

Controllable Synthesis and Characterization of Silver Nanoparticles Using *Sargassum Angostifolium*

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ABSTRACT: A simple, fast and one-pot synthesis procedure based on the bioreduction ability of an algal extract solution has been developed to produce silver nanoparticles (AgNPs). We focus especially on the influencing factors of AgNPs formation. The effects of various factors like pH, temperature, the concentration of metal ions and alga, the volume ratio of silver nitrate solution to alga extract, reaction time, mixing order and the mixing rate of the silver nitrate and the alga extract on the AgNPs synthesis are evaluated. The obtained colloids have been characterized by UV-Vis spectroscopy, TEM, XRD, and FT-IR. TEM image of the silver colloids shows that the AgNPs are predominantly spherical around average particle diameters of 32 ± 10 nm. FT-IR study indicates that the carboxyl ($-C=O$), hydroxyl ($-OH$), and amine ($N-H$) groups in alga extract are mainly involved in the reduction of Ag^+ ions to AgNPs. The XRD spectrum clearly shows that the AgNPs are crystalline in nature. This work may prove to be of great value in synthesizing nanoparticles with well-controlled sizes in chemical, pharmaceutical, and materials production processes.

KEYWORDS: Silver nanoparticles; *Sargassum Angostifolium*; Alga.

INTRODUCTION

The synthesis of nanomaterials is of current interest due to their wide variety of applications in fields such as electronics [1,2], photonics [3,4], catalysis [5,6], medicine [7,8], etc. Most of the applications are due to the fact that matter at the nanometer scale has different properties as compared with the bulk state. For this reason, many research groups around the world are trying new methods of synthesis of different materials at the nanoscale.

Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, catalytic activity, optical properties,

electronic properties, and antibacterial properties [9,10] they are gaining the interest of scientists for their novel methods of synthesis.

Silver nanoparticles have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, sensing/optical, antimicrobials, and biomaterial production [11,12]. In medicines, silver and silver nanoparticles have a wide application including skin creams containing silver to prevent infection of burns and open wounds [13], medical devices and implants prepared with silver-impregnated polymers [14,15]. Also, AgNPs are excellent components for the Surface Enhanced Raman

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Scattering (SERS) to probe single molecules [16], in addition to their desirable role as catalysts for speeding up some chemical reactions [17].

Metal nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Chemical methods employ toxic chemicals as reducing agents, organic solvents, or non-biodegradable stabilizing agents and are therefore dangerous to the environment and biological systems [18]. Moreover, most of these methods are quite complicated, expensive and involve non-standard conditions. The biosynthesis of nanoparticles is now established as a cost-effective and environmentally friendly alternative to chemical and physical methods. Many sources such as microorganisms [19] and plant materials [18] were explored in the past. The use of plant extracts for the synthesis of nanoparticles is advantageous over microorganisms because it does not involve the elaborate process of maintaining cell cultures, or bio hazards and is easy to scale-up [20]. Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. Much of the published research is limited to terrestrial plants, but relatively few publications exist for the synthesis of nanoparticles from marine plants [21-24] Marine macro algae produce a great variety of secondary metabolites that showed therapeutic potential [25]. *Sargassum angostifolium* is a brown alga and is known to have beneficial properties such as antioxidants, antibacterial, immune-stimulating and also Acetylcholinesterase activity) AChEI) which encouraged us to carry out the present investigation on the synthesis of AgNPs using it [26]. However, it is very desirable to devise alternative, 'green' methods of nanomaterial preparation that use environmentally friendly reactants. The silver nanoparticles obtained by the green synthesis method are candidates to be used in biological systems. The present work is part of this new line of research. To the best of our knowledge, there is no report in the literature on the synthesis of silver nanoparticles using the extract of the *S. Angostifolium*. Also, the influences of the synthesis conditions including the reaction temperature, pH, volume ratio of silver nitrate solution to alga extract, reaction time, mixing order and mixing rate of the silver nitrate and the alga extract were examined.

EXPERIMENTAL SECTION

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade. Silver nitrate (AgNO_3) was purchased from Merck. The pH adjustments were performed with 0.1 M HNO_3 and 0.1 M NaOH solutions.

Apparatus

UV-Vis spectrophotometer (Analytik-Jena) equipped with a 1-cm quartz cell was used for recording the visible spectra and absorbance measurements. The XRD measurements were performed on the XRD Bruker D8 Advance. The FT-IR spectra were recorded on a Perkin Elmer FT-IR (Spectrum RXI). A Transmission Electron microscope (Zeiss-EM10C) was used for recording of TEM images. The freeze-drying process was performed on an Operon model freeze-dryer with the temperature maintained at $-55\text{ }^\circ\text{C}$ (Seoul, Korea). An InioLab WTW 730 pH meter was used for monitoring the pH values.

Preparation of alga extract

The alga extract used for the reduction of silver ions to silver nanoparticles was prepared by placing 10 g of washed dried fine cut alga in 250 mL glass beaker along with 100 mL of distilled water. The mixture was then boiled for 15 minutes until the color of the aqueous solution changes from watery to light yellow color. Then the extract was centrifuged at 6000 rpm for 30 min to remove the heavy biomaterials before filtering with Whatman No. 1 filter paper. The filtrate was collected and stored at $4\text{ }^\circ\text{C}$ in order to be used for further experiments.

Synthesis of silver nanoparticles

The Round-bottom flask containing 1 mM of AgNO_3 was reacted with the prepared alga extract. The alga extract was added to the aqueous AgNO_3 solution and incubated in dark under optimum conditions. The samples changed their visual appearance shortly after addition of the extract, indicating that a reduction reaction took place.

RESULTS AND DISCUSSION

Characterization of Silver nanoparticles

The AgNPs were centrifuged at 10000 rpm for 30 min to isolate the silver nanoparticles from alga materials or other compounds present in the solution, and the residue

was collected, washed three times with double-distilled water and freeze dried. The dried powder was used for XRD and FTIR analysis.

UV-vis absorbance study

Synthesis of the AgNPs has been confirmed by measuring the UV-vis spectra of the reaction mixture. The analysis was performed at room temperature using quartz cuvettes, and the blank was the corresponding *Sargassum* extract aqueous solution. The addition of alga extract to 1 mM AgNO₃ solution resulted in the color change of the solution from light yellow to brown due to the production of silver nanoparticles. The characteristic brown color of silver solutions provided a convenient spectroscopic signature to indicate their formation. As apparent from Fig. 1, the absorption peak appeared at about 428 nm is corresponding to the characteristic surface plasmon resonance of the resulting AgNPs [27].

TEM analysis of silver nanoparticles

TEM image of AgNPs synthesized using *Sargassum* extract is given in Fig. 2. TEM picture clearly shows that the AgNPs are spherical in shape and not in contact with each other. The average size of the AgNPs was 32±10 nm.

FT-IR spectroscopic study

In order to understand the formation mechanism of AgNPs, FT-IR spectroscopy was used to probe the chemical composition of the surface of the AgNPs, and the local molecular environment of the capping agents on the nanoparticles. Curves a, b in Fig. 3 show the FT-IR spectra of the alga extract and the AgNPs, respectively. The FT-IR spectra of the nanoparticles and the extract are similar to one another, indicating that the organic molecules have indeed become a part of the nanoparticles. However, a difference in the peak intensity is found between the peaks in parts a and b of Fig. 3. Those peaks correspond to the amide I and amide II bond of proteins (1655, 1421 cm⁻¹) which arise due to carbonyl (C=O) and amine (-NH) stretching vibrations in the amide linkages and OH stretching in alcohols and phenolic compounds (3385 cm⁻¹), respectively [28]. The absorption peak at 1655 cm⁻¹ is close to that reported for native proteins [29] which suggest that proteins are interacting with biosynthesized nanoparticles and also

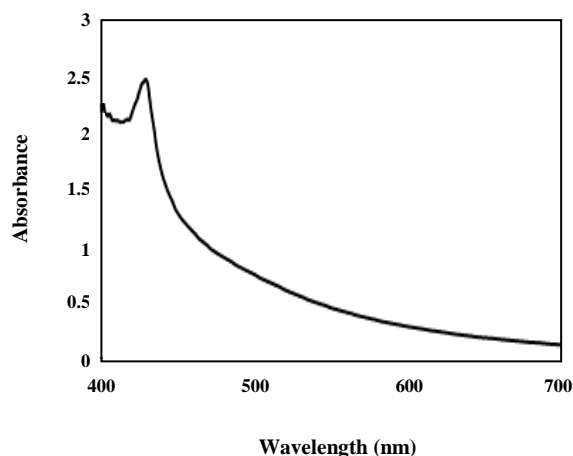


Fig. 1: UV-vis spectrum of synthesized silver nanoparticles.

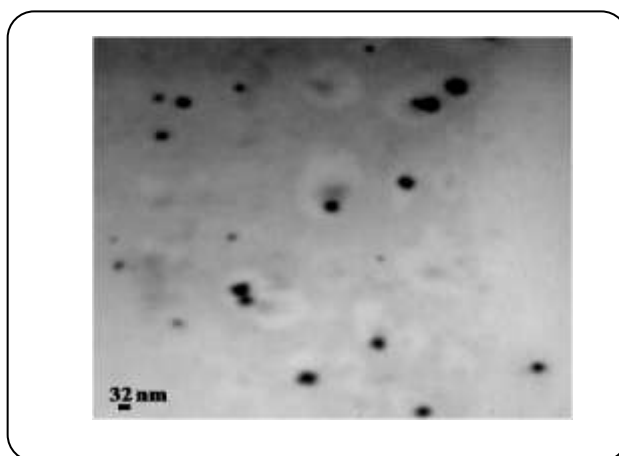


Fig. 2: TEM image of synthesized silver nanoparticles.

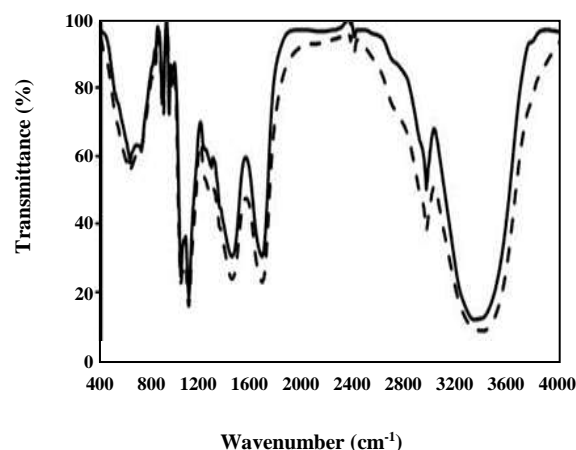


Fig. 3: FT-IR spectra of (a) alga extract and (b) the synthesized silver nanoparticles.

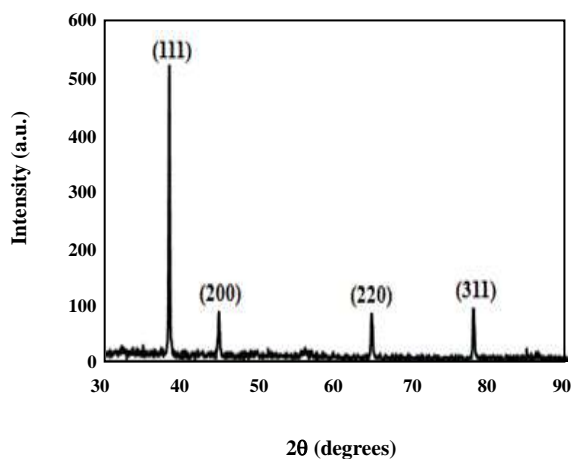


Fig. 4: XRD pattern of silver nanoparticles synthesized using alga extract.

their secondary structure was not affected during reaction with Ag^+ ions or after binding with AgNPs [30]. FT-IR study indicates that the carboxyl ($-\text{C}=\text{O}$), hydroxyl ($-\text{OH}$), and amine ($\text{N}-\text{H}$) groups in alga extract are mainly involved in the reduction of Ag^+ ions to AgNPs. In 2010, *Sathyavathi et al.* confirmed that carbonyl group of amino acid residues have a strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as a capping agent to prevent agglomeration and providing stability to the medium [31]. These results confirm the presence of possible proteins acting as reducing and stabilizing agents for silver nanoparticles. Thus, it is reasonable to consider that AgNPs are capped by biomolecules in the *Sargassum* extract.

XRD studies

Analysis through XRD was carried out to confirm the crystalline nature of the silver nanoparticles (Fig. 4). A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.45° , 44.80° , 64.25° , and 77.25° corresponding to (111), (200), (220), and (311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver. X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the *Sargassum* extract are crystalline in nature.

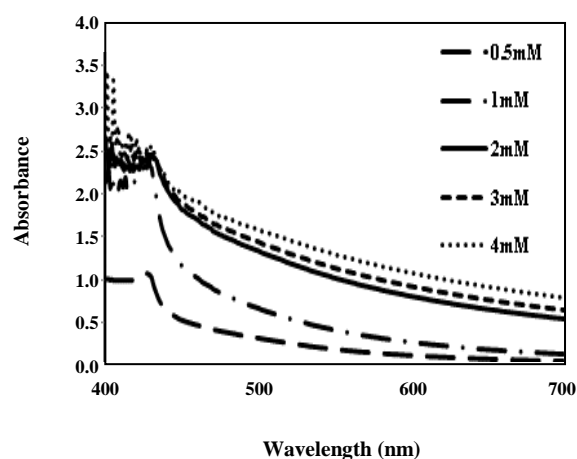


Fig. 5: UV-Vis spectra of silver nanoparticles obtained at different AgNO_3 concentration.

Effect of different factors on the synthesis of silver nanoparticles

Different factor like pH, temperature, concentration of metal ions and alga, volume ratio of silver nitrate solution to alga extract, reaction time, mixing order and the mixing rate of the silver nitrate and the alga extract play a major role in the synthesis of nanoparticles.

Effect of AgNO_3 concentration

Effect of AgNO_3 concentration on the formation of AgNPs was studied with the increase of the concentration of AgNO_3 from 0.5 to 4 mM. As shown in Fig. 5, the intensity of SPR band was increased while increasing the concentration of silver ions, and then kept constant. Increasing intensity indicates increasing concentration of nanoparticles. But, the plasmon peak shifts toward higher wavelengths (red shift) from 426 to 431 nm, meaning an increase of the particle size [33], when AgNO_3 concentration changes from 0.5 to 4 mM. At a higher concentration, the AgNPs begin to aggregate and form into large particles [33]. *Zhang et al.* were believed that this phenomenon come from two possible reasons: (1) The formation rate of nuclei increases more significantly than the growth rate of silver nanocrystals with increasing AgNO_3 concentration [34]. As a result, large numbers of tiny nanoparticles with the high special surface area are formed. Due to the adsorption of capping agents on the particle surface, aggregation between the particles is inhibited, which is favorable to form fine nanoparticles. (2) A large

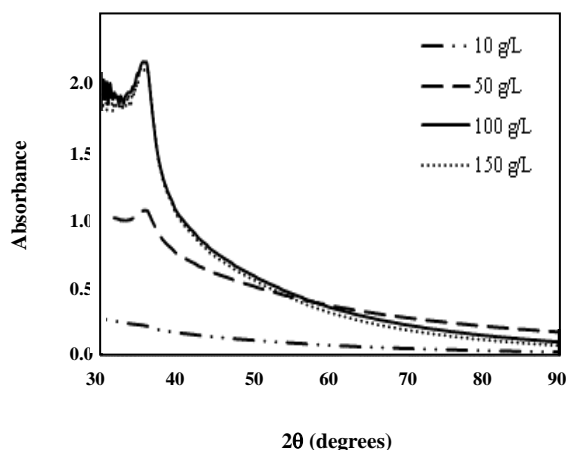


Fig. 6: UV-vis spectra of silver nanoparticles prepared using 10, 50, 100 and 150 g/L of alga as the reductant.

a number of silver nanoparticles are formed at a higher concentration of AgNO_3 . As a result, the collision frequency increases significantly, which results in aggregation of the particles. This investigation concludes that the optimum AgNO_3 concentration of 1 mM is suitable for nanoparticles synthesis.

Effect of concentration of reducing agent

The effect of different concentrations of reducing agent (10-150 g/L^{-1}) on AgNPs synthesis is shown in Fig. 6. The observed increase in the plasmon absorbance with increasing alga concentration indicates a greater amount of Ag^+ reduction. The decrease seen in the maximum absorbance in lower concentrations (10,50 g/L) shows that a fraction of the Ag^+ ions is likely to remain unreduced at low alga concentrations. Fig. 6 shows that by increasing the concentration of alga up to 100 g/L , the absorbance increased. This could be explained by this fact that more algae became available for reducing the Ag^+ ions to Ag colloids. Further addition of the reductant did not show any significant change in absorbance. Thus, 100 g/L^{-1} of the algae was chosen as the optimum concentration for performing the following steps of the optimization procedure.

Effect of volume ratio of silver nitrate solution to alga extract

To investigate the effect of the volume ratio of "AgNO₃ solution: alga extract" on the formation of AgNPs, the reagent mixing volume ratios was varied in such a way

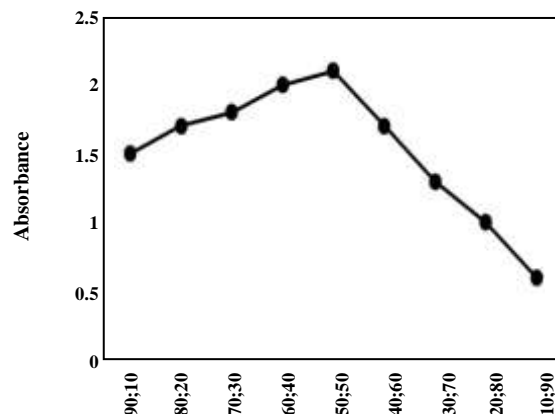


Fig. 7: Absorbance intensity of silver nanoparticles at 428 nm at the different volume ratio of the extract and the AgNO_3 solution.

that the final volume of the solution remained constant. Different volume of the extract and the AgNO_3 solution were mixed thoroughly (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10) with a stirring time of 90 minutes and temperature of 70 °C. As shown in Fig. 7, with increasing volume of extract, the intensity of the SPR band increased, with further higher volumes of extract the particles were not stable. According to literature, an excess of reducing agent may result in instantaneous particle precipitation [35]. The uncoated particles undergo uncontrolled growth and aggregation phases. The 50:50 volume ratio corresponding to the stability condition with the optimum reagent ratio. Within this range, a complete coating of the particles occurs.

Effect of temperature

In order to see the effect of temperature on the formation of AgNPs, 50 mL of the extract was added to 50 mL of 1 mM aqueous silver nitrate solution and the reaction temperature was varied from 35 to 85 °C for 90 minutes. Fig. 8 displays a plot of the plasmon intensity at 428 nm against temperature. With the increase of temperature, the reduction occurred very fast and the intensity of SPR band also increased. However, due to energy saving temperature 70 °C was chosen for performing of the optimization procedure.

Effect of reaction time

In order to determine the kinetics of formation of AgNPs, we followed the reduction of Ag^+ using alga extract

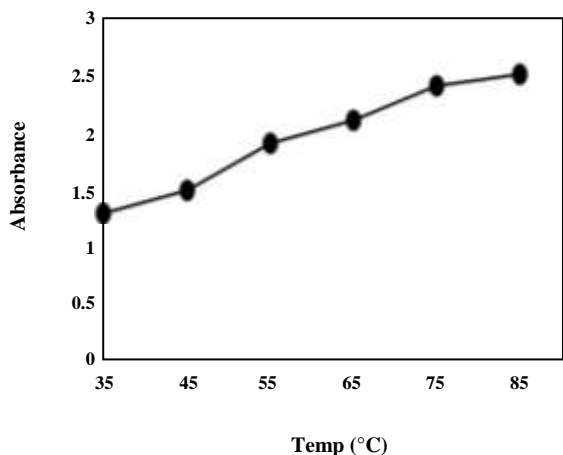


Fig. 8: Absorbance intensity of silver nanoparticles at 428 nm at different temperatures.

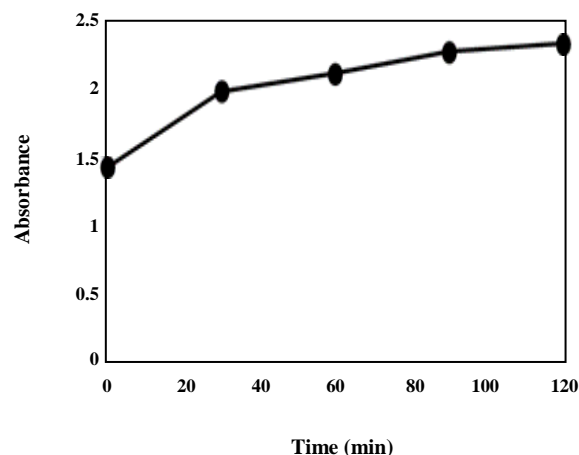


Fig. 9: Absorbance intensity of silver nanoparticles at 428 nm at different reaction times.

by monitoring the absorbance changes at different time intervals. Samples were withdrawn at different reaction times for recording absorption spectra. Fig. 9 displays a plot of the plasmon intensity at 428 nm against reaction time. At the beginning of the addition of reducing agent, spectra were taken from 0 till 120 min to find when the formation of silver nanoparticles starts. It was also observed that the production of nanosize silver particles starts almost immediately on addition of the reducing agent, and continues throughout the investigated period, as indicated by the emergence and the progressive increase in the intensity of the well-defined plasmon band. The increase in absorbance is observed when the rate of silver nanoparticles formation has increased and more particles are formed during the same time. It can be seen easily that the plasmon intensity at the reaction time of 90 minutes is near to that at 120 min, meaning completion of the reaction.

Effect of mixing order and the mixing rate of the silver nitrate and the alga extract

It was found that colloids with different particle size could be obtained by changing the mixing order and the mixing rate of the silver nitrate and the extract solution involved in silver colloid synthesis. In a typical experiment, an aqueous solution of AgNO_3 was heated up to 70 °C. The reducing agent, alga extract was then added (direct method). The same experiment was repeated by maintaining the amounts of reagents but changing

the order of reagents addition (inverse method). All reactions were maintained at 70 °C for 90 minutes. Fig. 10A shows the TEM and UV-Vis spectra of final AgNPs synthesized following the above-detailed conditions. Surprisingly, the effect of exchanging the order of reagent addition resulted in a blue shift of the SPR peak from 429 nm (inverse synthesis) to 427 nm (direct synthesis) together with an increase of the maximum absorbance value. Considering that the SPR absorption peak is indicative of particle size [32], this result indicates that AgNPs synthesized by the direct method are smaller than those synthesized by the inverse one. TEM images (Fig. 10A) clearly confirmed a decrease of the mean particle size from ~40 nm (inverse synthesis) to ~32 nm (direct synthesis).

To find out if the method of mixing affected the resulting silver particles, the reactant solutions were combined rapidly and drop wise, while keeping all other conditions the same. As can be seen in Fig. 10B, an increase of the maximum absorbance value was obtained when 50 mL of extract solution was added to 50 mL of a silver nitrate solution (10^{-3} M) drop wise. The drop wise addition of the reducing agent probably leads to a seeding effect resulting in a growth of the silver particles [35,36]. It is also worth noticing that during the synthesis of the colloids stirring is not necessary (to check the effect of stirring on the synthesis of AgNPs, all colloids were prepared under vigorous stirring and without stirring). However, the colloids produced in this study were stirred to ensure a reproducible preparation protocol.

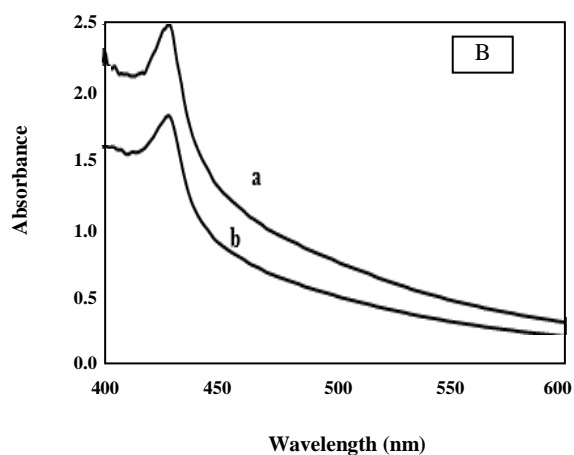
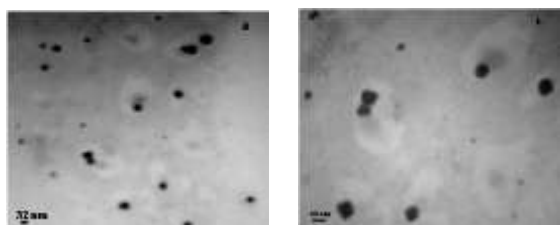
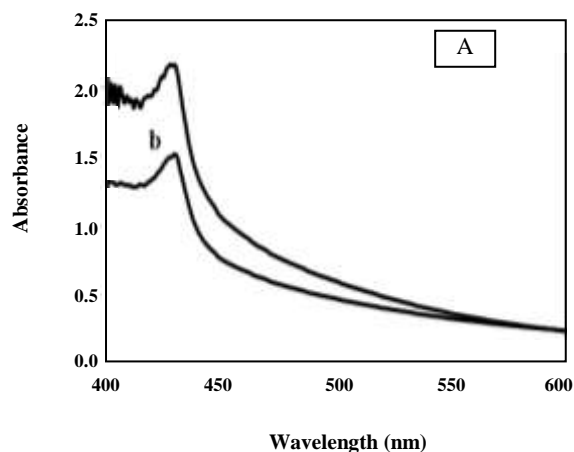


Fig. 10: (A) TEM and UV-Vis spectra of silver nanoparticles synthesized under (a) direct, (b) inverse sequence of reagents addition; (B) UV-vis spectra of silver nanoparticles synthesized under (a) drop-wise addition of extract, (b) rapid addition of extract.

Effect of pH

To see the effect of pH on the formation and the stability of AgNPs, the reactions were carried out at different pH ranging from 2 to 10 by adjusting with 0.1 M HNO₃ and NaOH. In the variation of pH, first, the pH of the extract was adjusted before adding to the silver nitrate solution. The acidic pH suppresses the nanoparticle formation. At low pH, agglomeration took place because of the over nucleation and formation of larger nanoparticles [37]. In alkaline pH, the formation of AgNPs was instantaneous and an intense SPR band was observed. At pH 10 the maximum of the absorption band is shifted to 424 nm that is indicative of smaller size silver particles. At high pH, a large number of nanoparticles are present due to the bioavailability of functional groups in the extract. The increase of pH presumably facilitated the ionization of -OH and -COOH groups of biomolecules (probably proteins) present in the extract. Thus formed -O⁻ could reduce Ag⁺ ions into AgNPs, which in turn got oxidized to -C=O. The AgNPs thus formed are stabilized by -COO⁻ groups present in the molecules, which is evidenced by the better stability of particles formed under alkaline pH. *Andreescu et al.* reported a similar pH effect and rapid reduction at high pH [38]. So the alkaline pH 10 was favorable for the nanoparticle formation (Fig. 11). It was proposed that the silver ions undergo electrostatic interaction with the proteins present in the extract which leads to the formation of the silver complex.

CONCLUSIONS

Our investigation has demonstrated the high importance of a comprehensive study of nanoparticle synthesis conditions, which has not been reported previously. The data obtained show that the proper selection of the reagent ratio, synthesis temperature, pH, concentration of reactants, mixing rate and mixing order makes it possible to obtain outstanding results on such qualitative parameters as the mean particle size. Synthesis of AgNPs could be achieved in one step, which simplifies operations and reduces the cost. This is an economic method and excludes the use of external stabilizing/capping agents. The data obtained will allow the acquired knowledge to be extended to other systems and will provide researchers with a more convenient, practical, and cost-effective method of nanoparticle synthesis. The controllable synthesis of

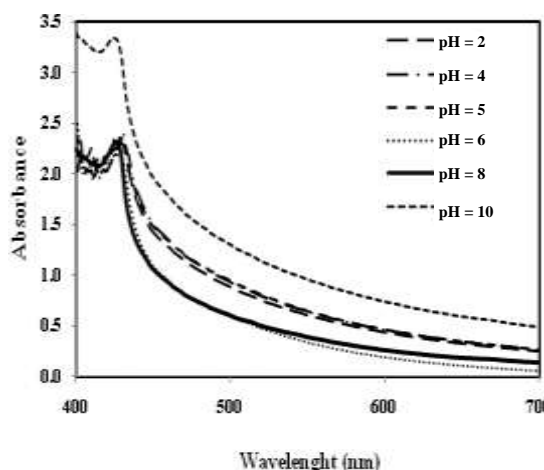


Fig. 11: UV-Vis spectra of silver nanoparticles obtained at different pH.

silver nanoparticles was obtained by this method, opening a new route to study antibacterial properties of this nanomaterial.

One interesting area of further research will be to investigate the antibacterial properties of AgNPs. Another subject of interest that calls for a subsequent, is synthesizing magnetic nanoparticle coated with these AgNPs. Combination of magnetic and antibacterial features, exhibited by magnetic@silver nanoparticle, predestinates to exploit them in medicine where they can be used for a targeted transport of antimicrobial agent and its subsequent removal by an external magnetic field.

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