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Biosynthesis Of Ag Nanoparticles For The Detection Of Pathogenic Bacteria In Food

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Abstract. In today's world due to the outbreak of infectious diseases caused by different pathogenic bacteria and development of antibiotic resistance the pharmaceutical companies and the researchers are searching for new antibacterial agents. The synthesis, characterization and application of biologically synthesized nano materials have now become an important factor of nanotechnology. In this paper, we report the synthesis of highly dispersed silver nanoparticle detection using the extra cellular enzymes of Escherchia Coli as the reducing agent. The identification of pathogens still relies on conventional culturing techniques, which is not suitable for onsite applications. Therefore this field is focussed on the need to develop reliable, rapid, sensitive and specific measures to detect the pathogens (bacteria) at an economical rate. This technique is to combine function of Surface Enhanced Resonance Raman Scattering (SERRS) and Silver nano particles to produce antigen-antibody interaction onto biological products. This paper also also deals with extracellular biosynthesis of "silver nanoparticles" using Escherichia coli and characterization of nanoparticles by UV-visible spectroscopy, FTIR, SEM of nanoparticles against different human pathogenic bacteria in food.

Keywords: Extracellular Enzyme, Silver nanoparticles (Ag nanoparticles), *Escherchia Coli*, Surface Resonance Raman Scattering (SERRS), FTIR, SEM

1. Introduction

The field of nanotechnology is one of the most active areas of research in modern material science. Nano particles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route.

Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and thus have proved to be better methods due to slower kinetics[1].They offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The uses of environmentally benign materials like plant extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead with presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals[1][2].

2. MATERIALS AND METHODS

2.1 PREPARATION OF NUTRIENT AGAR

Take a 100ml of distilled water in a conical flask and add yeast, peptone and NaCl to it. Set the pH to 7.2±. And then 2gm of agar is added to this solution and mixed well .Cover the conical flask and keep it at

autoclave for 30 minutes. Pour the agar medium into the Petri plate in front of chamber. The Petri plates are kept for 15 minutes in chamber for solidification of agar and then the organism of Escherichia coli is swabbed into the Petri plate using loop. It is then subjected to incubation for 24 hours at room temperature[9].

2.2 PREPARATION OF BROTH MEDIUM

Take 100ml of distilled water in a conical flask and add yeast -5mg,, peptone-3mg and NaCl -5 mg to it. And set the pH to 7.2±. The mixture is mixed well. Cover the conical flask and keep it at auto clave for 30minutes.Now the culture of Escherichia coli are inoculated into the broth.The resultant is now allowed to settle in the shaker for overnight. Then centrifuge the medium at 5000rpm.The supernatant is collected and stored in 40 degree celcius[7][8][9].

2.3 PREPARATION OF SILVER NITRATE SOLUTION

This step is carried out using the formula given below.

$$\text{Molarity} = \frac{\text{Molecular Wt.} * \text{Required Molarity} * \text{Req.volume}}{1000}$$

According to the above formula 1mM, 3mM, and 5mM concentration of silver nitrate solution is prepared in 100ml of double distilled water in three separate conical flasks. The silver nitrate solution is prepared and exposed to sunlight. The colour of the solution changes from light white to orangeish red. The time taken for the colour change is noted[4][6].

2.4 SYNTHESIS OF SILVER NANOPARTICLES

The conical flasks containing the solutions are kept overnight in room temperature for the formation of the silver nanoparticles. Then the readings were taken for 1mM by using UV-VIS Spectrophotometer at every 1 hour interval, the graph is plotted and the λmax (max wavelength) is calculated. The same procedure was repeated for 3mM and 5Mm concentrate of silver nitrate. Among the three concentration 3mM extract resulted in good silver nanoparticle formation[2],[3].

2.5 PREPARATION OF BULK PRODUCTION

Among the three concentrates, 1mM of extract had shown good silver nano particle formation. So the bulk solution for 3mM concentration. of extract is prepared in 100ml of silver nitrate with 10ml extract of Escherichia coli. The bulk solution is kept overnight for silver nitrate extract (for bulk formation of silver nanoparticles).

2.6 EXTRACTION OF SILVER NITRATE

The extract is centrifuged the next day in 8 centrifuge tubes at 5000 rpm for 30 mins. The supernatant is discarded and the pellets are filled with a small quantity of Toluene. Now it is subjected to the mixer for complete mixing and then spread by using micro pipette in the Petri dishes which was wiped with toluene and later dried. After drying it is collected in the ependorf tube by using blade and then analyzed [4].

3. RESULTS AND DISCUSSION

3.1 UV-VISIBLE SPECTROSCOPY

The reduction of the Ag ions by the supernatant of the test bacteria in the solution and formation of silver nanoparticles were characterized by UV-visible spectroscopy monitored by sampling the aqueous component (2 ml) and measuring the UV–Visible spectrum of solutions at various time intervals as shown in Figure: 1. The UV–Visible spectra of these samples were measured on ELICO SL 159 Ultraviolet visible spectrophotometer operated at a resolution of 1 nm. The surface plasmon band in the silver nanoparticles solution remains close to 440nm through out the reaction period indicates that the particles are dispersed in the aqueous solution with no evidence for aggregation [5], [6], [7], [8].

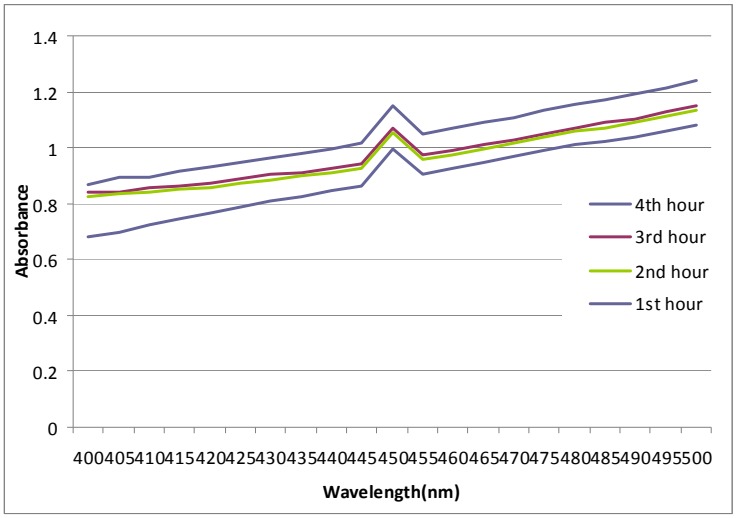

Figure1: UV VISIBLE spectra for Escherichia coli

3.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR spectrum of silver nanoparticles synthesized from Escherichia coli extracts are shown in Figure: 2. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by Escherichia coli extracts shows peak at 3787 cm-1, 3445 cm-1 assigned to O-H stretching & aldehydic C-H stertching respectively. The peaks 2359 cm-1 , 2338 cm-1 , 2063cm-1 corresponds to C-N stretching of amine[11],[12],[13]. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium [14][15]. Plot was drawn between Wave number and % Transmittance as shown in Figure: 2.

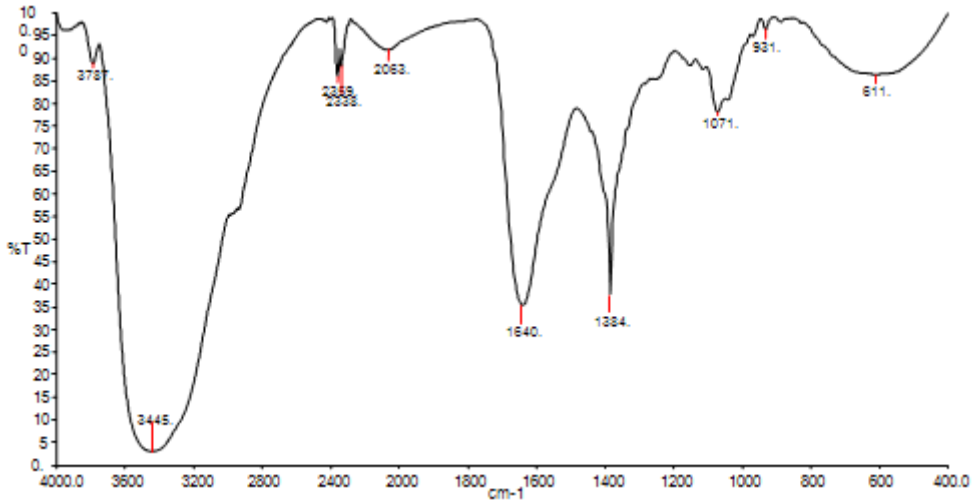

Figure 2: FTIR after synthesis of silver nanoparticles from Escherichia coli

3.3 SCANNING ELECTRON MICROSCOPY (SEM)

Type of electron microscope that images a sample by scanning it with a high-energy beam of electrons. Signals produced by an SEM include secondary electrons, back-scattered electrons, light (cathodoluminescence), specimen current and transmitted electrons. Magnifications are possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine [12]. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. SEM analysis showed the cubic structure of silver nanoparticles (Figure: 3).

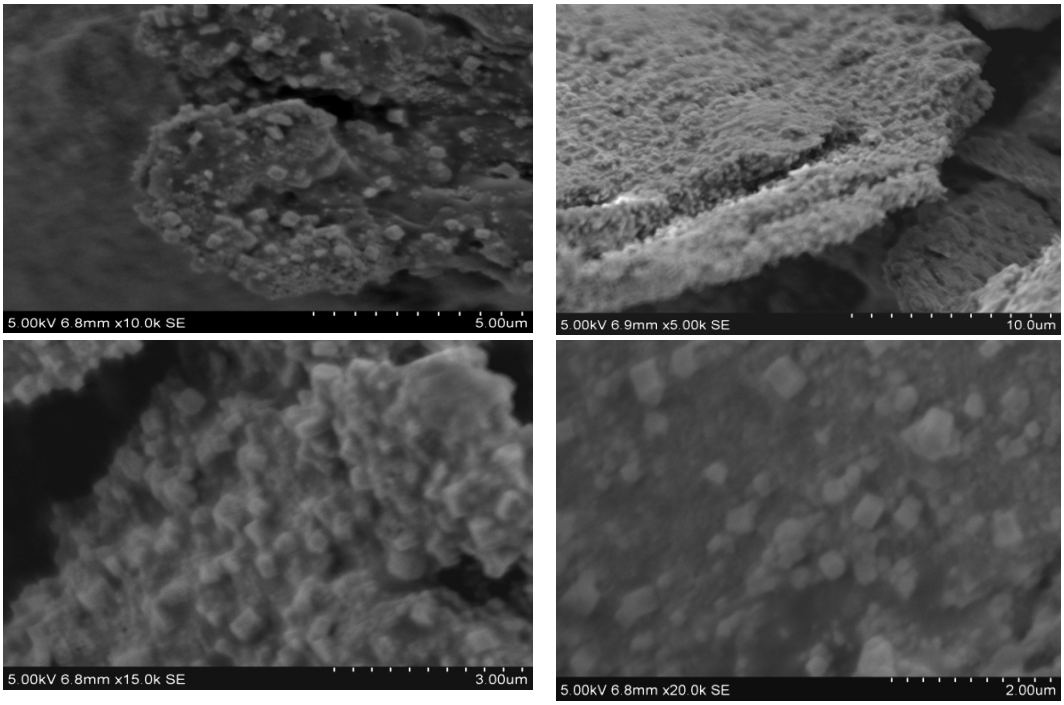

Figure 3: SEM micrograph of silver nanoparticles by Escherichia coli

4. CONCLUSION

In conclusion, we introduce a simple,fast and economical,biological procedure to synthesize silver nanoparticles using Escherichia coli. We characterized these nanoparticles using UV-Visible spectra, FTIR and SEM techniques [7],[8],[9]. Optical non-linearity of these silver nanoparticles was comparable or superior to those synthesized through other procedures[15]. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, drug discovery makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents[16].

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