

Biogenic Synthesis of Silver Nanoparticles by the Action of Anthocyanin Extracted from Rose Petals, Soybeans, Red Cabbage and Rice and their Characterizations

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ABSTRACT

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The biogenic route is used for the synthesis of silver nanoparticles in such a way that anthocyanin was extracted from the rose petals, soybean, red cabbage, and rice treated with 0.1M AgNO₃. Silver nanoparticles are the center of attention of researchers from all around the globe due to their reminiscent properties (Physical & Chemical). Herein this reports a fixed ratio of the extract was prepared and mixed with a solution containing metal ions. The color of the solution was changed which confirms the preparation of silver nanoparticles. The color change indicates the formation of silver nanoparticles at initial stages while structural studies and characterization with UV-Vis, FT-IR, and SEM provided further details about the prepared silver nanoparticles. SEM analysis provided the details about the size and morphology of the synthesized silver nanoparticles. The size of prepared nanoparticles was found to be 34 nm and the nanoparticles are having spherical morphology.

Keywords: Nanomaterials, Silver Nanoparticles, Biological Synthesis, Characterizations, Anthocyanin, Green Synthesis of Nanoparticles

I. INTRODUCTION

Nanotechnology is one of the emerging fields of material science in recent years and it is being used in almost every aspect of daily life. Nanoparticles are the substance having the size in between 0-100nm so they are having a very small size also they have a high surface to area ratio [1]. It is, for this reason, they are nowadays the most important material having extraordinary physical and chemical properties.

Nanoparticles can be prepared by various methods mainly categorized in chemical, physical, and biological methods [2]. Among all of the methods, the biological method is a safe, economic, and non-hazardous one as in this method natural products are used for the synthesis of nanoparticles like microorganisms and plants. It is therefore also termed as green synthesis [3]. The use of the plant for the synthesis of the nanoparticle is the most common route used for the synthesis of nanoparticles, the plant

is either used as a whole or some parts of the plant are used to synthesize nanoparticles of various metal and metal oxides [1, 4-6]. In this method extract of some plant is used for the synthesis of nanoparticles the extract can be prepared from the whole plant or leaves, stem, roots, flower, and fruit depending on the number of biomolecules present in the plant. These biomolecules are actually phytochemicals such as flavonoids, fatty acids, terpenoids, polyphenols, etc they have excellent reducing power and can reduce metal ions into metal nanoparticles [7]. Thousands of studies have been reported showing the synthesis of nanoparticles of different elements by the use of different plant extracts as reducing agents. Studies have also shown that the plant extract not only functions as a reducing agent but it can act also as a capping agent in addition to its function as a reducing agent because the biomolecules present in the extract of plant surround the synthesized nanoparticles to prevent them from agglomerations [7].

Different plants and different parts of the plants can be used for the synthesis of nanoparticles because of the availability of phytochemicals. Each plant has a different concentration of phytochemicals and each part of the plant is enriched with different phytochemicals, so every plant, as well as every part, has its own importance and role in the synthesis of nanoparticles [2]. The temperature and pH have a very important role in the synthesis of nanoparticles so it's necessary to perform the experiment in the controlled pH level and temperature in order to obtain the nanoparticles of the desired size and shape [6]. The reason behind this is that the nature of phytochemicals in plant extract is different and temperature-sensitive so it can disturb the size as well as the morphology of nanoparticles [8].

As discussed earlier that nanoparticles can be synthesized by several chemical and physical methods but those methods have several drawbacks such as the use of harsh and toxic chemicals during the process. Toxic and harmful gases are evolved and chemical by-

products are formed during the synthesis of nanoparticles through physical and chemical processes[9]. These gases are not only harmful to humans but also to plants and animals in fact they are harmful to the whole ecosystem. The loophole of these methods is that they involve the use of some expensive chemicals and instruments to prepare nanoparticles which is a challenge to the developing countries [9]. The demand of nanoparticles had been increasing day by day because of their wide application in almost every modern technological aspect so the researchers focused on cost-effective novel methods for the preparation of nanoparticles to meet the need of nanoparticles of the modern world and they introduced the biological synthesis of nanoparticles which is a non-expensive and non-toxic method and excellent solution for developing countries to produce nanoparticles in cheap ways.

Nano-silver (Ag-NPs) is one of the most widely studied noble metal nanoparticles and has a wide range of applications in almost every field of the modern world. Ag-NPs are the most common part of electric batteries, widely used in ceramics pigment and glass [10]. Silver nanoparticles have the most important applications in the devices used to treat deleterious diseases such as HIV, TB, cancer, malaria, and diabetes mellitus [11-13]. Silver nanoparticles can be used in the detection of biological molecules and also they have excellent catalytic properties so they can be used in various reactions as a catalyst [14-17]. Silver nanoparticles are plentifully being used in the dermatological sector as they can be used in creams and skin ointments for the prevention of infection in open wounds and burns [18-19], silver-impregnated polymers are used to manufacture implants and medical devices [20]. Sports equipment now came with silver embedded fabrics, which is also a major use of Ag-NPs in the textile industry [21].

We reported in this paper the synthesis and characterization of silver nanoparticles for which we used four different extracts i.e. the extract prepared

from rose petals, extract prepared from soybean, the extract prepared from rice, and the extract prepared from rice powder. Each extract was treated with a fixed amount (fixed ratio) of silver nitrate to prepare silver nanoparticles. The Ag-NPs synthesized by the use of each extract were later on subjected to structural characterization to study in more detail.

II. METHODS AND MATERIAL

2.1. Materials and Techniques:

Silver nitrate (AgNO_3), Anthocyanin, Methanol, Ethanol, Salt, Nitric Acid, Base, Rose petals extract, Soybean extract, Rice extract.

Beakers, Digital balance, volumetric flask, Pipette, Whatman filter paper no. 1, Dropper, Test tubes, Sample holders, UV cuvette.

2.2. Preparation of extracts:

2.2.1. Preparation of rose extract:

Fresh rose petals (*rosa indica*) were collected from different departmental lawns of Kohat University of Science and Technology, Pakistan. 15g of rose petals were washed with distilled water several times to remove the entity of small impurities and dust particles and they were placed under sunlight for 10 minutes after which the petals were put into a 500 ml clean & dry beaker and 250 ml of deionized water was poured in that beaker. The supernatant was heated for 30 minutes the color of the water turned deep red and then it was left for cooling to room temperature. At last, the mixture was filtered with whatman filter paper 1 to obtain clear and fine extract.

2.2.2. Preparation of red cabbage extract:

The leaves of red cabbage were collected from the B1 hostel of Kohat University of Science and Technology, Pakistan. They were washed and placed under sunlight after removing dust and other impurities. 10g of red cabbage leaves were taken out into a clean 500ml beaker into which 150ml of double distilled

water was added and shake to mix well. The mixture was heated constantly for 25 minutes and the color of the mixture turned reddish-green. The heating was stopped after 25 minutes and the mixture was filtered with whatman filter paper 1 after cooling to room temperature. The extract prepared is enriched by many phenolic compounds but especially anthocyanin as red cabbage is a rich source of it. The useful impact of this is that it is a local natural product and non-expensive.

2.2.3. Preparation of soybean extract:

Soybean is also proven to be a rich source of phenolic compounds especially anthocyanin but the use of soybean for nanoparticles have a small deficiency that soybean is a little bit expensive as compared to red cabbage. For the preparation of extract of soybean 55g of soybean was used, the extract was prepared after crushing and grinding of soybean into fine powder form. The powdered soybean was dissolved in 150 ml of methanol by constant stirring for 20 hours by placing the mixture on a magnetic stirrer. The extract prepared by filtration of the mixture and the filtrate was placed for 1 day so that the remaining precipitate or impurity would be settled down and after this, it was once again filtered with whatman filter paper to make sure there is no impurity or solid particle is present. The prepared extract was separated into different components by the process of centrifugation. In this process, a small amount of extract was epi dripped and centrifuged for about 1 minute after which the extract was finally separated into two components of low density and high density so that anthocyanin could be separated which was done and the separated anthocyanin was collected in a clean and dry bottle, stored for future use.

2.2.4. Preparation of rice extract:

Rice is also known for its rich phenolic contents such as anthocyanin which is used here to synthesis silver nanoparticles. The rice used in our reports is known as kurrami chawal locally and they were collected from a

local area i.e Kohat, Pakistan. 35g of rice was taken for the preparation of extract, for this purpose they were grounded into a fine powder and the powder was then put into a 250ml beaker following the addition of double-distilled water. The mixture was placed on a magnetic stirrer for better mixing for about 10 hours after which filtered and separate the extract into different components by the process of centrifugation.

III. SYNTHESIS OF SILVER NANOPARTICLES

3.1. Rose petals extract and silver nitrate

First of all 100ml of stock solution of silver nitrate was prepared and extracts of rose petals, red cabbage, soybean, and rice were prepared as described earlier. Now several sets of experiments were carried out in which the extracts of each source were mixed with silver nitrate solution in a different ratio. The initial mixing of rose petals and silver nitrate solution was done to prepare a total of 3 samples in the following way;

The 1st sample was prepared when 10 ml of silver nitrate solution was taken in a separate beaker and now rose petal extract (anthocyanin) was allowed to put into silver nitrate solution drop-wise. The rose petals extract's drops were added in a sequence of 2 drops, 3 drops, and 5 drops making 11 ml total solution volume. After the mixing of silver nitrate solution with the extract of rose petals, the whole solution was take taken into a separate bottle and covered with aluminium foil so that no solar light could interact with the solution and to prevent it from photodegradation.

In the same way, 2nd sample was prepared but the amount of silver nitrate solution was reduced from 10 ml to 5 ml and similarly, 2 drops, 3 drops, and 5 drops of rose petals extract (anthocyanin) were added and covered with aluminium foil.

The 3rd sample was prepared a little bit differently it was made in observation to see that the synthesis of silver nanoparticles is influenced by the amount of

reducing agent. Finally when all 3 samples were prepared then they were incubated for the last three days to see what happened to silver nitrate with the addition of anthocyanin so a very clear change was observed which was the color change of the solutions which was totally different from the initial stage colors, this was the first indication that silver is oxidized and then UV-Vis spectroscopy was carried out for further confirmation of nanoparticles. Next is rice extract

3.2. Soybean extract with silver nitrate:

Soybean is among the richest sources of natural flavonoids especially anthocyanin the main component of our research to synthesize silver nanoparticles. The procedure of extract preparation is described earlier now 3 separate test tubes were taken and 10ml of silver nitrate was added into each of the test tubes after which the soybean extract was added in a drop-wise manner i.e. 2 drops, 3 drops, and 5 drops of the extract was added in each of the test tubes by the help of dropper now these 3 samples were wrapped with aluminum foil and kept in dark for 2 days. The next sample was prepared in the same manner with the only difference was that the concentration of silver nitrate was reduced from 10 ml to 5 ml in each of the test tubes while the 3rd sample was prepared differently in such a way that 3 test tubes were taken and a different ratio of extract to silver nitrate was used i.e. in 1st test tube 5 ml of silver nitrate was mixed with 1 ml of extract and in second the concentration of the extract was increased to 1.5 ml and in 3rd beaker 2 ml of soybean extract while keeping a 5 ml constant concentration of silver nitrate solution.

All of the samples were kept in dark condition for 2 days for incubation afterwards the samples were studied with the help of UV spectroscopy which confirmed the formation of silver nanoparticles as the absorption spectrum showed peaks at different wavelengths and the color of the extract which was

yellow initially turned to fume type after the addition of silver nitrate solution.

3.3. Rice extract with silver nitrate:

Rice is not a good source as compared to the other extract used in our report just because the amount of anthocyanin is less in rice extract than others used here in this report but we used to understand the effect of different extracts on the synthesis of silver nanoparticles. A constant volume of silver nitrate was taken into 3 test tubes and rice extract was added drop-wise in the sequence of 5 drops, 7 drops, and 10 drops respectively and the samples were placed in a dark condition to prevent them from photodegradation. The second attempt was quite different from all; by taking a small amount of stock solution and an excess amount of rice extract to observe the effect, for this purpose addition of plant extract was done such a way that 2 ml, 3 ml, and 5 ml of extract were mixed a fixed volume of silver nitrate i.e 5 ml of each and again to prevent these sample they were kept in dark condition for two days. UV studies were carried out after two days which clearly indicates the formation of silver nanoparticles.

3.4. Red cabbage extract and silver nitrate

Red cabbage is one of the most favorable sources of anthocyanin because it is commonly available, cheap, and non-harmful. The extract was prepared by the procedure as described earlier now the extract was mixed with silver nitrate in a different ratio to obtain silver nanoparticles. Here two sets of solutions were prepared each containing 3 solutions making a total set of six solutions. The 1st set sample was prepared in such a way that the concentration of silver nitrate was kept fixed which is 7 ml while extract of red cabbage was added in the following manner of 2 ml, 3 ml, 4 ml in each of the silver nitrate solution. The solutions were shaken well and then kept for incubation with a proper cover of aluminum foil.

The 2nd set sample was prepared by keeping the amount of extract constant while changing the

concentration of red cabbage extract i.e. 2 ml of extract in and 4 ml, 5 ml, and 6 ml of extract respectively. The effects caused by decreasing the concentration of extract are observed by UV studies which are discussed in detail below.

IV. RESULTS AND DISCUSSION

4.1. UV spectroscopy of silver nitrate and anthocyanin (1st day)

Freshly prepared silver nitrate solution and anthocyanin were mixed and subjected to UV-Visible spectroscopy as a result silver nanoparticle formation was observed at a wavelength range from 320 nm to 600 nm but lambda maximum for silver nanoparticle was observed at 460 and 480 nm as shown in fig 1.

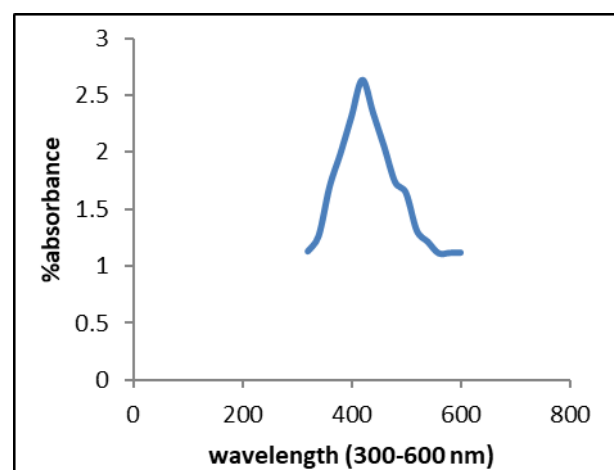


Fig: 1. Day 1 UV spectrum of silver nanoparticles.

4.2. UV spectroscopy of silver nitrate and anthocyanin (2nd day)

The following fig 2 is obtained by studying the same solution of anthocyanin and silver nitrate UV-Visible spectrophotometer after two days while the extract had been added to silver nitrate solution. The spectrum showed peaks at different wavelengths here the peak corresponding to silver nanoparticles is observed at range 320 – 600 nm while the maximum absorption spectrum was observed at 440 nm.

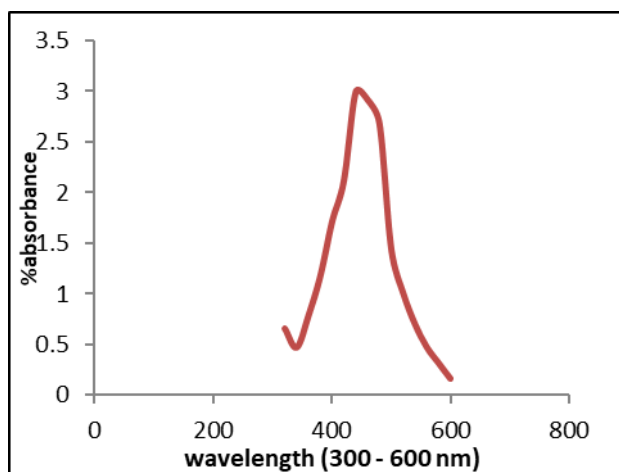


Fig. 2: Day 2 UV spectrum of silver nanoparticles.

4.3. UV spectroscopy of silver nitrate and anthocyanin (1st week)

The following fig 3 is quite interesting because as long as time passed at a lower wavelength and at higher wavelength maximum number of particle showed parallel but a gradual increase was observed at wavelength ranges from 420 nm to 480 nm whereas lambda maximum was observed at 460 which is a good result because silver nanoparticles having lambda maximum at 435 nm are very near to the ideal ones proved from numerous pieces of literature previously.

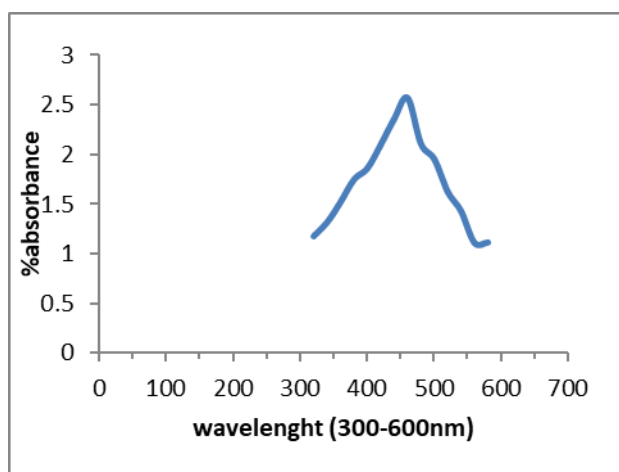


Fig: 3. Week 1 UV spectrum of silver nanoparticles.

4.4. UV spectroscopy of silver nitrate and anthocyanin (2nd week)

The sample was incubated for two weeks in order to obtain stable nanoparticles and to understand the

capping effect of extract upon silver nanoparticles so the sample was once again subjected to UV-Visible spectrometer and the absorption spectrum was obtained as shown in fig 4 which clearly indicates that lambda max for nanoparticles of silver is reduced and plenty of particles show absorption at shorter wavelength.

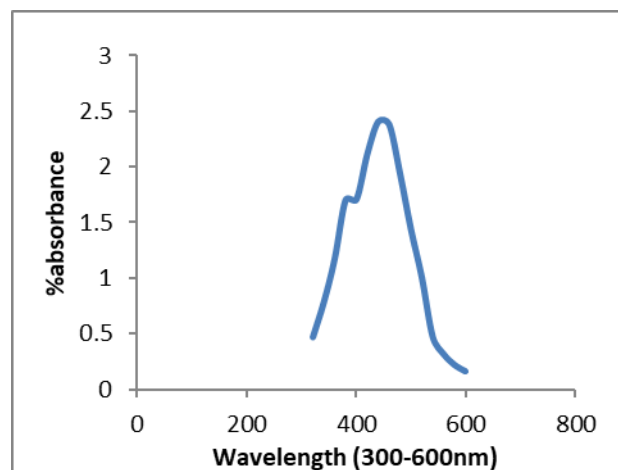


Fig: 4 Week 2 UV spectrum of silver nanoparticles.

4.5. UV spectroscopy of silver nitrate and anthocyanin and salt

The effect of salt on silver nanoparticles is determined by UV spectroscopy which indicated that the maximum number of nanoparticles showed absorption at a higher wavelength. The graph gives a very clear image to understand.

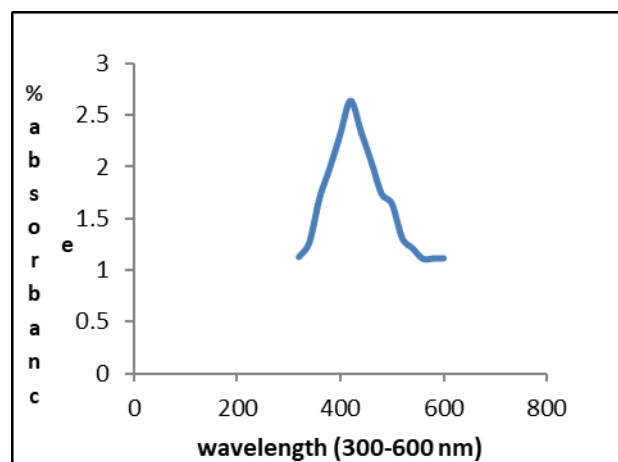


Fig. 5: UV spectrum of silver nanoparticles after the addition of salt.

4.6. UV spectroscopy of silver nitrate and anthocyanin and nitric acid

The study of nitric acid with silver nanoparticles is studied with UV spectrophotometry showed in fig 6. Here we observed that the lambda the maximum for nanoparticles is reduced from 480, 460 nm to 440 nm.

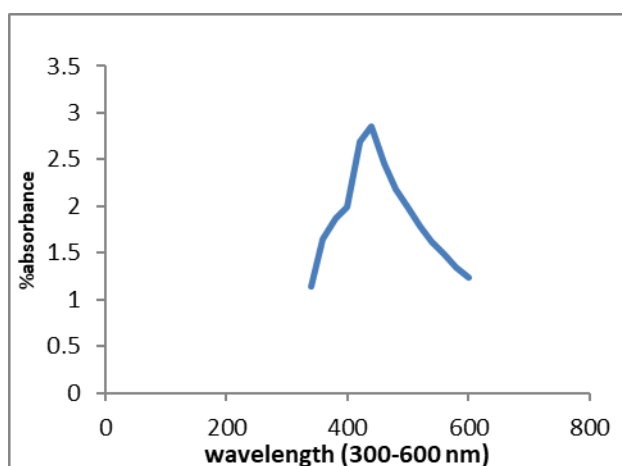


Fig: 6. UV spectrum of silver nanoparticles with the effect of nitric acid.

4.7. FTIR study for silver nanoparticles:

To understand and identify the phytochemicals or biomolecules in the extract and their role in efficient stabilization of synthesized silver nanoparticles FTIR spectrometry is carried out by using (Perkin Elmer spectrometer). The FTIR absorption spectrum of Ag-NPs in the case of both 0.1 M silver nitrate solution and anthocyanin showed an absorption band between 3490 – 3500 cm^{-1} associated with the O – H stretching and H bonded phenols & alcohols. A peak at 1600 – 1800 cm^{-1} was observed, which is responsible for the carbon-carbon double bond (C = C). The main stretch for synthesized silver nanoparticles was observed at 450 – 500 cm^{-1} . The presence of all of the peaks and stretches are responsible for the presence of phytochemicals also surrounds the prepared silver nanoparticles hence capping them giving us more stabilized nanoparticles.

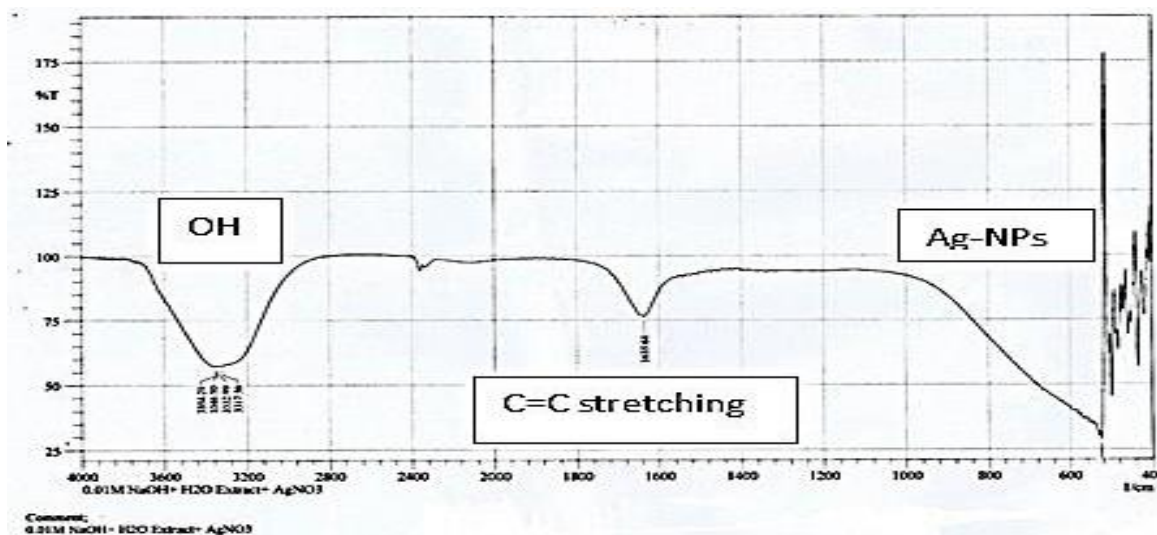


Fig: 7. FTIR Spectrum of biosynthesized silver nanoparticles.

4.8. SEM analysis of silver nanoparticles:

The synthesized silver nanoparticles were now characterized and studied by scanning electron microscope which was done by JSM5910 SEM at centralized resource laboratory (CRL) university of

Peshawar (UoP). The results of a different set of the experiment reported in our research when compared it was observed the prepared silver nanoparticles are of several μm diameters. The size of our prepared silver nanoparticles was in the range stated in earlier pieces of literature i.e. 1- 100 nms, most of the silver

nanoparticles prepared were observed having a diameter of 34 nm lying in between the ideal range. The SEM analysis also gave details about the morphology of silver nanoparticles having a regular spherical shape. The SEM images detailing the size and morphology of silver nanoparticles are shown in fig 8, 9, and 10.

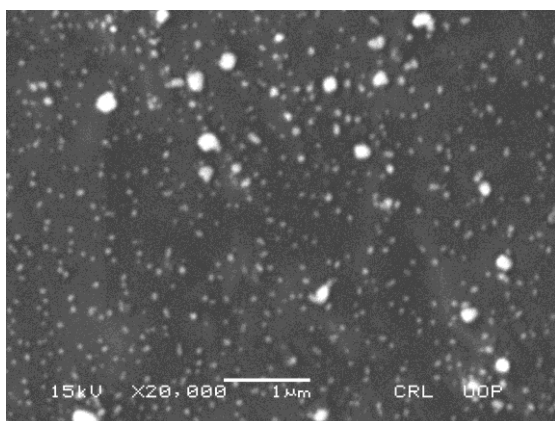


Fig. 8: SEM micrograph of silver nanoparticles at 15kV and 20000 × magnification

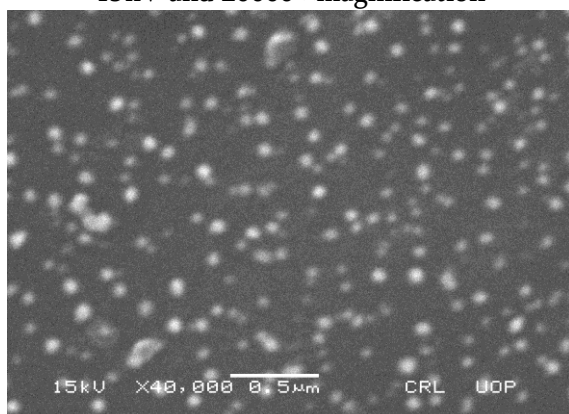


Fig. 9: SEM micrograph of silver nanoparticles at 15kV and 40000 × magnification

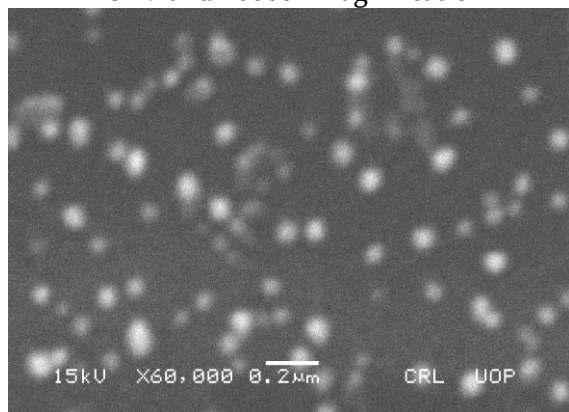


Fig. 10: SEM micrograph of silver nanoparticles at 15kV and 60000 × magnification

V. CONCLUSION

The methods used for the synthesis of silver nanoparticles in our research are rapid, cost-effective, efficient, and eco-friendly having a high ratio of repeatability. From the structural studies i.e. characterization by UV, FTIR, and Scanning electron microscope it was observed the prepared nanoparticles were of a spherical and sheet-like structure having an absolute diameter of 34 nm lying in between the ideal range of silver nanoparticles size. It was found in our findings that the ratio of plant extract to the metal ion solution plays a very important role in the size and shapes determination of our desired nanoparticles and the characterization techniques also evident this statement. The size of prepared silver nanoparticles was found to be different with the different concentration of plant extracts because of the number of phytochemicals present in the plant extract which are the main items in the reduction of metal into its nanoparticles and further also the stabilization of nanoparticles also depend on the concentration of phytochemicals as they also act as capping agents. Technologically silver nanoparticles synthesized by green synthesis have wide application in the biomedical field and this rapid simple method have much more advances like it is feasible cost-effective and various applications in the dermatological and pharmaceutical sector and can be produced commercially on large scale without even polluting the environment.

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