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GREEN SYNTHESIS/BIOSYNTHESIS OF SILVER NANOPARTICLES BY USING
ORANGE PEEL EXTRACT

A Thesis

by

LEELA JOSHI

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2018

Major Subject: Chemistry

GREEN SYNTHESIS/BIOSYNTHESIS OF SILVER NANOPARTICLES BY USING
ORANGE PEEL EXTRACT

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LEELA JOSHI

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December 2018

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ABSTRACT

Joshi, Leela, Green Synthesis/Biosynthesis of Silver Nanoparticles by Using Orange Peel

Extract. Master of Science (MS), December, 2018, 39 pp., 23 figures, references, 36 titles.

In this work, we report synthesis of silver nanoparticles (AgNPs) with an average size of ~6 nm. The aqueous solution of Ag^+ ions is reduced using an aqueous extract from orange peel (*Citrus Sinensis*) at varying pHs. Reduction of Ag^+ ions in solution was monitored using UV–visible absorption spectroscopy, and the corresponding surface plasmon resonance of AgNPs at 435 nm. The solution changed color from yellow to reddish-brown upon bioreduction of silver nitrate in orange peel extract. This eco-friendly nanobiotechnology synthesis route is preferred over toxic and expensive chemical and physical methods. The synthesis performed using a 3 mM concentration of silver nitrate at two different temperatures-- room temperature and 80 °C. The resultant products were characterized by UV-vis spectroscopy, X-ray diffraction, scanning electron microscopy, energy dispersive spectroscopy, high performance liquid chromatography, and transmission Electron Microscopy techniques.

DEDICATION

I would like to dedicate this to my daughter Atreyee Mukherjee and my husband Pinaki Mukherjee. My sweet little daughter supported by all her means in my academic endeavors in USA. The completion of my studies would not have been possible without the love and support of my family. Thanks for all your love and support.

ACKNOWLEDGEMENTS

I would like to thank my research advisor Dr. Jason G. Parsons whose supervision allowed me to complete my master's degree. I am grateful for all his advice, insightful scientific discussions, words of encouragement and above all his politeness and generous nature.

I would like to extend my deepest gratefulness to my committee members. I want to give thanks to all instrumentation center personnel for their help with using the instruments and understanding the collected data. I would like to acknowledge all chemistry stock room personnel for all their help in the stockroom.

Lastly, I would like to give thanks to all my friends and lab members for all their support. I would like to give an extra special thanks to my lab mates. I would like to thank my husband and my daughter, family members back home for their encouragement and love.

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CHAPTER I

INTRODUCTION

Silver Nanoparticles

Metal nanoparticles are of great scientific interest as they considered to bridging the gap between the bulk and atomic dimensions. High surface area, tunable particle size, and particle morphology provides high reactivity to nanoparticles. From available literature noble metal nanoparticles Ag, Au, Pd, and Pt are the most extensively studied. Among the reported various noble metal nanoparticles, silver are extensively researched and of great interest in the fields of biological and medicinal research. The chemical stability and catalytic effect of silver nanoparticles have an advantage for antibacterial, antiviral, anticancer, antifungal and to anti-inflammatory activities and are a popular choice due to their nontoxicity towards the human. Silver nanoparticles have been researched in a diverse range of fields for example sensing, catalysis, medicine, and metallic inks.^[1-9] Chen et al. ^[10], stated that Ag nanoparticles are good, highly efficient and stable photocatalysts under ambient temperature with visible light illumination for degrading organic compounds and dyes. Four different oxidation states of silver are the most abundant mainly Ag^0 , Ag^{1+} , Ag^{2+} and Ag^{3+} . Among which Ag^{3+} is unstable in the aquatic environment. Environmental acute toxicity of silver is dependent on the availability of free silver ions. From (WHO, 2002) ^[11] investigations the concentrations of Ag^+ ions are too low to lead to toxicity. Silver nanoparticles are used in numerous consumer products such as textiles, medical products, cosmetics, and food containers.^[12] Ag Nanoparticles can be synthesized using

various approaches including chemical, physical, and biological methods. Among reported methods; the chemical synthesis route is performed using a variety of organic and inorganic reducing agents is well documented. There is growing attention in using environmentally friendly green methods to avoid hazardous and toxic materials to the environment.

Synthesis Methods of Silver Nanoparticles

Synthesis of Silver Nanoparticles by Chemical approaches:

Chemical reduction of silver nitrate by organic and inorganic reducing agent is the most common way for the synthesis of silver nanoparticles. From literature variety of reducing agents such as sodium borohydride, sodium citrate, elemental hydrogen, N, N-dimethylformamide and poly (ethylene glycol)-block copolymers are reported in both aqueous or non-aqueous solutions. Sodium borohydride (NaBH_4) is one of the most applied reducing agents which has been used for chemical synthesis of AgNPs. The reducing agents reduce silver ions (Ag^+) to metallic silver (Ag^0). To protect these nanoparticles from agglomeration and sedimentation surfactants having functional group such amines, thiols, acidic functionalities, and alcohols are used. Some other polymeric protecting agent such as poly (vinyl alcohol), poly (ethylene glycol), polymethylmethacrylate, poly(methacrylic acid) and poly (vinylpyrrolidone) have also been reported to be effective protective agents to stabilize nanoparticles.^[13] Organic compound can act as both reducing and capping agent.^[14-17] Chemical synthesis has been one of the first approaches in this regards and biosynthesis is a novel approach to eliminate disadvantages of chemical synthesis mainly lead to non-ecofriendly byproducts.^[18]

Synthesis of Silver Nanoparticles by Biological approaches:

The development of efficient green chemistry methods is major attention for researchers nowadays. The biological method employed using natural reducing, capping, and stabilizing agents without the use of any toxic and expensive chemicals. Huge varieties of biological molecules such as carbohydrates, proteins, alcohols, aromatic compounds phenols, and polyphenols have been used. Reported biological methods for nanoparticle synthesis involve bacteria, fungi, algae, and plants.^[19-21] This approach reduces hazard exposure to the environment. Green synthesis explores plant sources in the development of silver nanoparticles and having an advantage regarding biocompatibility, cost-effectiveness, single step synthesis, and reduced environmental toxicity. To achieve greater stability, controlled size-shape nanoparticles and high yield; different reaction parameters such as temperature, pH, solvent medium, reaction time have also been shown play a vital role in green synthesis.^[22-23]

Synthesis of Silver Nanoparticles by Plants and Extract:

More recently, the use of plants and plant extracts are the most adopted method of eco-friendly synthesis of nanoparticles due to easily available, safe to handle and mostly nontoxic starting materials. Plant extracts for the biogenesis of AgNPs are advantageous as they are easily available, safe, and nontoxic. The whole plant or their parts such as fruit, seed, root, leaf, and stem are reported for green synthesis of silver nanoparticles. Presence of phytochemicals plays an important role in plant-assisted reduction. It is reported that phytochemicals are involved directly in the reduction of the ions and the formation of silver nanoparticles. The important phytochemicals involved are flavones, aldehydes, ketones, carboxylic acids, and terpenoids.^[24-25] Water-soluble phytochemicals such as flavones, organic acids and quinones are responsible for the immediate reduction of the ions. Several plant extracts from their fruits, leaves, seeds and

roots are used to synthesize AgNPs. Production of AgNPs occurs with the ionic interactions of functional groups and silver salts. Solvent medium, reducing, capping and stabilizing agents are the major constituents in the formation of AgNPs. Generally, the bio-reduction mechanism of metal nanoparticle in plants and plant extracts includes three main phases.^[26] First is the activation phase, in which metal ions reduced and nucleation of reduced metal atoms takes place. Next, is Ostwald ripening or the growth phase, here spontaneous coalescence of the small adjacent nanoparticles occurs to form larger size particles which provides the thermodynamic stability to the nanoparticles. Third is the termination phase in which the final shape of the nanoparticles is generated. The aqueous extract is considered more eco-friendly over organic extracts and found more appropriate to green synthesis of AgNPs. Figure 1 is showing schematic of silver nanoparticles synthesis by (a) physical, (b) chemical, and (c) the biological approaches.

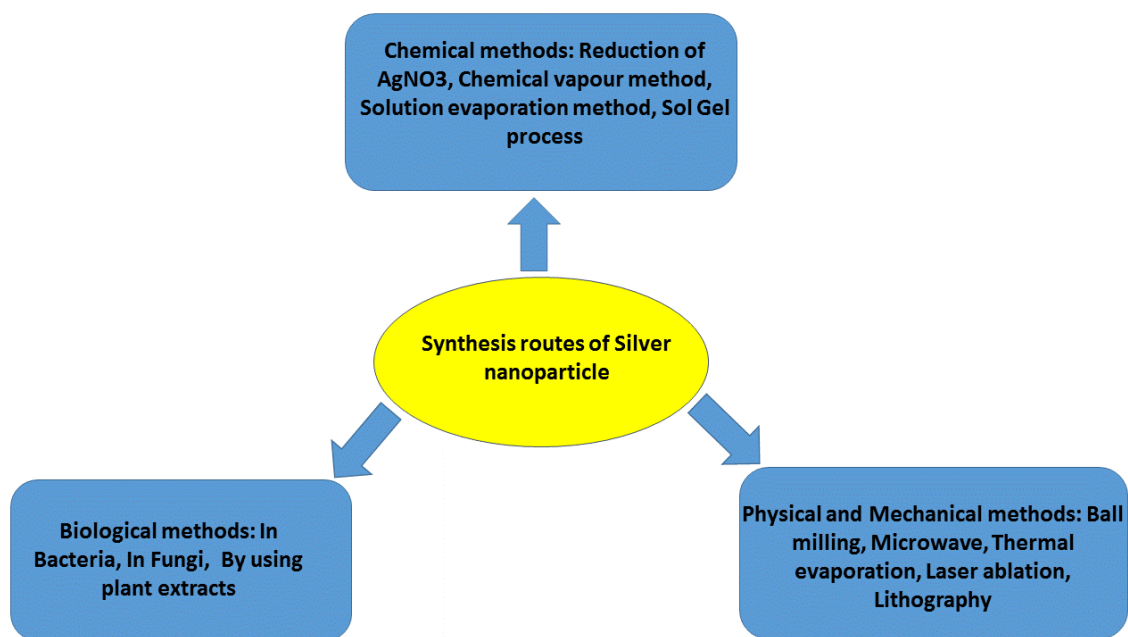


Figure.1 General procedures to synthesize silver nanoparticles and schematic of the synthesis of silver nanoparticles using (a) the physical, (b) the chemical, and (c) the biological approaches.

Here in my work using orange peel, a common waste material is used for nanoparticle synthesis. The first aim was to use the aqueous extract of orange peel to synthesized “green” silver nanoparticles. Producing nanoparticles using various plants parts and their extracts is advantageous over other biologicals routes such as maintaining microbial cultures which involve complex multi steps. Thus the plant extract approach is more advantageous since it does not need any special, complex, and multi-step procedures such as isolation, culture preparation, and culture maintenance.^[27]

CHAPTER II

MATERIALS AND METHODS

The present study entitled “**Green synthesis of silver nanoparticles by using orange peel extract, their characterization**” was performed in Department of Chemistry “University of Texas Rio Grande Valley”. The detail of materials used, methodologies employed, experiments and techniques have been elaborated below. Silver nitrate of analytical grade was purchased from Sigma- Aldrich Chemicals and was used without any further purification. All of the solutions and chemical were prepared in Millipore water (18M Ω).

Preparation of Orange Peel Extract

The Navel Oranges were brought from the local market. First, fruits were rinsed with tap water followed by distilled water to remove dirt. The peel was removed from fruit and dried in an oven at 60 °C. The dried peels were further grounded to a fine powder using a blender. Approximately 10 g of powder was refluxed in 100 mL millipore water for 30 min. After cooling the solution was centrifuged at 3000 rpm and the supernatant was saved. The supernatant was used for Ag NP synthesis and stored in a refrigerator until further use.

Preparation of AgNO₃ solution

A 3mM solution of AgNO₃ (0.509 gm) was prepared by dissolving in 1000ml millipore water and stored in an cool, dark in an amber coloured bottle.

Syntheses of AgNPs using orange peel extract

Detailed procedure is as follows: 100 mL of 3 mM solution of AgNO₃ was taken and drop by drop orange peel extract solution was added in a ratio of 10:1 (v:v). 100 mL 3mM AgNO₃ solution was placed in an erlenmeyer flask and different pHs (2,5,7 and 9) were maintained for each set of experiment. The resulting solutions were protected from light using aluminum foil and kept in stirring. After maintaining different pH for each set, 10 mL orange peel solution was added drop by drop to each set and stirred for 3 hours. Formation of the AgNPs was confirmed by color change of the reaction solution from transparent to pale yellow and eventually to reddish brown as steps shown in Figure 2.



Figure 2. Steps of silver nanoparticle synthesis (a) AgNO₃ stock solution (b) AgNO₃ and aqueous orange peel extract mixture after 30 min of reaction (c) reaction mixture after 1 hour and (d) reaction mixture after 3 hour.

Characterizations

Characterization of materials was performed using UV-Vis spectrophotometer, x-ray diffraction (XRD), Scanning Electron Microscopy (SEM), electron dispersive x-ray spectroscopy (EDX), Transmission Electron Microscopy (TEM) and High Performance Liquid chromatography (HPLC) techniques.

UV-Vis Analysis

U.V analysis was performed using a Perkin Elmer Lambda 950 UV/VIS/NIR spectrometer. Spectra were recorded from 200 to 800 nm was used to characterize the plasmon resonance of the silver nanoparticles. All spectra were obtained in solution phase using quartz cuvettes.

XRD Analysis

XRD analysis was performed on Rigaku Miniflex II. The diffractometer was fitted with a copper x- ray source using the Cu K α 1.54 Å, with a corresponding Ni filter. Composite samples were analyzed from a scanning angle of 20-80° in 2θ , using a step size of 0.05° with a count time of 2 seconds per step.

SEM Analysis

A Sigma VP FE-SEM from Carl Zeiss scanning electron microscope was used to obtain all SEM images. Under accelerating voltage, which ranged from 10.75 to 20.71 keV and working distance from 6.0 to 6.5 mm were used for the collected electron micrographs.

EDS Analysis

EDS analysis conducted using an EDAX-EDS spectrometer attached to the SEM. Scans were obtained using an accelerating voltage of 20.71keV with a 120 second count time per sample.

TEM Analysis

The electron micrographs and Energy dispersive X-ray spectroscopy maps were obtained using a FEI Titan Themis aberration corrected scanning transmission electron microscope (S-TEM) operated at 200kV. The point resolution in this aberration-corrected mode is 0.08nm. The microscope was fitted with SuperXTM X-ray detector, which is a combination of 4 detectors for fast X-ray mapping in STEM mode. For extremely stable (mechanical and e-beam interaction) samples, atomic resolution maps are possible. For the present experiment, nm resolution EDX maps were taken with an average beam current of 100pA beam current. The size of the maps were 512X 512 pixels and 60 μ s/pixel dwell time were used for collecting the signal. All maps are generated by summing over 30 frames. The drift correction during data collection and subsequent analysis were performed using Velox software.

Chromatographic Analysis

A Thermo Scientific Ultimate 3000 HPLC, equipped with a reverse phase C-18 column, was utilized. Phenolic compounds present in orange peels such as flavonoids were determined using a high-performance liquid chromatography. HPLC is a separation method for characterization and quantification of the phenolic composition. The chromatographic conditions were: reverse phase column RP-18; and an oven temperature of 10°C. The mobile phase was water-Methanol. DI water (solvent A) and methanol (solvent B), with a flow of 1 mL·min⁻¹ ,

using a linear gradient (starting with 40% of solvent B, increasing to 90% in 3 minutes and reducing back to 40% of solvent B in 8 minutes remaining in this conditions until 15 minutes). The time of the analysis was 15 minutes and the detection was performed using a diode array detector and the wavelengths characterized were 288 nm and 310 nm.

CHAPTER III

RESULTS AND DISCUSSIONS

Growth and Mechanism of Ag Nanoparticles

In the Present study a cost-effective and eco-friendly technique for the green synthesis of AgNPs has been developed; from the reduction of AgNO_3 using orange peel extract as reducing as well as capping agent. The aqueous extracts of orange peel were used for both the reducing and stabilizing agent for the synthesis at different pHs 2, 5, 7 and 9. The aqueous extract is considered ecofriendly over organic extracts and found more appropriate for the green synthesis of AgNPs. The synthesis was performed at room temperature as well as at high temperatures. Further AgNP studied by using different instrumental techniques which included UV-Visible, XRD, SEM, TEM and HPLC techniques. Biologically-inspired steps for the synthesis of nanoparticles has been evolving as an important branch of nanotechnology. The biosyntheses of nanoparticles via green route does not employ toxic chemicals and hence proving to become an environment-friendly processes. Several functional groups present in orange peel extract such as flavonoids, citric acid, carotenoids, and aromatic compounds exhibit antioxidant properties, and consequently, can play an important role as reducing and/or capping agents in nanoparticle synthesis.^[28]

A possible mechanism of AgNPs synthesis was to proceed via ionic interactions of functional groups and a silver salt. A number of biomolecules present in the plant extract reduces the monovalent silver ion to uncharged atoms and these atoms aggregate to reach nano-size, other biomolecules of the plant extract act to the envelope to prevent their further aggregation.^[29] The rich source of negatively charged functional groups may responsible for the reduction of metal ions and efficient stabilization of synthesized NPs under natural conditions.^[30] In orange peel tannins and flavonoids are main compounds responsible for bioreduction. These are water-soluble polyphenolic compounds and cause proteins coagulation and are responsible for the chelation of metal ions. Oxidation of phenols results in quinones or quinoid structure in case of tannins. The higher total phenolic content in peel extract facilitates the reduction of Ag^+ ions to nanoscale size Ag^0 particles due to electron donating ability of these phenolic compounds. Moreover, the quinoid compound produced due to the oxidation of phenol group in phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension and stabilization. Possibility of large and dispersed AgNP is obtained due to a slower rate of reaction between plant extract and AgNO_3 .

UV-VIS Results

The growth and stability of AgNPs in an aqueous colloidal solution was investigated by using UV–vis spectral analysis. As reaction proceeds solution turned yellowish brown, which has been attributed to the excitation of surface plasmon resonance in AgNPs. Figure 3 shows UV absorption spectra from the reaction of reduction of silver ions, which have dispersed nanoparticles with broadening peak in the absorbance band at the wavelength of 448 nm. After adding the orange peel extract to silver nitrate solution the color of the solutions turned from transparent white to light yellow to brown. UV-vis absorption spectra Orange-Peel, silver nitrate stock solution and nanoparticles at different pH 2, 5, 7, and 9 were taken to understand the

differences. No absorption peak was observed in UV–vis spectrum of Ag^+ solution before adding peel extract. After addition of the reducing agent the gradual generation of silver nanoparticles started which is indicated by the increase in the absorbance intensity at 400–410 nm in UV spectra. The Orange peel extract showing absorption bands at 324 and around 284 nm Figure 3. At room temperature synthesis at different pH, a very clear absorption band is observed at ~413 nm at pH 9, which is completely missing at pH 2 but is present as a small hump at pH 5 and 7 as shown in Figure 4. The bands located at around 283 and 320 nm are associated with the formation of silver nanoparticle colloidal and cluster reported in some research.^[31] These bands may be from peel extract absorption band. The absorption band at 413 nm is due to localized surface plasmon resonance band related to the formation of silver nanoparticles. At room temperature the plasmon bands are not missing for pH 2, 5 and 7 though not observed. However, the concentration of these silver nanoparticles should be too low for the detection in UV-vis spectra. At higher temperature @80 °C plasmon bands are visible at pH 5, 7 and 9 but still missing at pH 2, as can be seen in Figure 5. Consequently, the orange extract is playing a role to reduce $\text{Ag}^+(\text{aq})$ ions to elemental silver and acting as a capping agent. Stability is further confirmed by overnight and two week study, which are shown in Figure 6 and 7. In these samples, synthesized nanoparticle were kept at room temperature and again Uv-Vis was performed. Monovalent silver ion is mainly reduced by –OH groups of the active components of the orange peel extract, which is itself oxidized to the corresponding carbonyl group. The change in color is due to the strong absorption of visible light due to excitation of the nanoparticle surface plasmon.

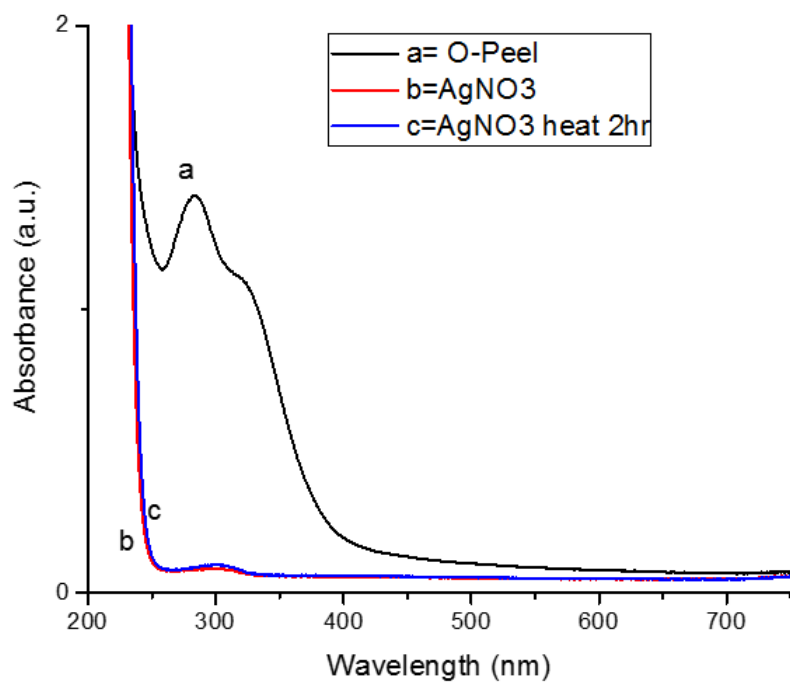


Figure 3: UV spectra of (a) Orange Peel (b) AgNO_3 stock solution (c) AgNO_3 heated solution.

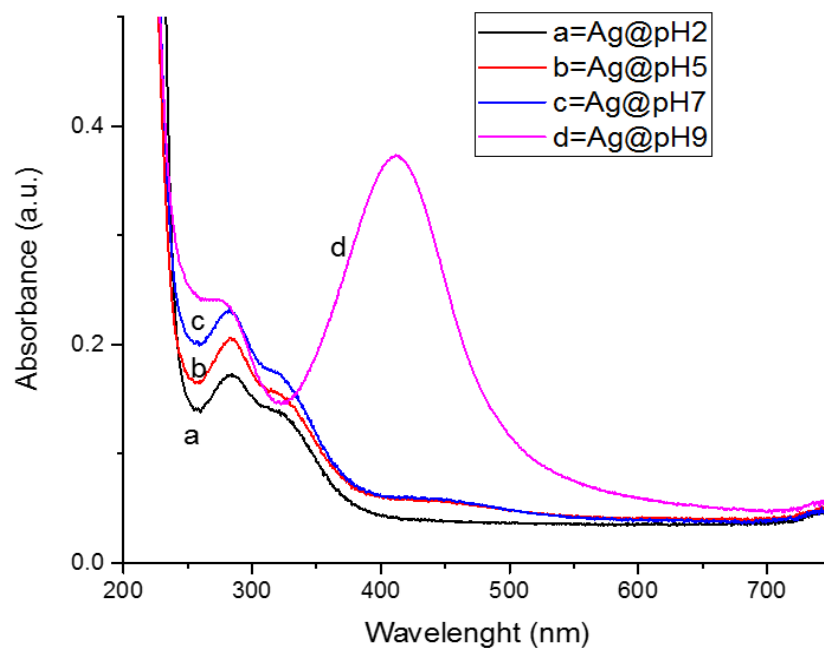


Figure 4: UV spectra of AgNPs synthesized using orange peel at room temperature at pH 2, 5, 7 and 9.

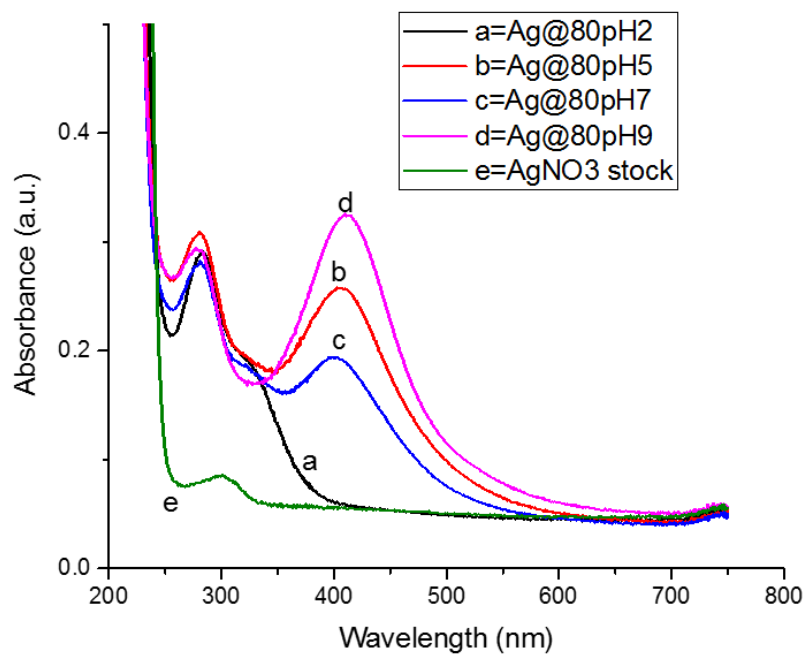


Figure 5: UV spectra of AgNPs synthesized using orange peel at high temperature $\sim 80^{\circ}\text{C}$ at various pH 2, 5, 7 and 9.

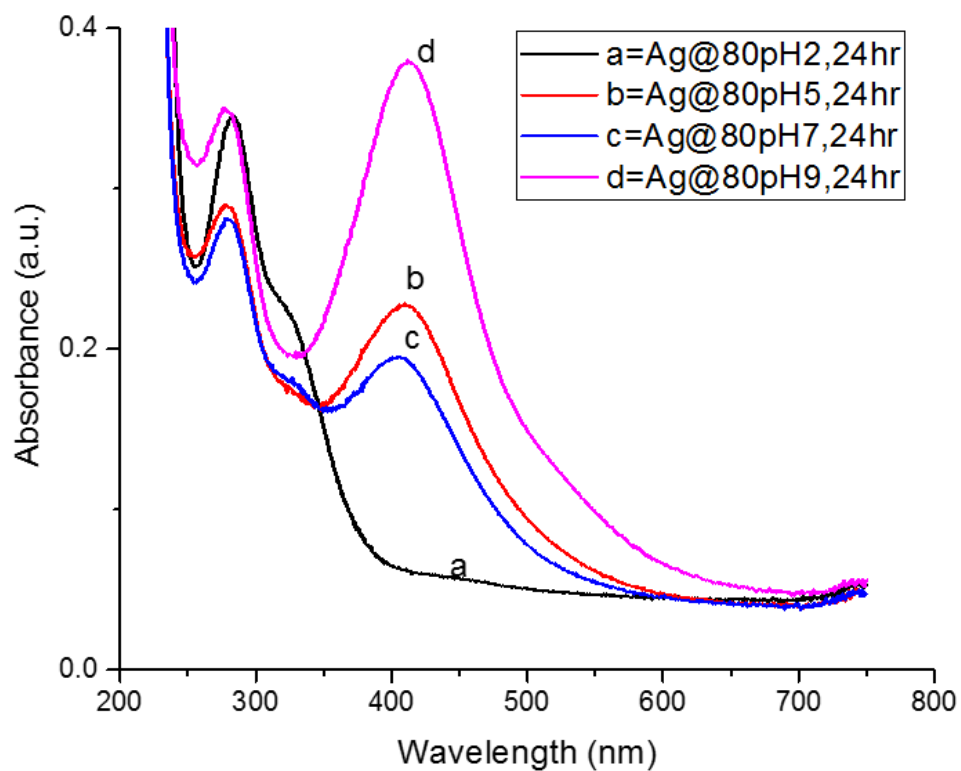


Figure 6: UV spectra of AgNPs to check stability (In this UV study, sample kept for overnight to check the stability of nanoparticle).

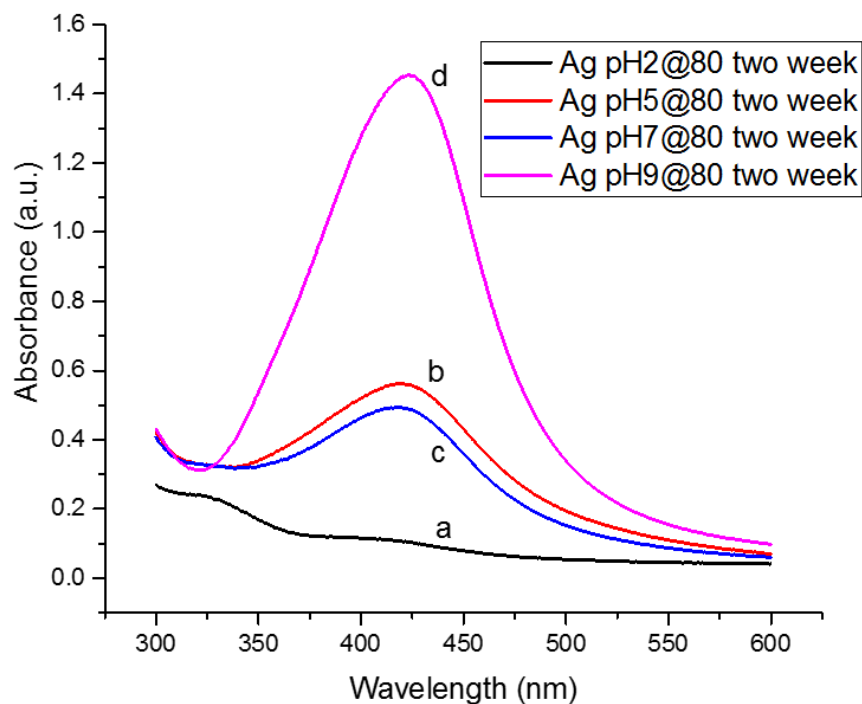


Figure 7: Two week study of UV-Vis of AgNPs synthesized using orange peel at high temperature.

Effect of Temperature

In this study, temperature was also an important parameter affecting the synthesis.

Figure 2 shows the UV-vis spectrum of the AgNPs prepared at two different temperature (25 °C and 80 °C). It is clearly shown from UV spectra that the absorbance increases with temperature. The reduction rate of the Ag^+ ions is faster at a higher temperature. At room temp study, broad peaks are observed due to large particle size, while the peak became sharp at a higher temperature synthesis, which clearly indicates the formation of small size nanoparticles.

Effect of pH

pH is also another most important parameter for determining the shape and size of AgNPs. Here synthesis is performed at various pH. Varying pH from acidic to basic the net charge on the biomolecules changes from positive to negative. Higher pH of the reaction medium produced smaller NPs compared to the low pH. At low pH or acidic solution less than pH 3, the synthesis was very slow. Here pH 5, 7 and 9 were found to be very effective. pH played an important role in the reducing potency of the orange extract. Under acidic pH the active components of the extract are not effective for the reduction of silver ion due to structural stabilization, through H-bonding. Also the nitrate group of silver nitrate salt is a stronger oxidant than Ag. At basic pH silver ion first form Ag_2O , which is then converted to metallic silver. At higher pH, a red shift of absorption max was observed which indicates formation of the larger particles. ^[26]

XRD Results

XRD was performed to confirm the crystalline nature of the AgNPs. XRD spectra of AgNPs showing the peaks corresponding to the (111), (200), (220) and (311) lattice planes, which are due to presence of crystalline phase of silver metal with a face centered cubic structure. ^[32] At room temperature mostly on amorphous background less intense peaks are visible shown in Figure 8, whereas sharp and well defined peaks are clearly visible at high temperature synthesis Figure 9. The reason for this can be formation of mixture of Ag NPs, silver oxide and unreacted AgNO_3 in product obtained at room temperature. Figure 10 displays XRD image of high temperature synthesis. It is clearly seen that even at high temperature pH2 does

not show well defined peaks. More than likely at very low pH the active components of orange peel extract are not able to act as a reducing agent.

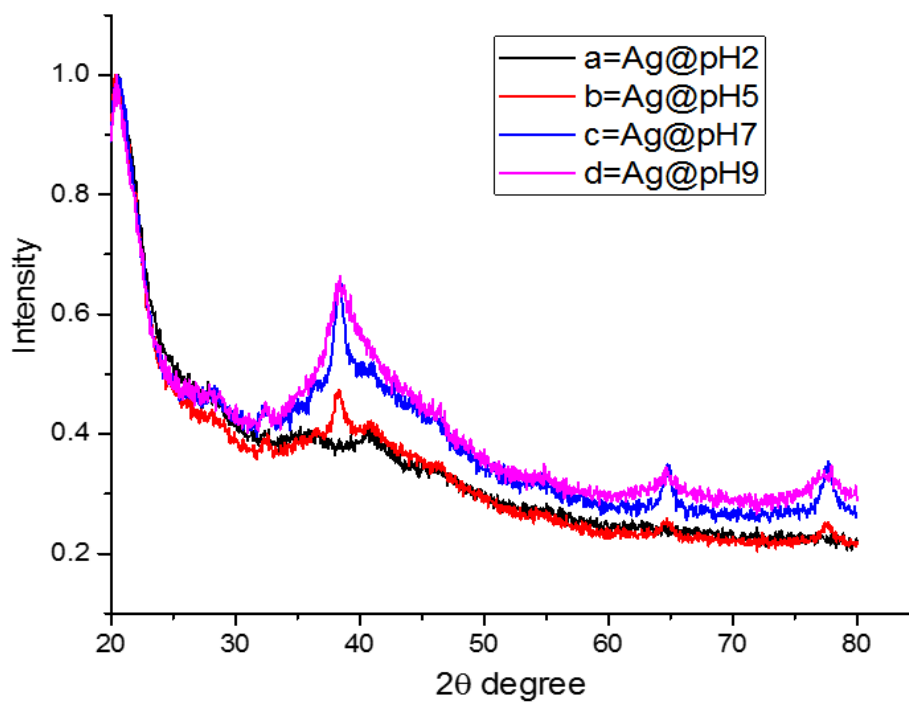


Figure 8: XRD spectra of AgNPs synthesized using orange peel at room temperature.

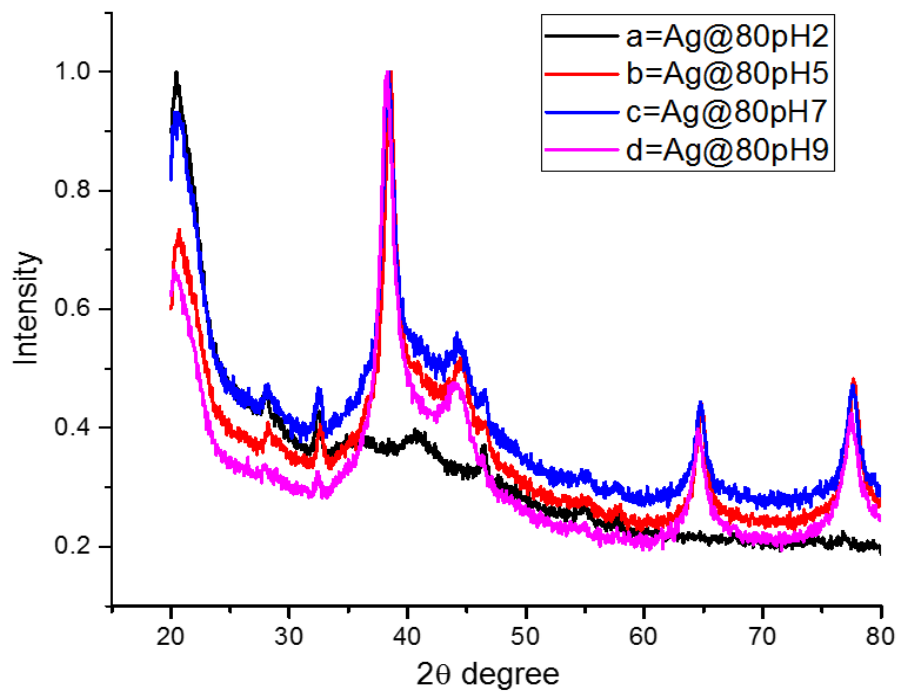


Figure 9: XRD spectra of AgNPs synthesized using orange peel at high temperature.

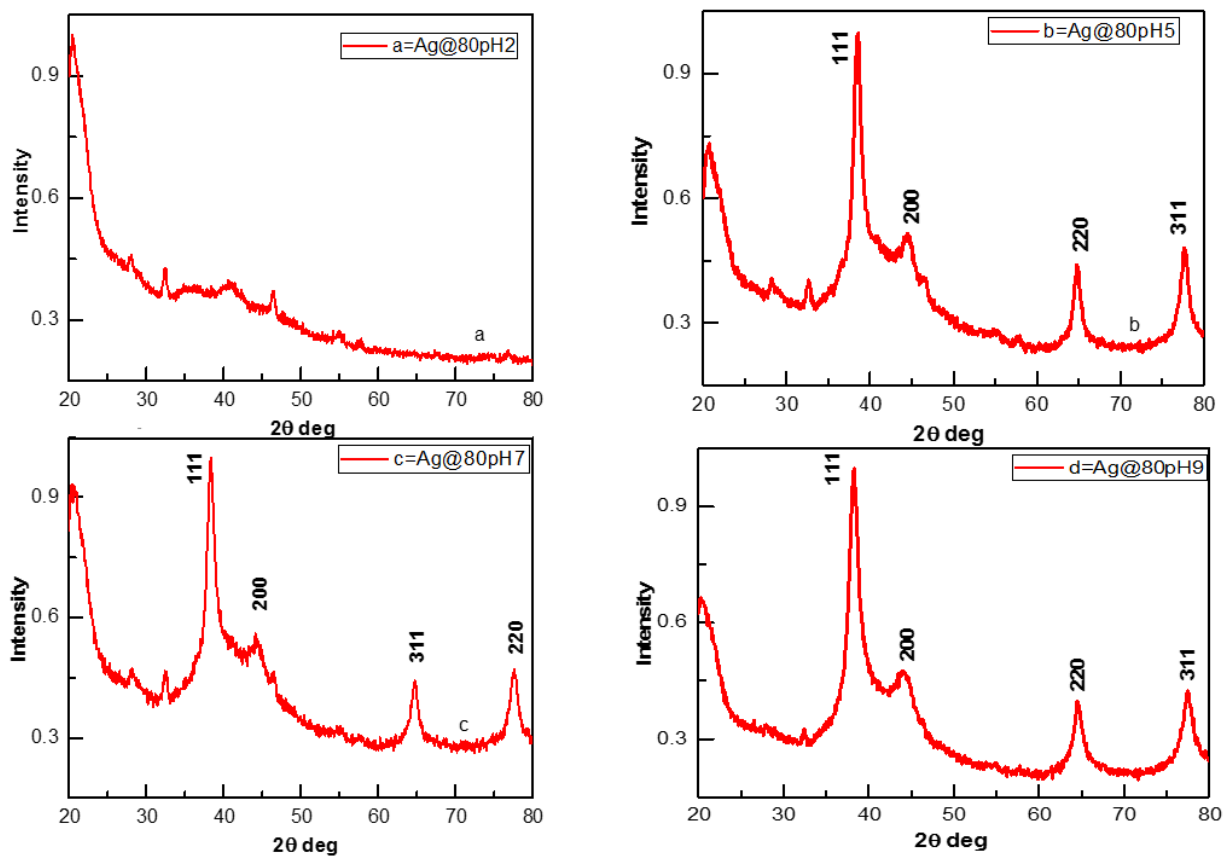


Figure 10: XRD spectra of AgNPs synthesized using orange peel at high temperature at various pH.

SEM and EDS Results

SEM microscopy shows that the AgNPs are approximately 100 nm in size with a predominant spherical shape. In these images the particles are well dispersed and the aggregation of the particles can be seen, as shown in Figure 11, 12 and 13. The EDX on the SEM analysis of the Silver Nanoparticles was performed for elemental study and are shown in Figures 14, 15 and 16. EDX elemental analysis show the same elemental composition for all the samples at different pH. Peaks located around 2.5 to 3.0 keV are characteristic of metallic Ag, due to Ag-L_a and Ag-L_b lines. Profile also showed signals for oxygen and carbon peaks which could be originated from

the biomolecules which are attached to the surface of nanoparticles. And the organic compounds present in Orange peel extracts.^[33] In addition, the oxygen signal may be a contribution from the atmosphere. The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of Silver Nanoparticles. The typical absorption peak at 3 KeV confirms the metallic nanoparticles due to surface plasmon resonance

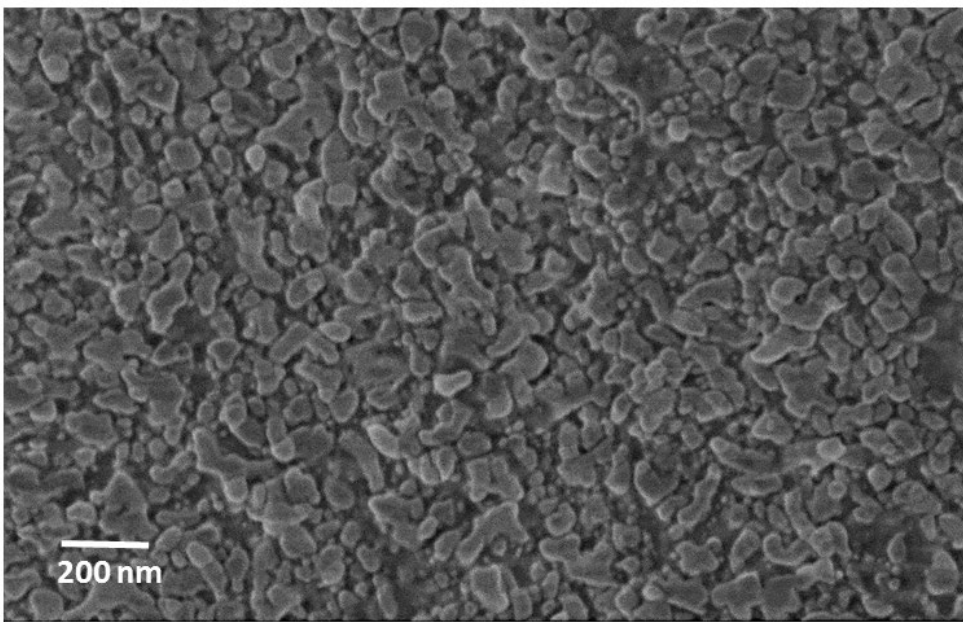


Figure 11 : SEM micrograph of Silver nanoparticles synthesized from the orange extract at pH 5@ 80 °C.

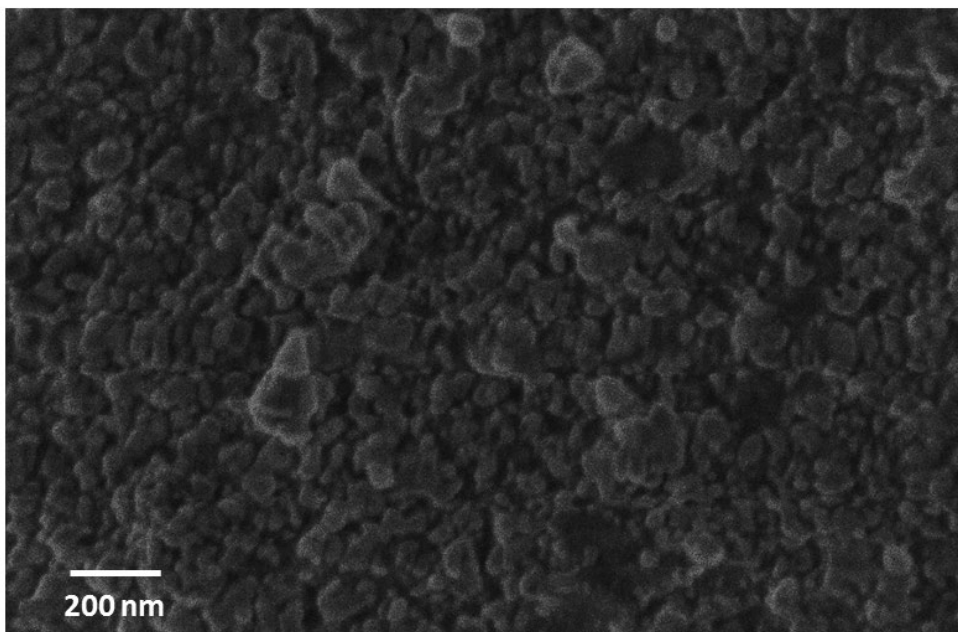


Figure 12: SEM micrograph of Silver nanoparticles synthesized from the orange extract at pH 7@ 80 °C.

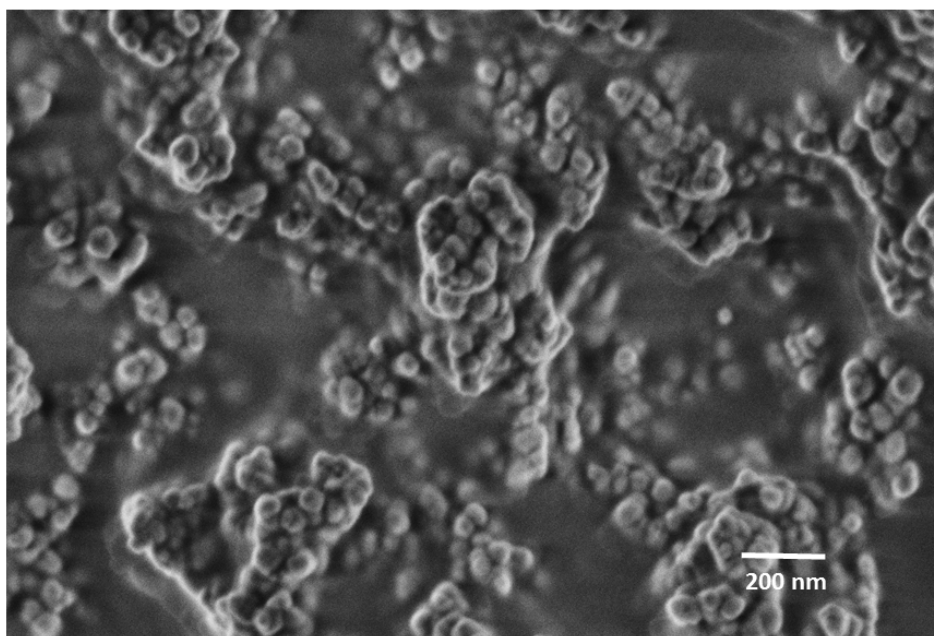


Figure 13 : SEM micrograph of Silver nanoparticles synthesized from the orange extract at pH 9@ 80 °C.

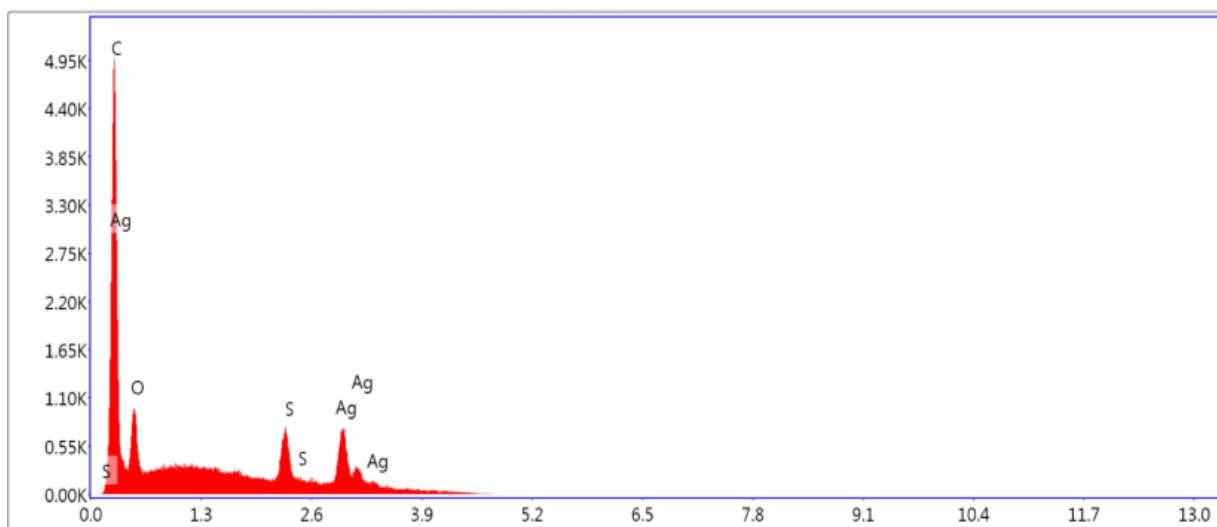


Figure 14: Energy dispersive spectrometer analysis of Silver nanoparticles synthesized from the orange extract at pH 5@ 80 °C.

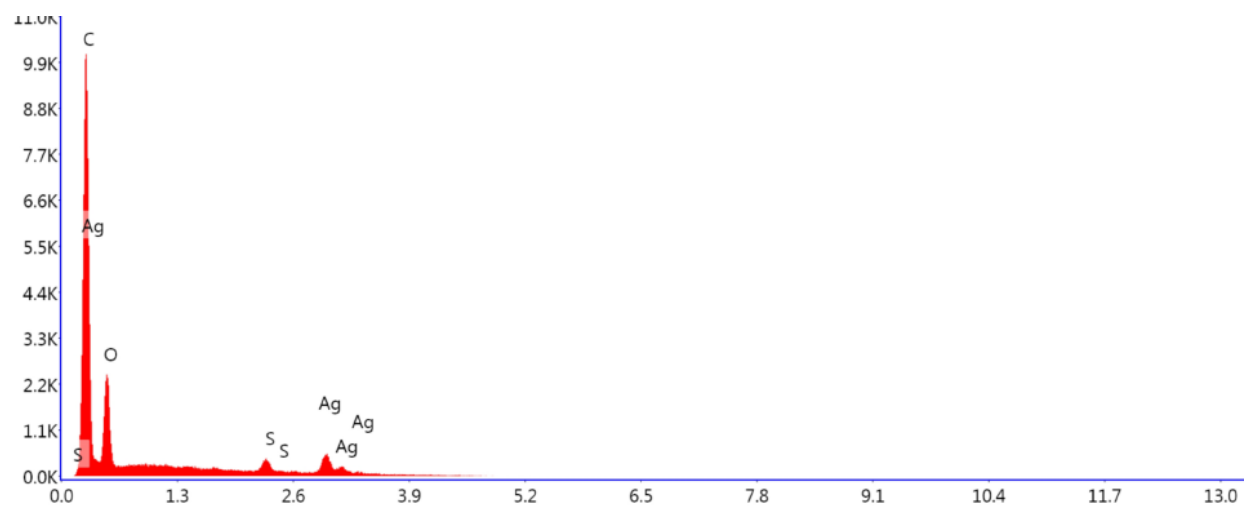


Figure 15: Energy dispersive spectrometer analysis of Silver nanoparticles synthesized from the orange extract at pH 7@ 80 °C.

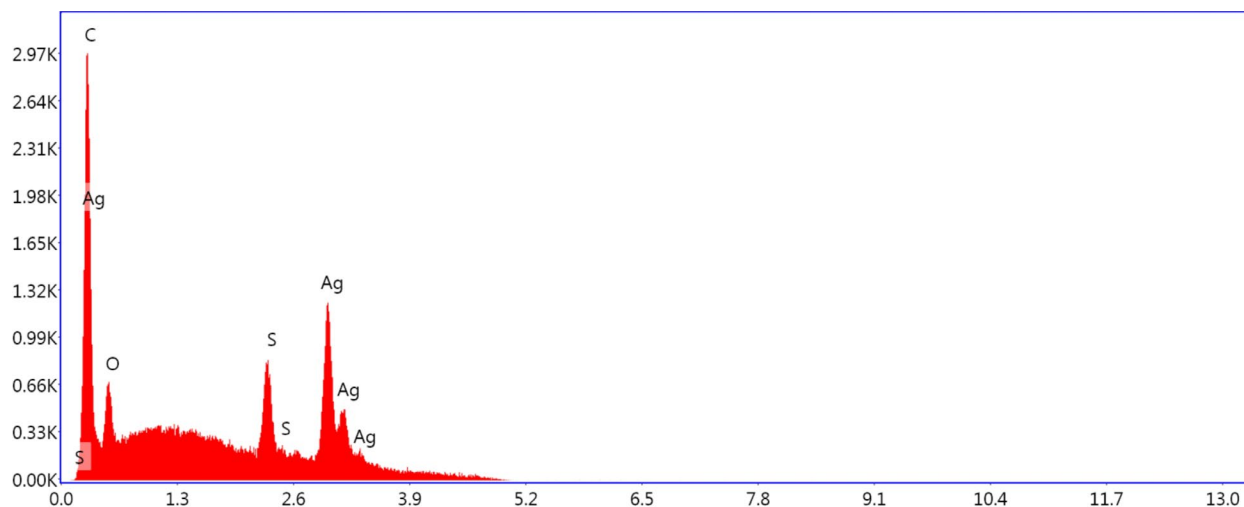


Figure 16: Energy dispersive spectrometer analysis of Silver nanoparticles synthesized from the orange extract at pH 9@ 80 °C.

STEM Results

The nanoparticles were also analyzed using scanning transmission electron microscope in high angle annular dark field (HAADF-STEM) mode. The contrast generated in this mode is due to atomic number of the elements present. Figure 17 shows the presence of round shaped well-dispersed nanoparticles with an average size of ~ 6 nm. The particle size differs from the SEM images^[32] because STEM experiments were performed on primary particles that were obtained after hand grinding of bigger agglomerates. These nanoparticles are crystalline as seen from figure 17b and the corresponding fast Fourier transform (FFT) of ROI 1(figure 17c).

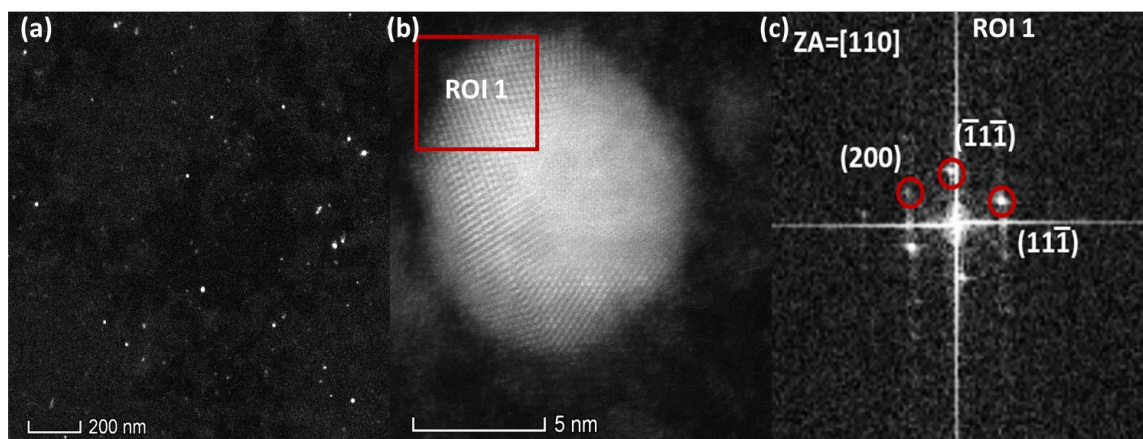


Figure 17 : (a) High angle annular dark field- scanning transmission electron microscope (HAADF-STEM) image of Silver nanoparticles synthesized from the orange extract at pH 7@ 80 °C. (b) HAADF-STEM image of a single nanoparticle showing lattice fringes corresponding to Ag. (c) Fast Fourier transform of ROI 1 shows the particle is oriented along [110].

Elemental maps obtained from energy dispersive spectroscopy in STEM mode shows that these particles are indeed Ag (figure 18b and d). O-K map is equally distributed over the particle and the substrate (figure 18c). The O-K EDS spectra corresponding to the particle (figure 18e) and the substrate (figure 18f) show no change in intensity. This shows that the nanoparticles are Ag and not some form of silver oxide.

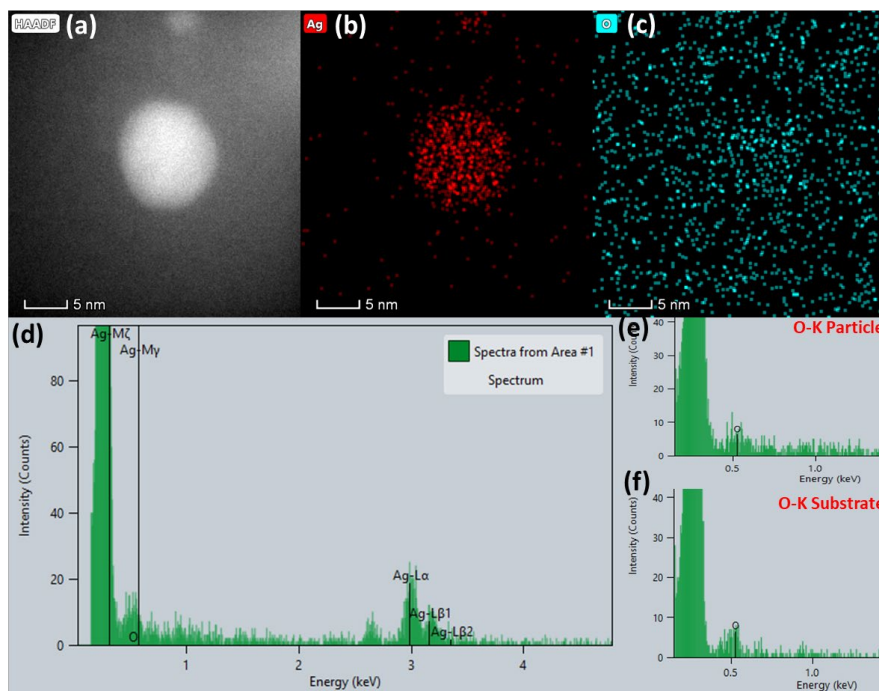


Figure 18 : Energy dispersive spectroscopy(EDS) and elemental mapping of a single Ag nanoparticle prepared at pH 7@ 80 °C. (a) HAADF-STEM image of the nanoparticle. (b, c) Ag and Oxygen map. (d) ED spectra showing Ag –L lines. (e, f) ED spectra corresponding to O-K line from the particle and the substrate, respectively.

HPLC Results

Different Flavonoids present in aqueous extract of navel orange peel solutions were determined using a high-performance liquid chromatography technique. Orange peels are high source of flavonoids. These are mainly glycosides and polymethoxylated flavones.^[34] Many researchers have reported HPLC analysis of glycoside flavanones of citrus family which are mainly hesperidin, hesperetin, naringin, and naringenin.^[35] Hesperidin, the most abundant

flavonoid in citrus family of fruits. The aim of this analysis is to identify for specific flavanones present in our navel orange which is responsible for the bioreduction of silver ions.

Reverse phase chromatographic analyses were performed under gradient conditions using C18 column. The chromatogram of orange peel extract is shown in Figure 18 and shows three different peaks at retention time (RT) of 4.1, 4.8 and 7, which corresponding to naringin, hesperidin and naringenin.^[35] Among these identified peaks concentration of hesperidin are found to higher in the extract and others are comparatively less. In order to compare which group in extract is playing major role in silver nitrate reduction to elemental silver, HPLC of extract was performed before and after the silver reduction reaction as can be seen in Figure 19. The chromatogram shows that hesperidin peak almost disappears and gradual decrease in naringin peak intensity at different pHs as can be observed in Figure 20, 21 and 22. Based on this result we can predict that hesperidin is major constituent in bioreduction of Ag(I) ion to Ag(0) and the stabilization of the Ag NPs.

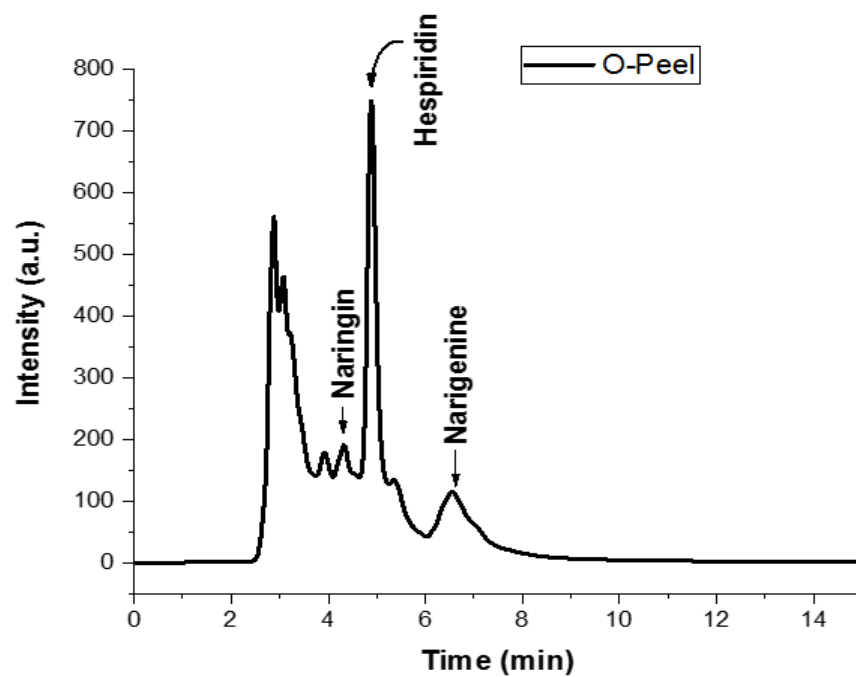


Figure 19. High performance liquid chromatography profile of Navel orange peel extract.

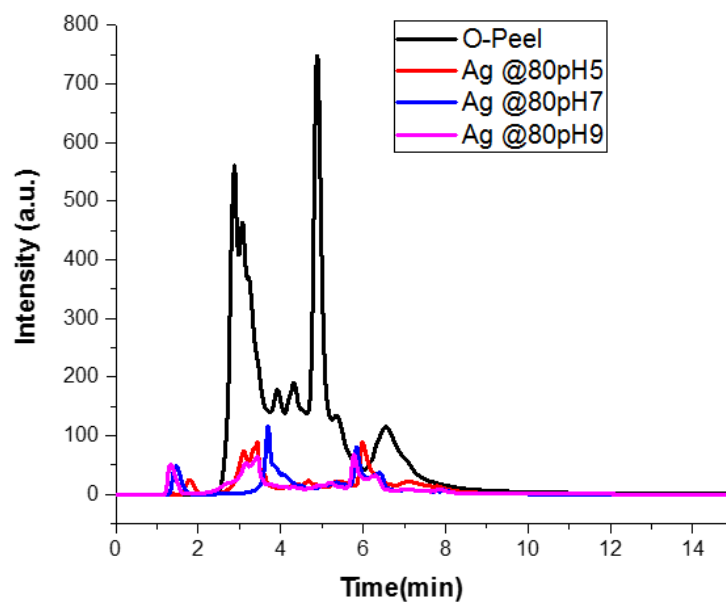


Figure 20. High performance liquid chromatography profile overlay of Navel orange peel extract before reduction and after reduction at different pH.

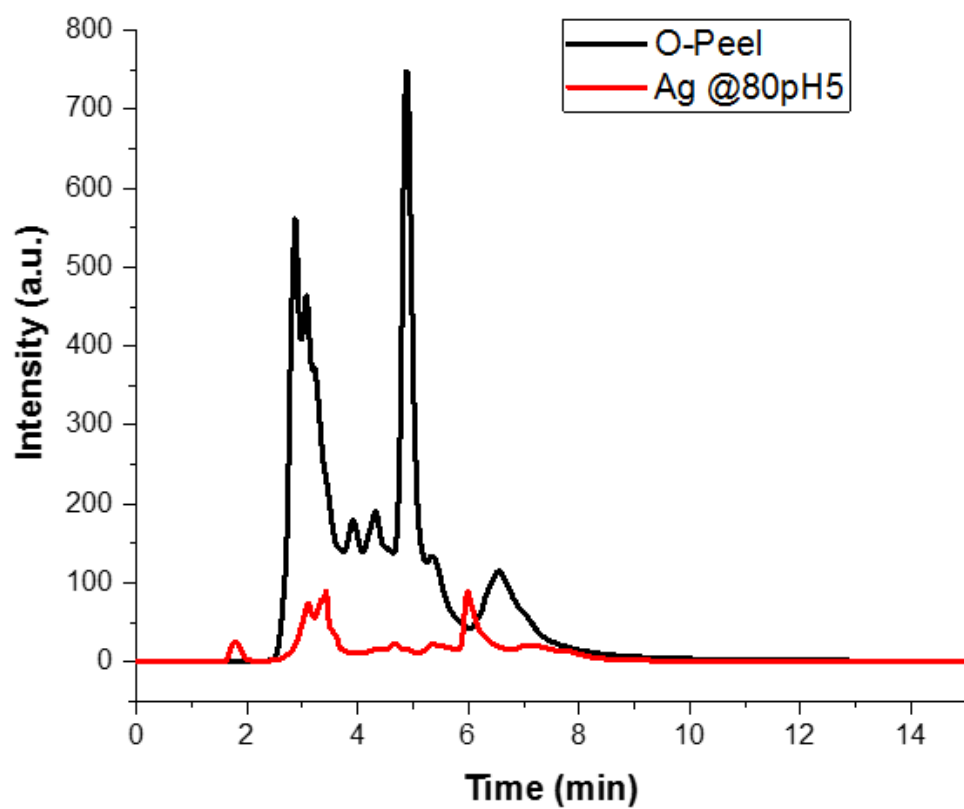


Figure 21. High performance liquid chromatography profile overlay of Navel orange peel extract before reduction and after reduction at pH5.

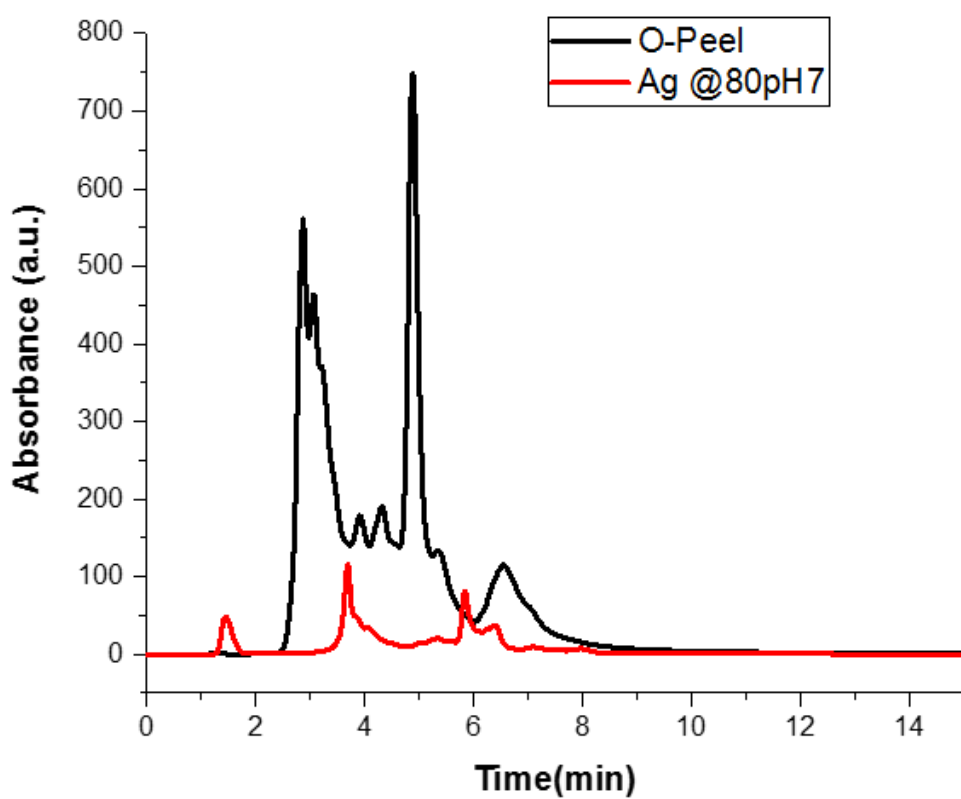


Figure 22. High performance liquid chromatography profile overlay of Navel orange peel extract before reduction and after reduction at pH7.

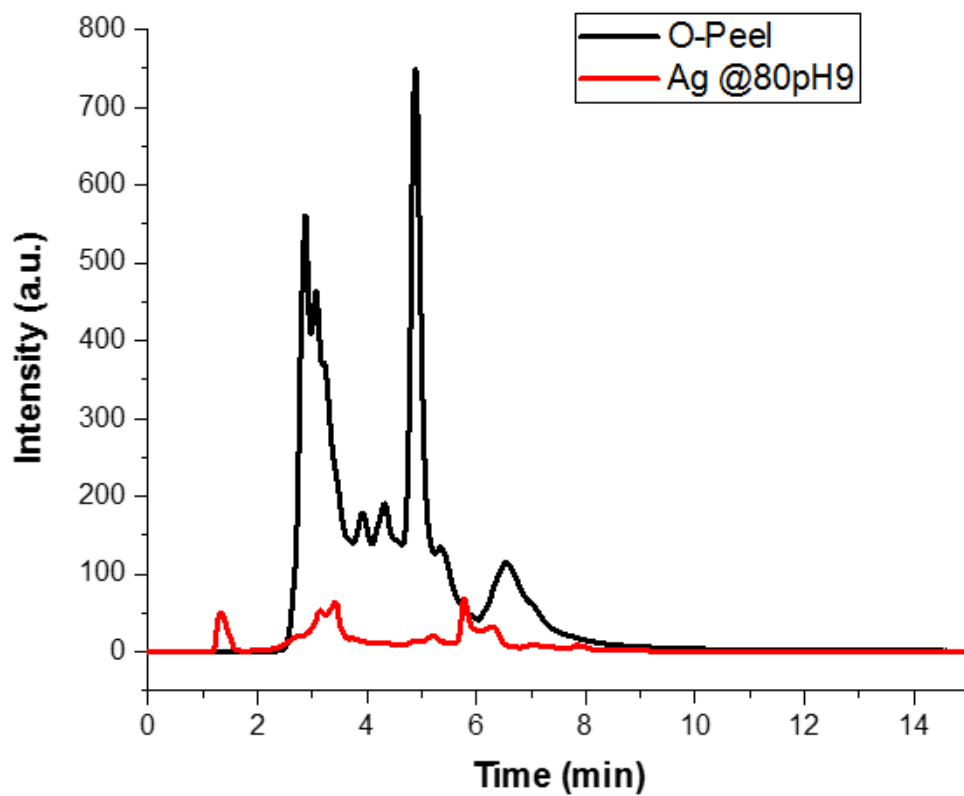


Figure 23. High performance liquid chromatography profile overlay of Navel orange peel extract before reduction and after reduction at pH9.

CHAPTER IV

CONCLUSION

In this work, we present a green synthesis approach to prepare silver nanoparticles using aqueous extracts of orange peels. Reduction of silver nitrate with orange peel extract is a simple, conducted at room temperature, efficient, and a clean method to synthesize silver nanostructures. After extensive literature survey protocol has been optimized for rapid and high yield of AgNPs. Proteins, flavonoids and phenols play an important role in the green synthesis of AgNPs as well as the stabilization of the NPs. For biological applications such as medicine nanoparticles should be free from toxic compounds and have good adsorptivity by biomolecules. So due to its huge potential in medicines these green or biologically friendly synthesis are important. As well characterization plays a key factor for the potential applications. This simple, low cost, and green method for synthesizing silver nanoparticles may be valuable in future biological studies and may be extended into catalytic applications.

Despite the environmental advantages of using green chemistry based biological synthesis over traditional methods as discussed in this thesis there are still some unresolved issues such as particle size and shape consistency, reproducibility of the synthesis process, and understanding of the mechanisms involved in producing metallic nanoparticles through biological compounds. In the case of plant extracts, nanoparticle formation mechanisms vary between different plant species. Therefore, there is a need for more studies to evaluate and

understand the actual plant dependent mechanism. Due to rich biodiversity of plants they are potentially used in the ecofriendly and compatible manner for nanoparticle synthesis which are explored in pharmaceutical and biomedical industries such as medicine, food industries and agriculture. This green chemistry approach has many advantages such as waste utilization and provide renewable stock to nanoparticle market. This work shows the successful synthesis of silver nanoparticles through a very simple, cost effective and green method.

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BIOGRAPHICAL SKETCH

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