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BIOINSPIRED SYNTHESIS OF SILVER NANOPARTICLES

UPENDRA KUMAR PARASHAR*, PREETI S. SAXENA[a], ANCHAL SRIVASTAVA
Department of Physics, Banaras Hindu University, Varanasi –05 India.
[a]*Department of Zoology, Banaras Hindu University, Varanasi –05 India.*

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides. Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes. In this work we have synthesized silver nanoparticles using environmentally benign material like Mentha Piperita leaf extract. In the process of synthesizing silver nanoparticles we observed a rapid reduction of silver ions leading to the formation of stable crystalline silver nanoparticles in the solution. Transmission electron microscopy and UV-Vis Spectroscopy analysis of these particles shows that they are ranged in size from 5 nm to 30 nm.

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1. Introduction

Drastically increasing industrialization and urbanization is damaging our environment throughing a great amount of hazardous and unwanted chemical, gases or substances, so now it is our need to learn about the secrets inherently present in the Nature and its products, lead to the development of biomimetic approaches for the growth of advancements in the synthesis processes of nanoparticles. Properties and attributes of biological molecules and systems make them highly suitable for nanotechnological applications. Biological molecules have the qualities by which it can undergo highly controlled and hierarchical assembly for making them suitable for the development of a reliable and eco friendly process for metal nanoparticles synthesis.

As nowadays synthesis and characterization of nanoparticles is being an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties[1,2], and their potential application in optoelectronics[3,4], recording media[5,6], sensing devices[7,8], catalysis [9] and medicine [10,11,12]. Processes which are available presently for nanoparticles synthesis are chemical, physical and a recently developed Biomimetics. It provides an advancement over chemical and physical methods as it is a cost effective and environment friendly method for nanoparticles synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [13]. Biomimetics is a technique in which we use biological systems such as yeast, fungi, bacteria and plants for the synthesis of nanostructures of biocompatible metal and semiconductors. Some well known examples are synthesis of gold nano triangles by using Lemmon grass extract and tamarind leaf extract [14,15], geranium leaf assisted biosynthesis of silver nanoparticles[16], synthesis of silver nanoparticles using Fungus[17,18], synthesis of silver nanoparticles by using soluble starch[19], extra cellular synthesis of silver nanoparticles by a silver tolerant yeast strain MKY3[20], synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta Indica) leaf broth[21], biosynthesis of silver based crystalline nanoparticles of well defined composition and shapes(such as equilateral triangles and hexagons) within the periplasmic space of bacteria Pseudomononas Stutzery AG259 isolated from silver mines[22,23].

Silver toxicity towards wide range of micro-organisms has long been known Silver can even destroy antibiotic resistant bacteria such as Methicillin resistant staphylococcus aureus [24, 25]. In fact, bacteria are not able to develop resistance against silver like they do with antibiotics [26, 27]. Among all the well known activity of silver ions and silver-based compounds, silver nanoparticles proved to be the material of choice as they kill microbes effectively [28]. Bacterial membrane proteins and DNA make preferential sites for silver nanoparticles interaction as they possess sulfur and phosphorus compounds and silver have higher affinity to react with these compounds [29].

In this paper we are reporting the synthesis of silver nanoparticles by reducing aqueous silver nitrate solution by the extract of *Mentha Piperita* leaves. By studying the previously synthesized silver nanoparticles by using different other extracts of distinguish plant species, we can conclude that reduction of silver ions from silver complex into silver nanoparticles is comparatively fast and we are able to get a highly dense and stable silver nanoparticles in the size range 5 nm – 30 nm. In our first step synthesis we have gotten nanoparticles of an average size of 50 nm then we have optimized some synthesis parameters and got nanoparticles which are very small in size and also about to homogenous in shape and size. So we have succeed to change silver nanoparticles appear in different shape and size into nanoparticles approaching to spherical shape of same size.

2. Materials and methods

Synthesis of silver nanoparticles using *Mentha Piperita* leaf broth

20 g of *Mentha Piperita* leaves were washed thoroughly with sterile distill water and air dried. Leaves were finely cut and were boiled for 2 min with 100 ml of sterile distill water in 500-ml Erlenmeyer flask. Leaf broth was sterilized by filtration (0.45 μm). Silver nanoparticles are prepared by reducing 1mM AgNO3 aqueous solution with freshly prepared leaf broth. All the following procedures were performed at room temperature and under atmospheric pressure. Part of the solution was preserved for further characterization and the rest was freeze-dried to be Ag powder. Ag powder was dispersed in sterile de-ionized water to achieve desired concentration

Characterization of silver nanoparticles

The bioreduction of pure Ag^+ ions was monitored by measuring the UV-vis spectra of the reaction medium at different time intervals after diluting a small aliquot of 100 μL of the sample by 10 times. UV-vis spectral analysis has been done by using a Perkin-Elmer Lamda-25 spectrophotometer operated at a resolution of 1 nm as a function of reaction time.

Samples for transmission electron microscopy (TEM) analysis were prepared by drop coating biologically synthesized silver nanoparticles solution (24 h reaction of the silver nitrate solution with the *Mentha Piperita* leaf broth) on to carbon-coated copper TEM grids. The films on the TEM grid were allowed to stand for 2 min, following which extra solution was removed using a blotting paper and grid allowed to dry prior to measurement. TEM measurements were performed on a Phillips EM-CM-12 model instrument operated at an accelerating voltage at 100 kV.

3. Result and discussion

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Mentha Piperita* plant leaf extract have been seen by the UV-Vis spectroscopy and found that morphology of silver nanoparticles and its polydispersity in the solution may be easily followed by the UV-Vis spectrograph, strong absorption of electromagnetic waves of visible wavelength is shown by the metal nanoparticles due to its induce polarization in the conduction electron with respect to the immobile nucleus, when the skin depth of a particular wavelength is matched to the size of a nanoparticle dipole oscillation is generated in the compensated form of the induce polarization and all the electrons in the nanoparticle resonates, introducing a strong absorption. As we mix the *Mentha Piperita* leaf extract in the aqueous solution of the silver ion complex, solution start to change color from pale water color to yellowish brown, it is an indication of silver nanoparticles formation as the color change observed is due to excitation of surface plasmon vibrations in the silver nanoparticles [30]. In Fig. (1) we have shown the optical photograph of colloidal solution of silver nanoparticles reduced by *Mentha Piperita* leaf extract with varying the concentration of silver ion complex solution and dilution of plant extract as a different reaction conditions,here we observed a change in the color of the colloidal solution of the nanoparticles due to variation in their size and shape. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time by using a quartz cuvette with water as reference. As about UV-Vis spectroscopy it is well known that it is used to investigate shape and size controlled of nanoparticles. Many experiments were carried out by taking silver ion complex (1mM) and leaf extract (100 times diluted) by varying the amount of the precursors and it was found that precursors in the ratio of 1:1 was giving best result of our interest. Fig. (2a) shows the absorption spectra of silver nanoparticles formed in the reaction media at different time like 5,10,15 minutes, evolution of absorption spectra of the particles has increasingly sharp absorbance at 480 nm with increase in time, broadening of peak indicates to the inhomogeneous shape and size of the particles. Fig. (2b) shows the UV-Vis. Spectrograph of the supernatant of the sample (after 15 min. reaction time) centrifuged at 1500 rpm up to 10 min., sharp absorbance peak at 420 nm is clearly showing the particle of small size and of homogeneous spherical shape. We had observed that the surface plasmon resonance band occurs at 420 nm and steadily increases in the intensity as a function of time of the reaction without any major shift in the peak wavelength. Analysis of this spectra with time is represent either the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with time.

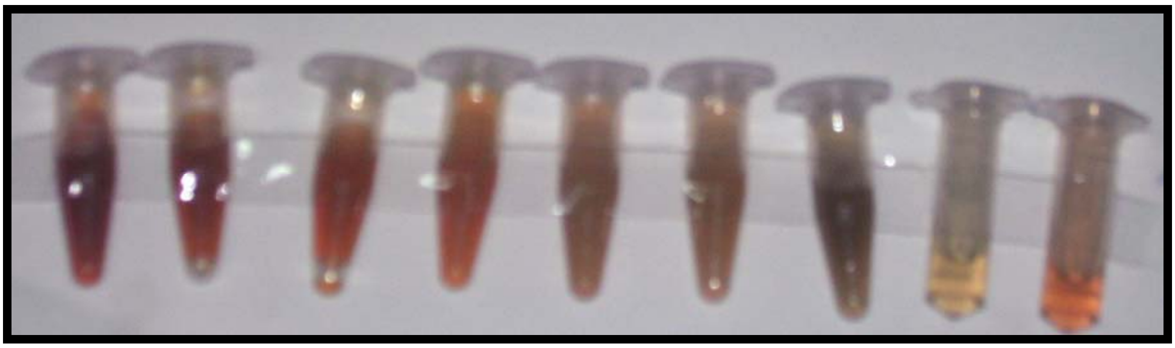
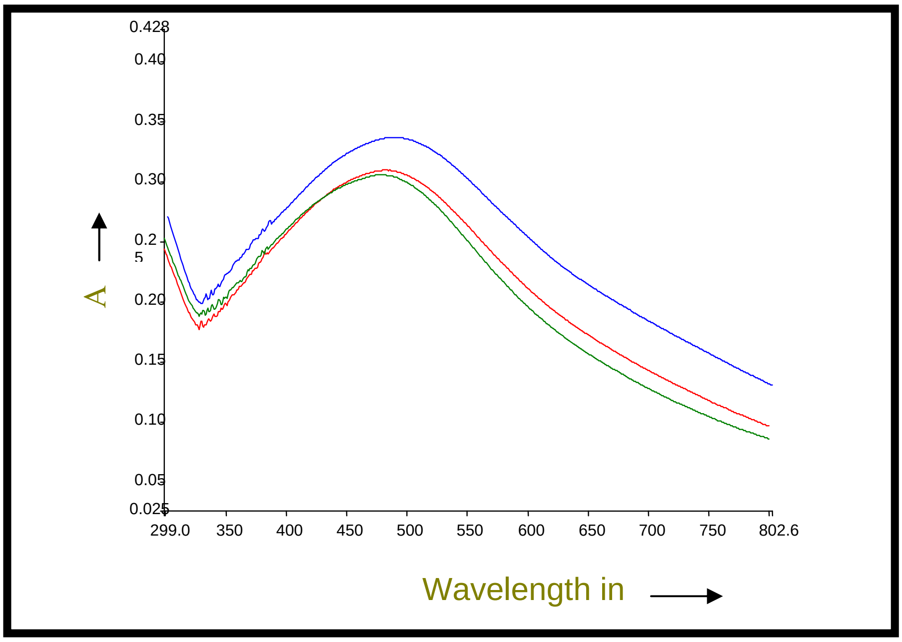
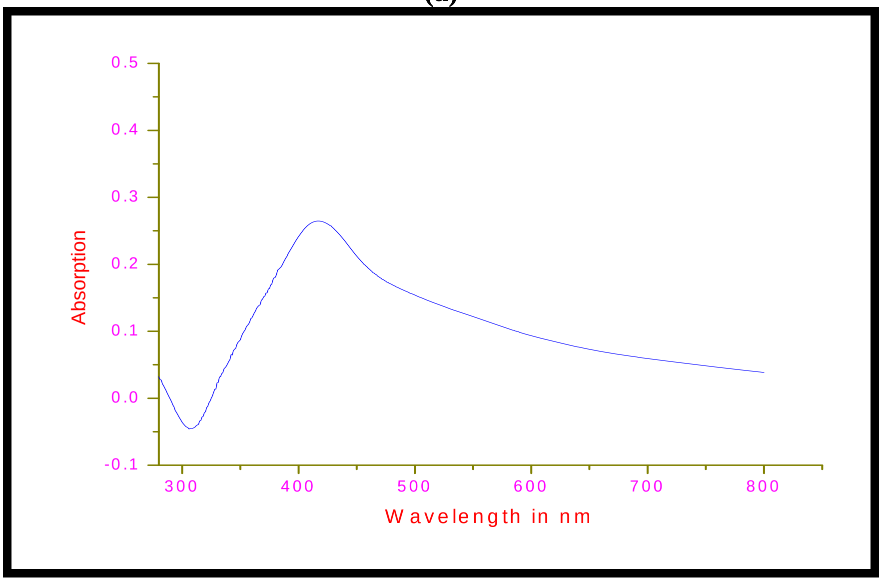


Fig. 1. Optical photograph of the colloidal solution of silver nanoparticles reduced by Mentha Piperita leaf extract with different concentrations.

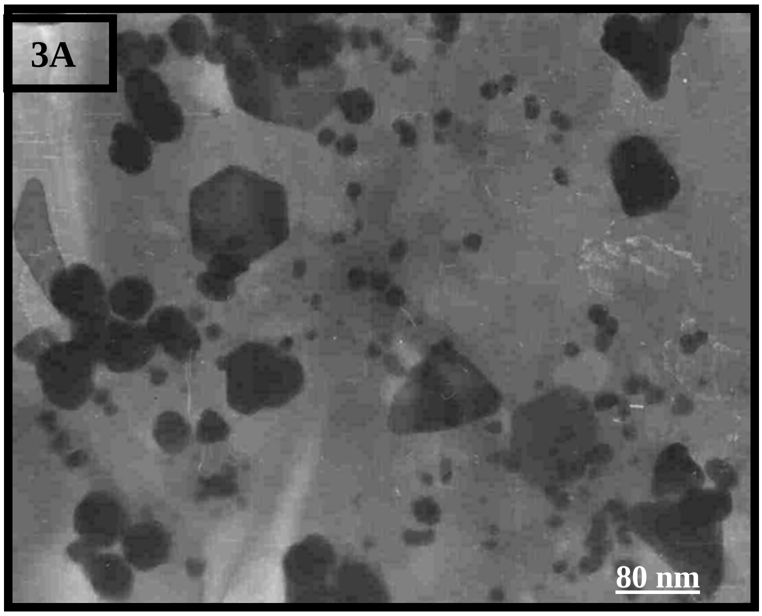


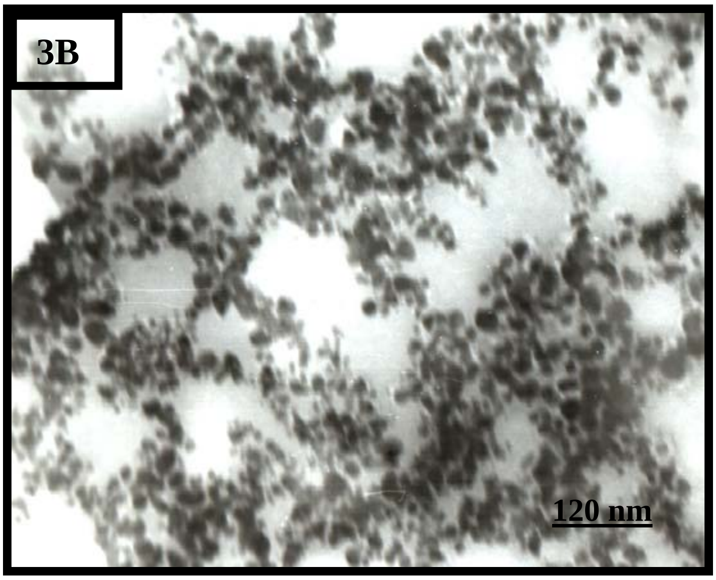
(a)



(b)

Fig. 2(a). UV-Vis. Absorption spectra recorded as a function of time of reaction of 1:1 solution of silver ions by Mentha Piperita leaf extract in the range 300 nm to 800 nm after 5,10,15 minutes reaction kinetics (curves 1-3 respectively).(b). UV-Vis. Absorption spectra of the supernatant of the sample obtained after 15 minutes reaction kinetics centrifuged at 1500 rpm up to 10 min.


3A
80 nm


3B
120 nm

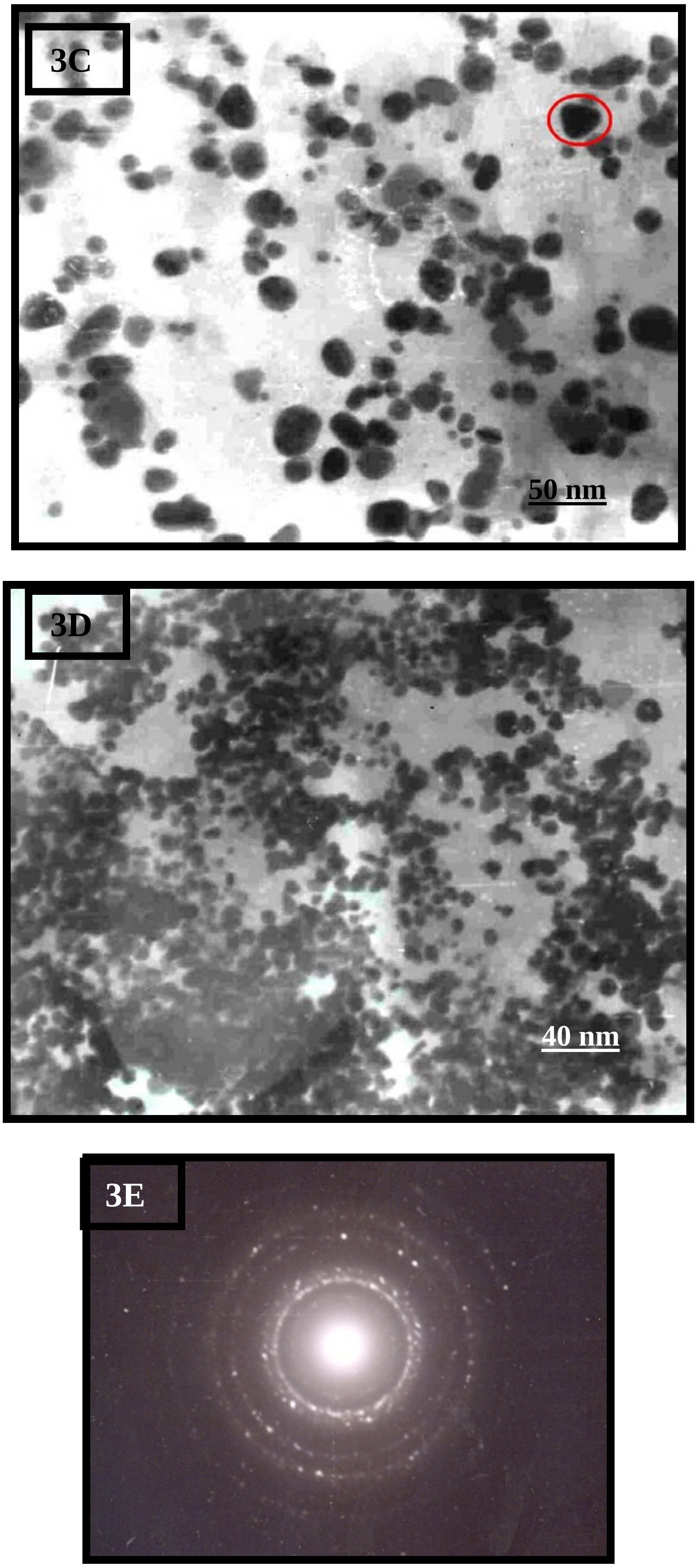


Fig. 3(a). TEM Micrograph of the sample after the 05 minute reaction kinetics with treating leaf extract(diluted 100times) with silver ions complex(1mM) in the ratio of 1:1, showing particles of irregular shapes which varies in size from 30 nm to 80 nm(average particle size is 50 nm). (b).TEM micrograph of the sample after 10 minute reaction *kinetics, showing the particles of less irregularity in shape as compare to 05 min reaction kinetics and variation of size is from 15 nm to 65 nm(average particle size is 31 nm).(c). TEM micrograph of the sample after 15 min reaction kinetics shows the particles of nearly spherical shape with average size of 22 nm.(d). TEM Micrograph of the supernatant of the sample (after 15 minute reaction kinetics) centrifuged at 1500 rpm up to 10 min shows the particles of spherical shape with nearly homogeneous size of 10 nm. 3(e) SAED-Pattern of the Silver nanoparticles.*

One of the main advantages of reduction of silver ions into nanoparticles by using Mentha Piperita leaf extract is this; in this case reduction occurs very fast as within 15 min reaction becomes stable and more than 90% of silver ions reduced into nanoparticles within this time. Therefore we can say that this is faster reduction process as compare to earlier studies on the production of silver nanoparticles using microorganisms (such as bacteria, yeast and fungi etc) and other plant extracts [14-22]. This reduction process provides advancement over advantages in the direction of the biosynthetic process that needs to be addressed if we compare Biomimetics with chemical methods for nanoparticles synthesis. Transmission Electron Microscopy was utilized to characterize the particles and their sizes and distribution by taking micrograph from drop-coated films of the silver nanoparticles synthesized by the treatment of silver complex solution with *Mentha Piperita* leaf extract for15min. Nanoparticles observed from the micrograph majority are spherical with a small percentage of elongated particles and ranged in size of 10 nm to 25 nm with an average size of 14nm. Peculiar thing is that the nanoparticles appearing into quasilinear, superstructures rather than very dense. Fig.(3a) shows the TEM Micrograph of the sample after 5 min. of the reaction kinetics, silver nanoparticles synthesized in this time of large size and irregular in shape, here we have triangular, spherical, ellipsoidal and many more shapes. Fig. (3b) shows the particle synthesized after 10 min.of reaction kinetics, here we obtained the particles smaller then Fig. (3a) but still particles are not much regular in shape. Fig. (3c) shows the particles after 15 min. of reaction kinetics, at this time we have the particles which is not only much smaller then Fig. (3a) & Fig. (3b) but they are not homogenous in shape of size range from 10 nm to 50 nm. But our interest was to get the particles, which would be homogeneous in shape and range in size from 1 nm to 10 nm. So we have done centrifugation of the sample obtained after 15 min of reaction kinetics at the rate of 1500 rpm up to 10 min. and then done the TEM analysis of the supernatant of the sample after centrifuged (Fig. (3d)). Particles which we have obtained now, was of our interest, ranges in size from 5 nm to 10 nm and have an average size of 7 nm. Fig 3(e) shows the selected area electron diffraction (SAED) pattern of the nanoparticles represented face centered cubic (fcc) crystalline structure of silver the different diffracting planes.

4. Conclusions

Thus we have reported successful synthesis of silver nanoparticles reducing silver ions present in aqueous solution of silver nitrate complex by the extract of *Mentha Piperita* leaves, concluded comparatively faster reduction rate of silver ions into nanoparticles. We were able to get highly dense and stable silver nanoparticles in the average size range from 50 nm to 7 nm.

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