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MICROWAVE ASSISTED RAPID COMBINATORIAL SYNTHESIS OF SILVER NANOPARTICLES USING *E.COLI* CULTURE SUPERNATANT

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ABSTRACT

Synthesis of nanoparticles is central to research and applications in nanotechnology. Silver nanoparticle is one of the most commercially successful nanoparticles due to their wide applicability in various areas such as electronics, catalysis, energy and medicine etc. Although,variety of physicaland chemical based synthetic methods of silver nanoparticles have been reported, these are either not cost-effective or uses chemicals, which are environmental toxicants. Though, biological synthesis of silver nanoparticles is a cost-effective and eco friendly method but also a time consuming one. The present study describes a rapid method of silver nanoparticle synthesis using *E. coli* culture supernatant along with microwave irradiation. This method is biological and physical based combinatorial method, capable of synthesizing silver nanoparticles of smaller size (8-9 nm) in about 10 minutes at alkaline medium in comparison to 24-36 hours and neutral medium used by conventional microbial method. This method is cost efficient, safe, consume less time and can be applied for up scaling and industrial use.

KEYWORD: Silver nanoparticles, *E. coli*, Microwave irradiation, Biological synthesis.

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INTRODUCTION

Nanotechnology is a new and innovative field of research and application which deals with materials of 1-100nm size. Nanoparticles exhibit unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from the bulk material which are again size dependent [1-3]. Silver nanoparticles are one of the medically important, commercially viable, biologically responsive nanoparticles. These are mostly being used in diagnosis [4], therapeutics [5-6], catalysis, , bio sensing devices [7], air and water purifiers, paints [8] etc. Although physical and chemical methods can produce well defined silver nanoparticles, these methods have their own disadvantages i. e. physical methods are often not cost effective and chemical methods needs application of chemicals that are not environmentally benign [9]. Biological synthesis of nanoparticles is a novel approach of nanoparticles synthesis. It involves the microorganisms or plant biomass as reducing agents [10] which are environmentally benign and non toxic. Biological synthesis of nanoparticles is thus an effective and economic approach which even can control the size and shape of the nanoparticles [11-12]. One important disadvantage of this approach is its relatively longer time consumption. In this context, the present study was carried out to reduce the time factor for nanoparticles synthesis by using culture supernatants of *Escherichia coli* where microwave irradiations assisted the process.

MATERIALS AND METHODS

Bacterial culture

The subcultures of *E. coli* were obtained from Infection Biology Laboratory, School of Biotechnology, KIIT University, Bhubaneswar and were revived in LB broth (1% tryptone [HiMedia, India], 0.5%yeast extract [HiMedia, India], 1% NaCl, pH 7.2). The bacteria were then cultured in 250 ml flasks. The culture flasks were incubated for 36 h at 37ºC with shaking at 150 rpm. After an incubation period of 36 hours, the cultures were centrifuged at 5,000 rpm for 10 minutes and the supernatants were collected. These supernatant were used as the starting material for synthesis of nanoparticles.

Synthesis of Silver Nanoparticles

50ml of 1mM aqueous silver nitrate (Sigma) solution was mixed with 50ml of culture supernatant of the bacteria in 250ml flasks separately. The pH of each reaction mixture was adjusted to 8.5, as synthesis of silver nanoparticles is favoured by alkaline conditions. Then the reaction mixtures were subjected to several short burst of microwave irradiation at power output of about 100W in a cyclic mode (on 15 s, off 15 s) using a microwave oven (Panasonic) for up to 10 minutes. Appearance of brownish colour indicated the synthesis of silver nanoparticles.

Characterization by UV-Vis spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium and the absorbance was recorded at 300-500 nm range using UV-Vis spectrophotometer (UV-1800, Shimadzu).

Characterizations by HR-TEM and EDX

A drop of aqueous solution from silver nanoparticle stock was placed on the carbon-coated copper grid, dried and kept under vacuum in desiccators before packing them at a specimen holder. The silver nanoparticle were characterized using HR-TEM (JEM 2100, JEOL) operating at 300 kV and elemental compositions of the nanoparticles were determined by EDX spectroscope (INCAx-Sight, Oxford Instruments) connected to the HR-TEM.

RESULTS & DISCUSSION

silver nanoparticles show brown color in aqueous solutions due to excitation of surface plasmon vibrations [13]. As the mixtures of bacterial culture supernatant and 1mM aqueous solution $AgNO_3$ were treated to several short bursts of microwave radiations, the color of the mixture started to change from yellow to brown indicating the formation of silver nanoparticles.

Characterisation of silver nanoparticles by Uv-Vis Spectra analysis

The silver nanoparticles showed the characteristic peak at 405nm attributable to the phenomenon of longitudinal surface Plasmon resonance [14, 15]. Additional peak at 370 nm can be attributed to the transverse surface Plasmon resonance of the nanoparticles [14, 16].

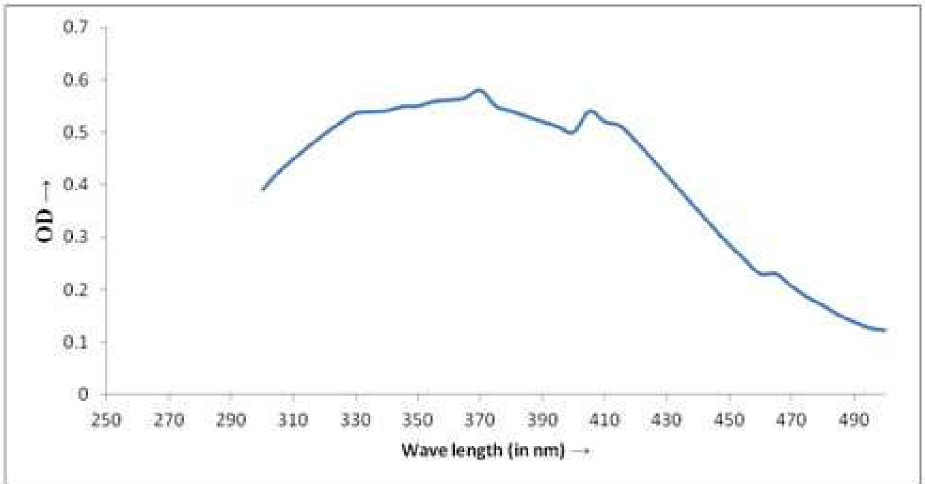

Figure 1
UV-Vis spectrum of the diluted aliquot of the silver nano particles synthesized using microbial culture supernatant.

Characterisation of silver nanoparticles by TEM, EDX & SAED

The Fig-2 (a, b, c) exhibited the micrograph, particle size distribution, selected area electron diffraction of the silver nanoparticles respectively. From these figures, it is evident that there is variation in particle sizes and the average size estimated was 4 to 9 nm and the particles are mostly polydisperse in nature. Gurunathan et al., (2009) [17] have synthesized silver nanoparticles from culture supernatant of *E. coli* and the average particle size of the nanoparticles in their study was 50 nm while those synthesized using the same microbe by Natrajan et al., (2010) [18] were of 40-60 nm in size. The nanoparticles synthesized in this study were much smaller than the earlier reports, which is an encouraging trend. Moreover, the time for synthesis is also considerably lower than the previously reported methods. Saifuddin et al. (2008) [19], have synthesized silver nanoparticles following similar methods using culture supernatant of *Bacillus subtilis,* the size of which were in the range of 5-100 nm, larger in comparison to that synthesized in the present study. The sizes of the nanoaparticles synthesized in this study were also smaller than those synthesized using some other microbes like *Bacillus licheniformis* [20], *Corynebacterium sp.* [21], *Staphylococcus aureus* [22], *Proteus mirabilis* [23], *Brevibacterium casei* [24]. The smaller size of the nanoparticles synthesized in this method can be due to additiveeffects of a number of factors like P^H, temperature and rapidity of the process. It has been reported that silver nanoparticles synthesized in basic environments are smaller than those synthesized in acidic environments. Similarly the nanoparticles synthesized at higher temperatures are smaller than those synthesized at lower temperatures [25]. It has been proposed that at lower temperatures and acidic pH, there will be less nucleation for silver crystal formation on which new incoming silver atoms deposit to form larger sized particles. But as the pH and temperature increase, the dynamics of the ions increase and more nucleation regions are formed due to the availability of –OH ions and increased temperature which leads formation of smaller nanoparticles [12]. In the present study, the pH of the reaction mixture was slightly basic as well as the temperature of the reaction mixture was also higher due to the microwave irradiations than the previously reported methods and can be the reason for production of smaller size nanoparticles.

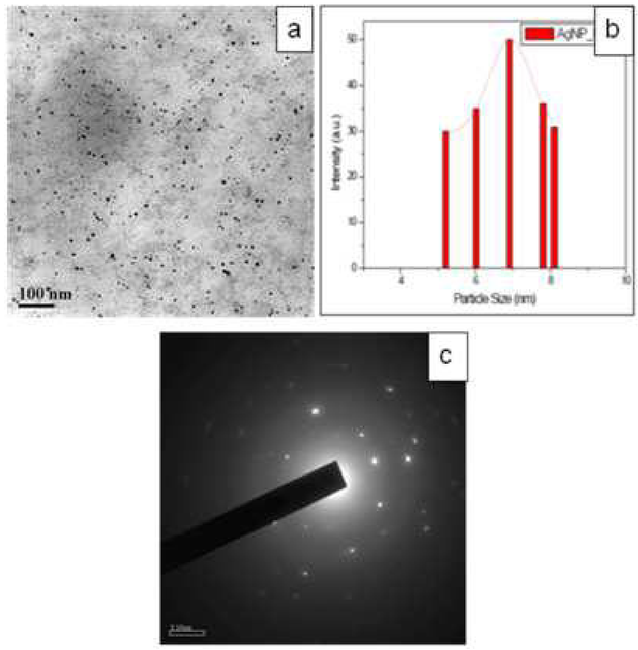

Figure 2
(a) Microphotograph of the silver nanoparticles synthesized from E.coli supernatant (b) Histogram of the size distribution of the nanoparticles (c) SAED structure.

The elemental compositions of the nanoparticles by EDX (Fig. 3) indicates a sharp peak at 2.9-3.0 keV which shows the presence of silver as base and dominant element. Additional peaks at 8.0-9.0 keV are due to copper which is the base material of the grid.

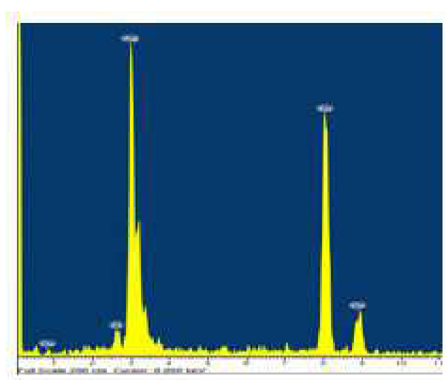

Figure 3
EDX spectroscope of the synthesized nanoparticles depicts silver as the base element.

Microbial culture extracts contain compounds that are not only capable of reducing the Ag+ ions but also act as natural capping agents. These biological extracts contain a combination of biomolecules like enzymes, proteins, amino acids, carbohydrates and vitamins that can be responsible for reduction and capping of the Ag+ ions [12]. Among the enzymes, "Nitrate reductase" is among the widely accepted enzymes responsible for synthesis of the nanoparticles. Nitrate reductase is an enzyme that is responsible for conversion of nitrate to nitrite and during this process of catalysis, an electron shuttles to the incoming silver ions [26]. Similarly synthesis of silver nanoparticles using microwave irradiations using starch as a capping agent has also been described which signifies that the microwave irradiations play a major role in the synthesis process [27]. Therefore, the mechanism of reduction of Ag+ ions can be attributed to the cumulative effect of both the biomolecules present in the bacterial culture supernatant and the microwave irradiations. However, the biomolecules present in the culture supernatant acts as environmentally benign capping agents.

CONCLUSION

The present study describes a cost-effective, rapid, combinatorial method of physical and biological based silver nanoparticles synthesis which not only uses environmentally benign chemicals, making the nanoparticles suitable for wide range of applications but also drastically reduces the time taken for synthesis in comparison to other biological and chemical processes. However, for bulk production of nanoparticles, further research like mathematical modelling and other steps of scaling-up are to be carried out. But this combinatorial approach will be proved to be a cost effective and efficacious in the long run.

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