (SCE) as reference electrode and a Pt wire as auxiliary electrode using an electrochemical work station Model 660c (CH Instruments).

2.3. Synthesis of silver nanoparticles

Ten grams of taxonomically authenticated, freshly collected healthy leaves of acacia melanoxydon were taken and washed thoroughly in water in the laboratory for 10 minutes in order to remove the dust particles. Fig. 1 shows the leaves of acacia melanoxydon plant and leaves are made into small pieces and rinsed in sterile distilled water. Then small leaves in to a 250 ml beaker containing 100 ml distilled water and boil the solution on a boiling steam bath for 5 to 10 min until the color of the solvent changes to light yellow. This extract was filtered through sterile filter paper and this solution was treated as source extract. The extract was stored at 5°C to inhibit the growth of fungus and utilized in subsequent procedures. The 5 ml of extract solution was treated with 50 ml of 0.01 M AgNO_3 solution and warmed again on the steam bath for 10 min until the color of the solution changes to reddish brown as shown in the Fig. 1b. Then it was allowed to cool and incubate in the laboratory environment.

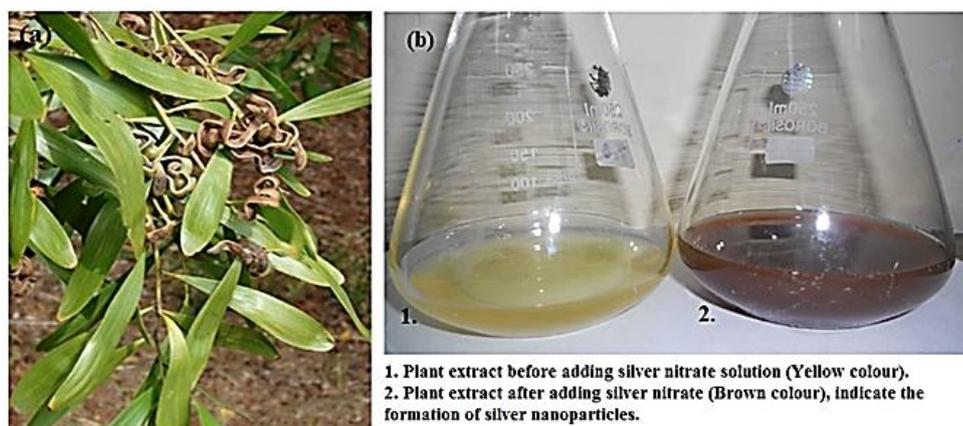


Fig. 1. (a) Acacia melanoxydon leaves (b) Colour change of the leaf extract before and after synthesis of Ag nanoparticles

2.4. Silver nanoparticles/carbon paste electrode

Carbon paste electrode (CPE) was modified by taking concentration of Ag nanoparticle (10-50 μL) in silicon oil (30%) and graphite powder (70%). This mixture was thoroughly mixed in an agate mortar for about 30 minutes and packed into a homemade Teflon cavity having a current collector and is polished by rubbing on a soft paper.

3. RESULTS AND DISCUSSION

3.1. X-Ray Diffraction study

Fig. 2 shows the XRD pattern of Ag nanoparticles confirms the crystalline characteristics. The diffraction peaks at angles 38.1° , 44.5° and 64.6° corresponds to facets (1 1 1), (2 0 0) and (2 2 0) respectively of the face centered cubic crystal structure. Among all the planes (1 1 1) plane is more intense and hence its width can be used to calculate the average crystal grain size using Scherer formula and it is found to be ~ 10 nm. With respect to XRD figure, among all the Bragg's peaks, two more but yet to be assigned peaks are there and are marked with stars. These unassigned peaks are due to the crystallization of bioorganic phase present in plant and it was reported by Shankar et al. [38] in Ag nanoparticles synthesized using geranium leaf extract. The intensities and positions of peaks are in good agreement with the reported values of Joint Committee of Powder Diffraction Standard (JCPDS) file.

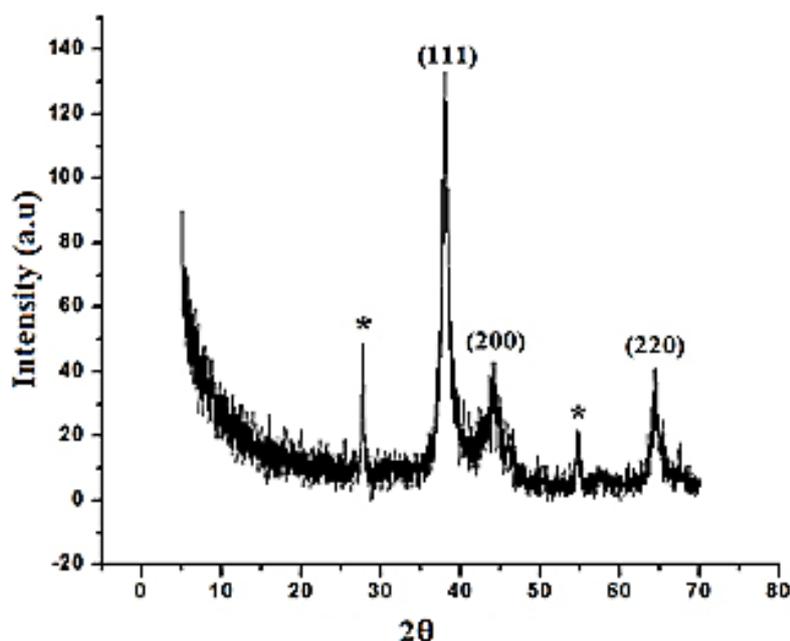


Fig.2. XRD of as prepared Ag nanoparticles

3.2. FTIR spectroscopy analysis

Silver nanoparticle prepared by acacia leaves does not requires any external capping agent, therefore FTIR measurement reveals the responsible factors which acts as capping agent and it also helps in identifying the possible interactions between silver and bioactive molecules. FTIR spectrum reveals two bands at 1573 and 1332 cm^{-1} that correspond to the bending vibrations of the amide I and O-H group of the proteins respectively [39] as shown in the Fig. 3. Absorbance band at 1021 cm^{-1} is due to stretching vibration corresponds to C-N group and the bands at 832, 723 and 683 cm^{-1} was due to bending vibrations. Absorbance band at 2926 cm^{-1} is associated with the C-H cm^{-1} stretching vibration. The bending vibration at 1332 cm^{-1} and broad absorption band at 3000-3500 cm^{-1} arises most probably from the O-H group of polyols such as hydroxyflavones and catechins [40].

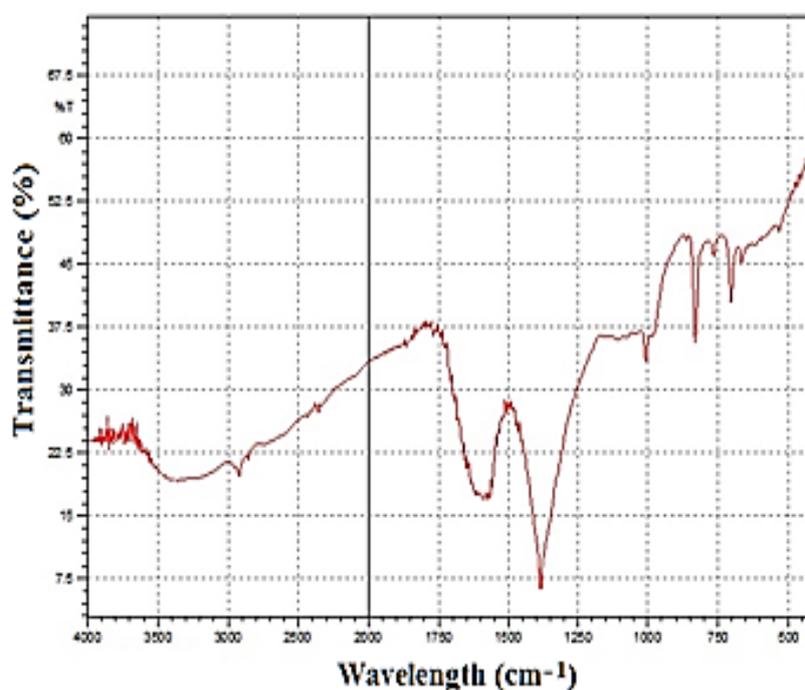


Fig. 3. FTIR Spectra of Ag nanoparticles

3.3. UV–Vis analysis of silver nanoparticles

UV–Vis spectroscopy is an important technique to ensure the formation of metal nanoparticles in aqueous solution. Colour of silver colloidal solution is wine red which is imputed to surface Plasmon resonance (SPR) arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field [9]. Fig. 4 shows the UV–Vis spectra of the silver nanoparticle obtained. The absorption peak is plausibly related

to the electronic transition taking place from valence band to the conduction band. Peak at 435 nm attribute to the formation of silver nanoparticles.

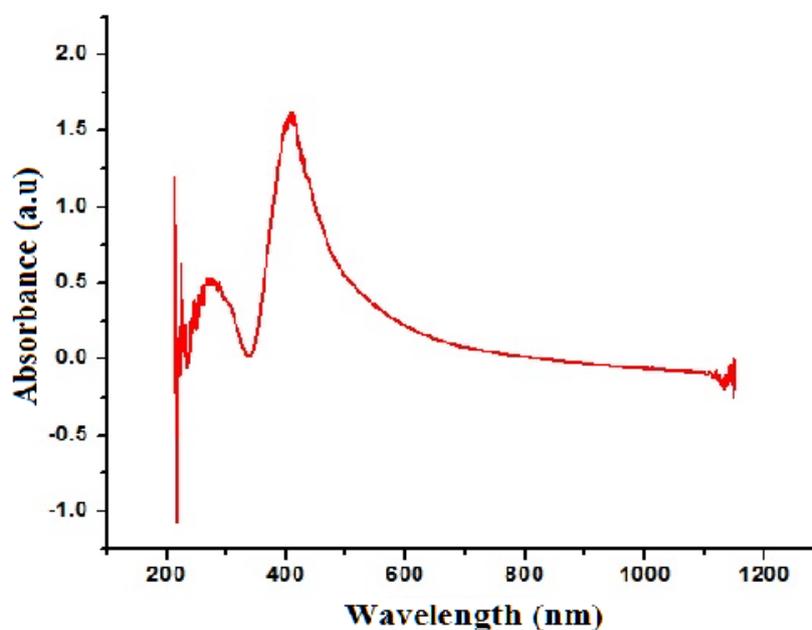


Fig. 4. Shows the UV–Vis spectra of the silver colloids obtained after 15 min of reaction

3.4. Zeta-potential analysis

Zeta-potential is the electrical potential developed at the solid–liquid interface in response to the relative movement of the nanoparticle and the solvent. In order to study the zeta-potential, the prepared silver nanoparticles were dispersed in double distilled water. The stability of nanoparticles depends upon the electrical potential and the surface charge; hence it is very important to know them. The surface potential of charged particle increases with increase in zeta potential. The zeta potential of prepared silver nanoparticle is -25.8 mV as shown in the Fig. 5.

3.5. Simultaneous determination of UA and DA using different concentrated silver nanoparticle /CPE

Simultaneous oxidation of both UA and DA was using carried out Ag nanoparticle /CPE in presence of PBS solution of pH 7.0 at a scan rate 100 mVs^{-1} using cyclic voltammetric technique. The Ag nanoparticle as modifiers enhances both the peaks of UA and DA as shown in the Fig.6a. Out of 10, 20, 30, 40, and 50 μl of silver nanoparticle, maximum oxidation peak current for 30 μl was obtained as shown in Fig. 6b plot of anodic peak current of UA and DA against concentration of Ag nanoparticles. Therefore 30 μl of silver nanoparticle/CPE was used for the determination of UA and DA simultaneously. UA and DA oxidize at an anodic peak potential of 314.8 mV and 205.1 mV respectively. Silver

nanoparticle/ CPE show an enhancement of current signals for the oxidation of both UA and DA (solid line) when compare with bare CPE (dashed line) shown in the Fig. 6a.

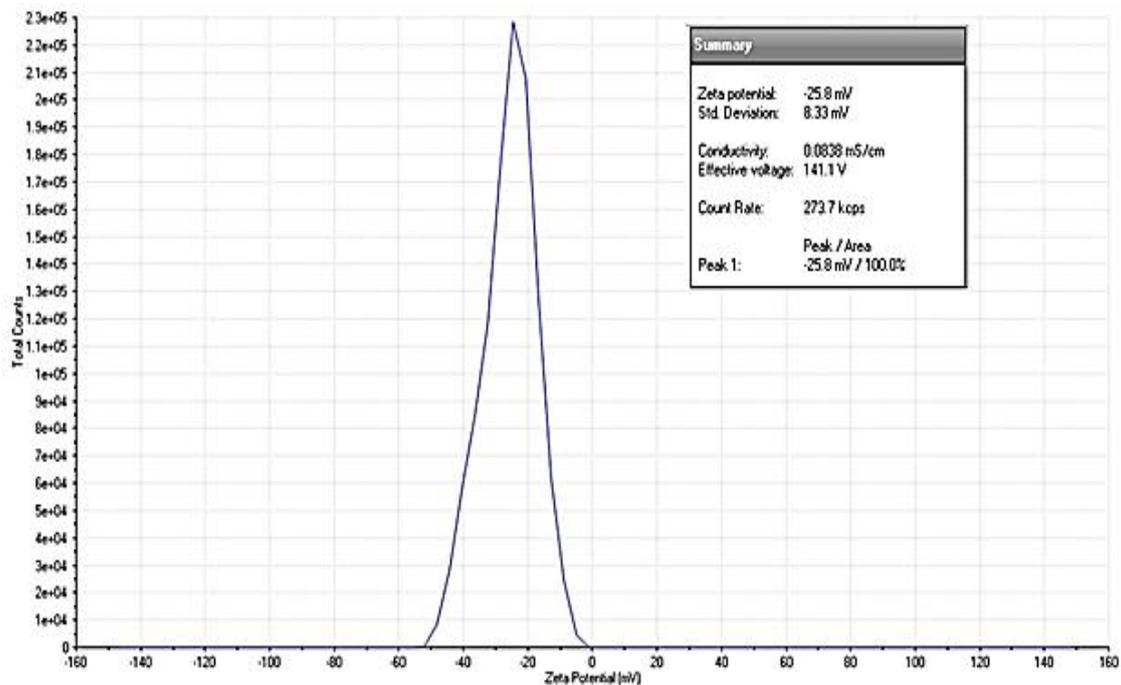


Fig. 5. Zeta potential of as prepared Ag nanoparticles

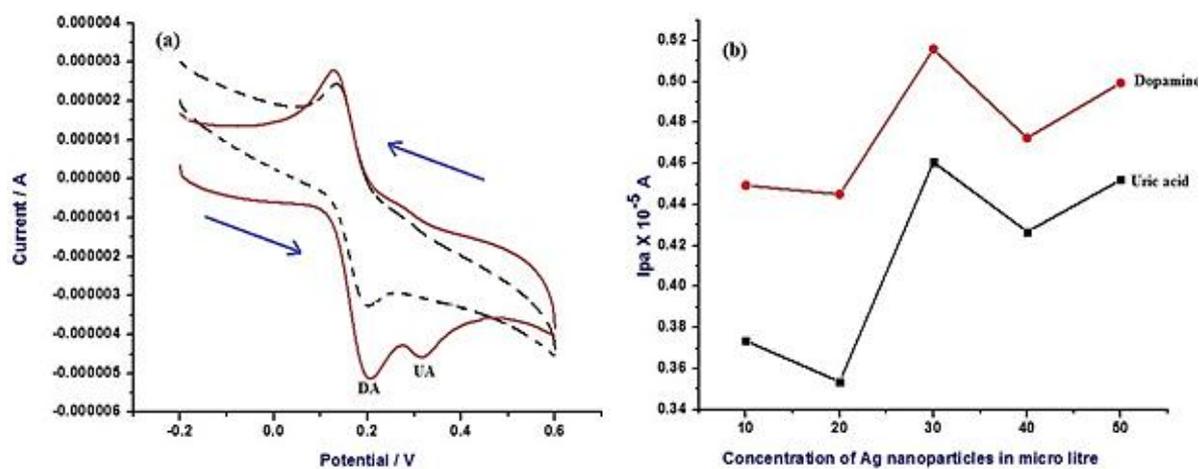


Fig. 6. (a) Cyclic voltammogram of BCPE and Ag nanoparticle MCPE for dopamine and uric acid (b) Plot of the concentration variation of silver nanoparticles from 10 to 50 μl vs. Anodic peak current at 0.1 V s^{-1} to determine dopamine

3.6. Effect of scan rate

Cyclic voltammograms of UA and DA were registered simultaneously for different scan rates. Fig.7a shows scan rate has a great effect on the peak current of UA and DA using 30 μ l of Ag nanoparticle/ CPE. The anodic peak currents of both UA and DA were increased with increase in scan rate from 100 to 700 mVs⁻¹. A plot of anodic peak current (I_{pa}) of UA and DA vs. square root of scan rate showed linear relationship as shown in Fig.7b. The correlation co-efficient of UA and DA were found to be 0.9997 and 0.9998 respectively. These results showed the electrode reaction process was diffusion controlled.

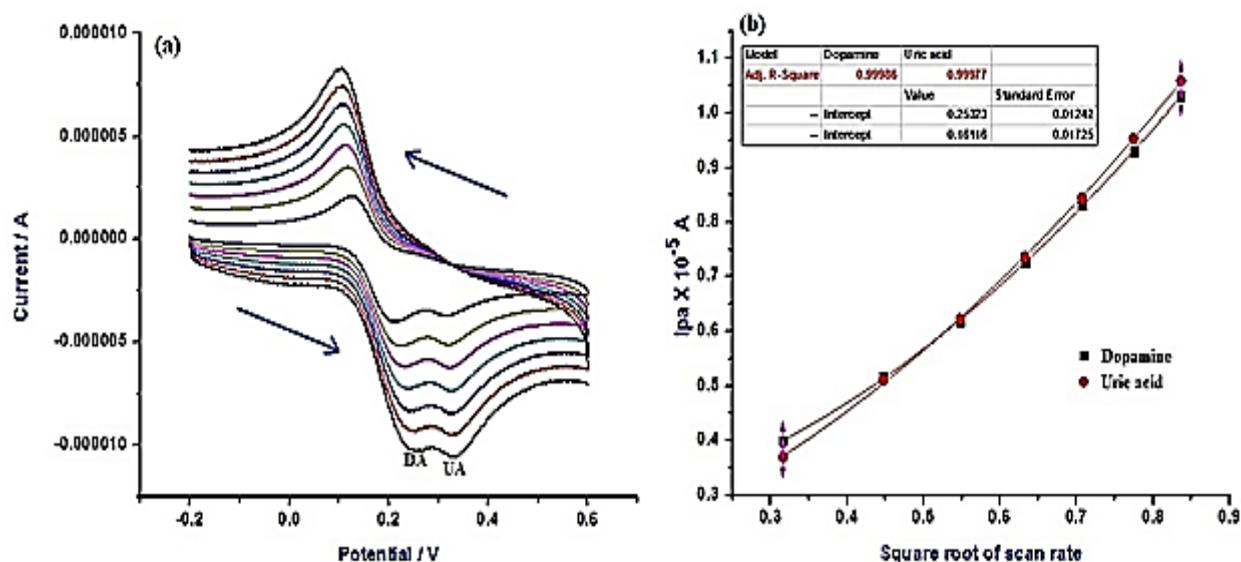


Fig. 7. (a) The effect of scan rate on cyclic voltammogram of BCPE and Ag nanoparticle MCPE of dopamine and uric acid **(b)** Graph of peak current vs. square root of scan rate of dopamine and uric acid

3.7. Effect of concentration variation of UA in presence of DA

The concentration effect of UA in presence of DA was studied at Ag nanoparticle/ CPE in phosphate buffer solution of pH 7.0 at a scan rate of 100 mVs⁻¹. Fig. 8a shows cyclic voltammetry peak current increases with increasing the concentration from 20 to 140 μ M of UA and keeping the DA concentration (50 μ M) constant and graph of anodic peak versus concentration of UA shows straight line with correlation coefficient of 0.9851 is shown in the Fig. 8b.

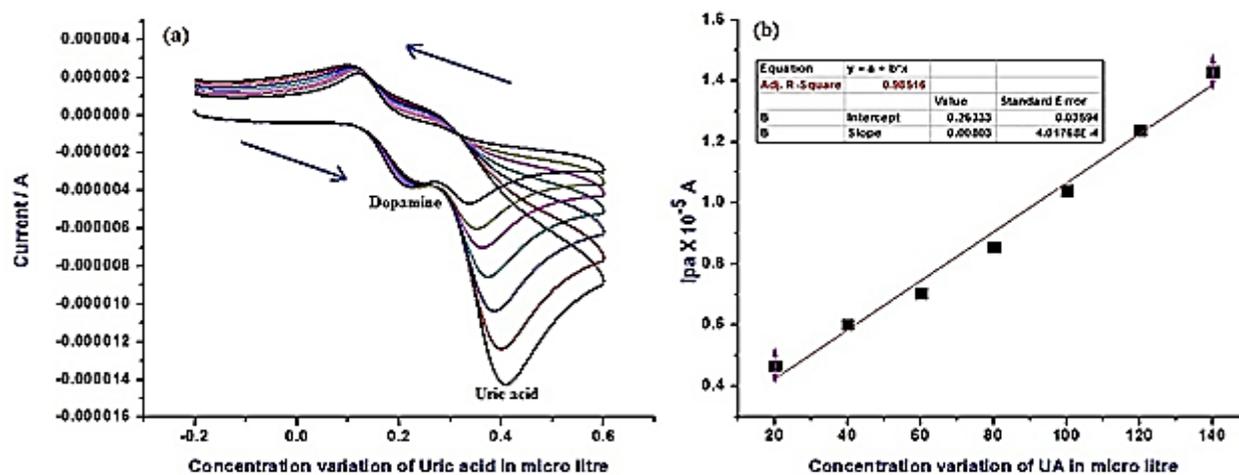


Fig. 8. (a) Cyclic voltammograms obtained for different concentration of uric acid (20 μM to 140 μM) and with constant dopamine concentration (50 μM) in PBS of pH 7 at scan rate 100 mVs^{-1} (b) Graph of concentration of the uric acid and dopamine vs anodic peak current

3.8. Antibacterial activity

Silver nanoparticles were synthesized using biological method and shows good antibacterial activity. Synthesized Ag nanoparticle antibacterial assay is aimed to just check the antibacterial activity of nanoparticles rather than to find minimum inhibitory concentrations as shown in the Fig. 9a, 9b and 9c of *E.coli*, *Bacillus subtilis* and *Staphylococcus aureus* respectively.

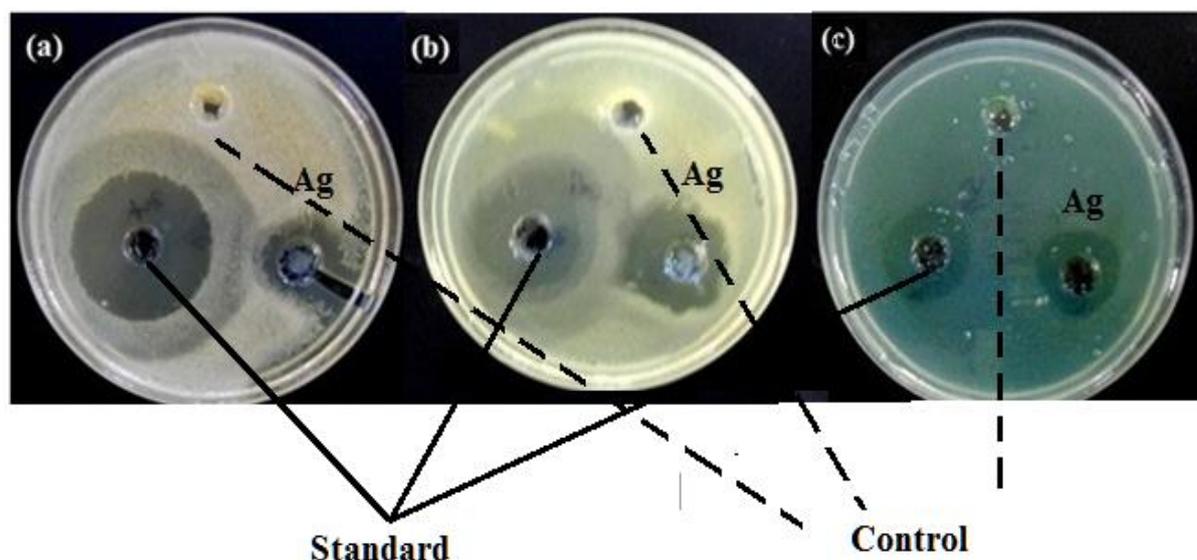


Fig. 9. Antimicrobial activity of silver nanoparticles on (a) *E.coli* (b) *Bacillus subtilis* and (c) *Staphylococcus aureus*

The method adopted was *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* were considered for checking the antibacterial activity. Glycerol stocks of these cultures were sub cultured in LB agar (2% agar) and incubated overnight to make the cultures viable.

3.8.1. Well diffusion method

Silver nanoparticles of concentration 1mg/ml in water were prepared by brief sonication for about 20 minutes and stored at 4°C until its use. LB agar (2% agar) plates were prepared and punched wells appropriately. Cotton swabbing of cultures was carried out subsequently from the dilutions. Sample, gentamicin (positive control) and water (negative control) were loaded into the appropriate well and incubated overnight at 30°C. Inhibition zone of above considered bacteria were tabulated in Table 1.

Table 1. Inhibition zone of prepared silver nanoparticles

Organism	Inhibition zone (cm)
<i>Bacillus subtilis</i>	1.8 ± 0.03
<i>Escherichia coli</i>	2.2 ± 0.03
<i>Staphylococcus aureus</i>	1.9 ± 0.03

4. CONCLUSION

Silver nanoparticles were successfully prepared by plant mediated biological method from the leaves of acacia melanoxylon plant. This method is simple, non-toxic and synthesizes nanoparticles rapidly. The synthesized Ag nanoparticles act as a modifier and electrochemical sensor to determine the concentration of UA in presence of DA using cyclic voltammetric technique. The antimicrobial study shows that silver nanoparticles have great promise as antibacterial agent against *E. coli*, *B. Subtilis* and *S. Aureus*. Therefore a silver nanoparticle shows wide applications in medical and electrochemical sensor fields.

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