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RESEARCH PAPER

Biological Synthesis and Characterization of Silver Nanoparticles Using *Bacillus Subtilis*

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Abstracts

Biological synthesis of silver nanoparticles using *Bacillus subtilis* was examined and reported. The quick reduction of silver (Ag+) ions was checked using UV-visible spectrophotometers and showed formation of silver nanoparticles within 1 hr. Transmission electron microscopy (TEM) investigation presented that the synthesized silver nanoparticle are 39.6 nm and has spherical shape. Additional the XRD analysis approves the Nano crystalline phase of silver with FCC crystal structure.

Keywords: *Nanoparticles; Silver; Bacillus subtilis.*

Introduction

Silver nanoparticles (AgNPs) have come to be the focus of abundant research attention due to their extensive diversity of uses[1, 2].Their capability to modify the physical, optical and the electronic properties of complexes[3-9].have bring into being applications in several fields including electronic devices[10].Chemical and biological sensing[11].In contrast, a favorable usage of AgNPs as antimicrobial agent is well known and has by this time found requests in antimicrobial paint coatings[12].Water treatment, textile and medical devices.

Old-style chemical procedures of synthesizing silver nanoparticles include the use of chemical solvents [13]. The chemicals used in these methodologies can be toxic and extremely reactive posing cause a risk to the humans and environment, and the procedures are too costly to be realistic at an built-up scale. For that reason there has been a search for cheap, dependable, harmless, and "green" style to the synthesis of stable metal nanoparticles with precise size and shape. As the result, some innovative techniques have freshly established using biologically derived reducing agents such as plants extract, microbes , polysaccharide and others [14] for synthesis of metal nanoparticles. Amongst them, bacteria mediated biological method has been extensively examined due to their low-cost and simple protocol.

Experimental design

Bacillus subtilis was initially grown at 37 ^{0}C for 24 hr in a flask that contained nutrient broth (100 ml) in a shaker incubator set at 200 rpm. Following bacterial growth, all the culture suspensions were incubated with aqueous 5 MM solutions of $AgNO_3$ at 37 ^{0}C in a shaker incubator at 200 rpm in the dark, the reactions were carried out for up to 120 hr (5 days).The extracellular synthesis of AgNPs was initially detected by visual inspection of the culture flask for a change in the color of culture medium from clear light-yellow to brown/green. Extracellular AgNPs were separated from bacterial cells by centrifuging aliquots of culture supernatants (1.5 ml) at 6000 rpm for 20 min at 25 ˚C[15].

Characterization of Silver Nanoparticles

To realize the nature of nanoparticles, detailed physic and chemical characterization of Ag NPs formed by *bacillus subtilis* was supported by using the frequent scans of the

optical absorbance between 200 and 800nm with a UV-visible spectrophotometer (Shimadzu -1800-Jaban).The Transmission electron microscope type- JEOL 100CX II (TEM) 100kV –Al-Sharif University-IRAN image was recorded by dissolving the synthesized powder sample in ethanol and then placed a drop of ethanol solution on the surface of copper grid. Synthesized silver nanoparticles were examined through powder x-ray diffraction(Shimadzu Xrd-6000-Jaban) using Cu Kα radiation operating between 10^{o} and 80^{o} at the scanning rate of 2^{o} per minute.

Results and Discussion

This study was done to conclude whether silver nanoparticles (AgNPs) creation by *Bacillus subtilis*. To carry out this study *Bacillus subtilis* isolate was exposed to 5 MM colorless $AgNO_3$ solutions *Bacillus subtilis* made dark brown colored solutions in 20 h of reaction fig.1the color of the solutions did not meaning fully alteration from that point forward (except in intensity),even after continuing the reaction for up to 5 days[16].

Fig.1:Medium with $AgNO_3$ (5mM) and controlled sets (Negative and positive) at room temperature

The UV-vis absorbance spectra of solutions gained after reaction of *Bacillus subtilis* with 5 MM $AgNO_3$ for zero, 24, 72 and 12hr.fig. 2.The existence of a characteristic Ag Surface Plasmon Resonance (SPR) between 400 and 500 nm is obviously palpable, hence approving the formation of AgNPs by *Bacillus subtilis*[16, 17].It is also remarkable to note as the result presented that the *Bacillus subtilis*ongoing synthesizing AgNPs as quick as in 1 hr. of reaction and the harvest of AgNPs augmented as the reaction movements over a period of time. On the other hand, the quantity of AgNPs formed by this bacterium reached to an overload state somewhere between 20 and 120 hr. of reaction. This proposes that *Bacillus subtilis* has the ability to reduce (Ag^{+}) ions to form AgNPs (Ag^{0}).And through this study we noted that the amount of AgNPs formation was found to be extreme at 5 mm $AgNO_3$ concentration, and was reduced by increasing the precursor concentration and over the 5 mm $AgNO_3$ the bacteria died and this result agree with [17, 18] result.

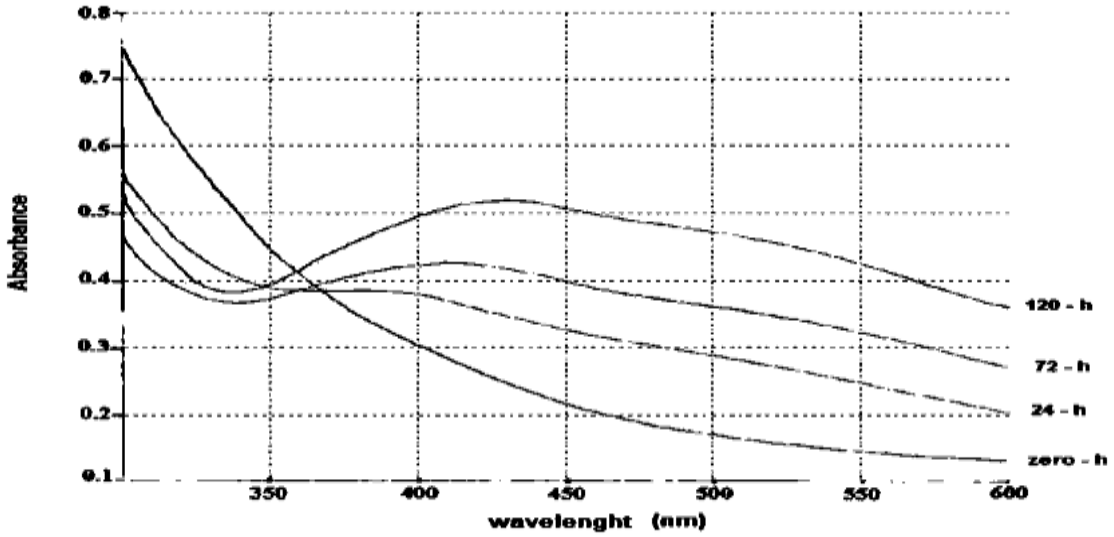

Fig.2: UV-VIS Absorbance spectroscopy for AgNPs from *Bacillus subtilis*

For TEM examinationFig.3,AgNPs sample achieved after 20 hr. of reaction were prepared by drop casting the suspensions of AgNPs onto carbon-coated Cu grids and drying under air for 24 hr. this test was done at Al-Sharif University-IRAN and the result display that AgNPs have a 39.6 nm size. This result agree with [17]result who found that TEM images that AgNPs formed by all bio groups were spherical shape, and have diverse size according to different bio groups.

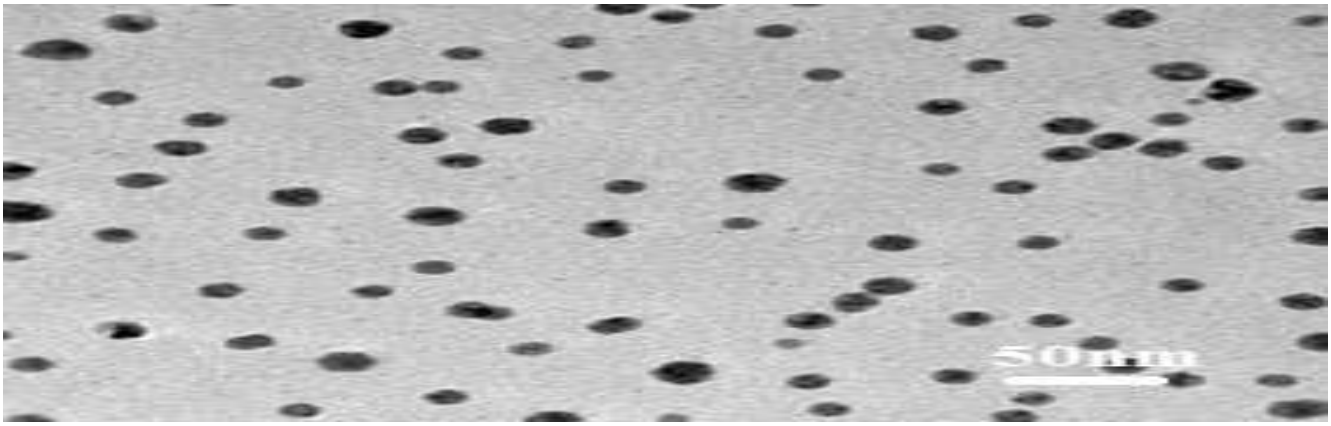

Fig.3: TEM images of the biosynthesized silver nanoparticles by *bacillus subtilis*

The XRD pattern of synthesized silver nanoparticles using *bacillus subtilis* were documented and characteristic XRD pattern is shown in Fig. 4. The peaks are indexed as (111),(200), (220), (311) and (222) plans of FCC silver. At a distance from these peaks responsible for silver nanoparticles the noted XRD pattern displays extra unassigned peaks, indexed as (142). This may be due to the development of the crystalline bioorganic compounds such as metalloproteinase that are existent in the bacterial broth. Parallel clarifications were described by S. Shiv Shankar *et al.*[16]for the silver nanoparticles synthesized using *P. grave lens* leaf broth.

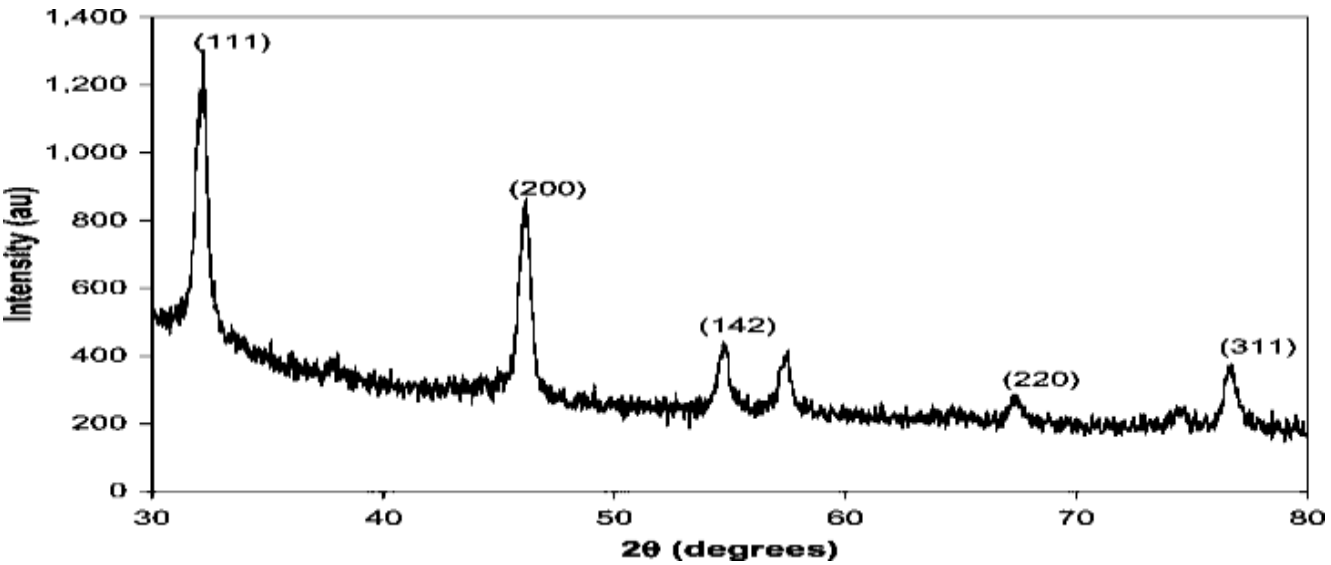

Fig.4: Typical XRD pattern of silver nanoparticles synthesized using

Conclusion

The biological syntheses of silver nanoparticles using *bacillus subtilis* was shown to be quick and create particles of properly identical size and shapes the synthesized particles size 39.6 nm and were spherical in shape, as shown by the TEM. The amount of AgNPs creation was found to be supreme at 5 mm $AgNO_3$ concentration, and was reduced by aggregate the precursor concentration and over the 5 mm $AgNO_3$ the bacteria was died.

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