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Biosynthesis of Silver-Phosphate Nanoparticles Using the Extracellular Polymeric Substance of *Sporosarcina pasteurii*

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***Abstract*—**Silver ions (Ag^+) and their compounds are consequentially toxic to microorganisms, showing biocidal effects on many species of bacteria. Silver-phosphate (or silver orthophosphate) is one of these compounds, which is famous for its antimicrobial effect and catalysis application. In the present study, a green method was presented to synthesis silver-phosphate nanoparticles using *Sporosarcina pasteurii*. The composition of the biosynthesized nanoparticles was identified as Ag_3PO_4 using X-ray Diffraction (XRD) and Energy Dispersive Spectroscopy (EDS). Also, Fourier Transform Infrared (FTIR) spectroscopy showed that Ag_3PO_4 nanoparticles was synthesized in the presence of biosurfactants, enzymes, and proteins. In addition, UV-Vis adsorption of the produced colloidal suspension approved the results of XRD and FTIR analyses. Finally, Transmission Electron Microscope (TEM) images indicated that the size of the nanoparticles was about 20 nm.

***Keywords*—**Bacteria, biosynthesis, silver-phosphate, *Sporosarcina pasteurii*, nanoparticle.

I. Introduction

NANOPARTICLES are ultrafine particles in the range of 1-100 nm. Nanoparticles may be either magnetic or nonmagnetic and they could be classified as inorganic (silver and gold NPs etc.) and organic (mainly carbon) [1]. Nanoparticle synthesis is appearing as an immensely developing field due to its application in science and technology for the purpose of constructing nanomaterials at nanoscale level [2]. Like many future areas of scientific exploration, nanotechnology and nanoscience exist on different borders between disciplines such as pharmaceutical industry, medicine, catalysis, drug delivery system, electronic, biotechnology, and environmental remediation [3], [4]. Recently, nanostructured materials have attracted considerable attention owning to their unique physicochemical and biological application in various science compared to their macro-scale ones [3], [5]. Many physicochemical methods are available for metallic or nonmetallic nanoparticle production, but most of these techniques are environmentally hazardous and require expensive chemicals [6]. So, there is a need to develop clean, toxicity-free, and environment-friendly methods to replace the conventional methods [7].

Nanobiotechnology is a new branch of nanotechnology that has appeared at the interface of biology and nanotechnology combined together for enhancing environmentally benign technology for the synthesis of nanomaterials with specific functions [8], [9]. It provides advantages over chemical and physical methods of nanoparticles formation. The production of nanoparticles of various composition, mainly metals, and sulfides or oxides can be either intra or extracellularly done by a variety of microorganisms.

Silver has been known since ancient times as an effective microbial agent. Silver nanoparticles have different applications as an extremely important metal from electronics, sensors and catalysis to medical diagnosis such as antimicrobials, and for biomolecular detection [10], [11]. Among these, the most important application of silver and its nanoparticles is in the medical industry [12]. Recently, many studies have reported the ability of different microorganisms to synthesis silver nanoparticles such as *Pseudomonas Deceptionensis* [13] and *Weissella oryzae* [14] which are able to synthesis silver nanoparticles extra and intracellularly, respectively. In addition to silver nanoparticles, other silver compounds such as silver-phosphate nanoparticles (SPNPs) [15] have antimicrobial properties. Several mechanisms were performed in foregone studies for the synthesis of SPNPs [16], [17]. However, the biological synthesis of these nanoparticles has not been reported yet.

In this study, a green method was applied for the biological synthesis of SPNPs using EPS of *Sporosarcina pasteurii* for the first time. It should be expressed that Nagarajan et al. [18] reported the green synthesis of silver nanoparticles using the biomass of *Sporosarcina pasteurii*. In this essay, different analysis was performed to characterize the nanoparticles.

II. Materials and Methods

A. Materials

The bacterium *Sporosarcina pasteurii* was purchased from Iranian Research Organization for Science and Technology (IROST). Silver nitrate ($AgNO_3$) and Tryptone water were purchased from Dr. Mojallali and Titrachem Chemicals, Iran, respectively.

B. Bacterial Growth

S. pasteurii which was previously grown on PDA (Potato, Dextrose, Agar) medium, inoculated into 100 ml autoclaved (121 °C for 15 min) liquid culture medium containing tryptone

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water (tryptone and NaCl), 10 g/L; and yeast extract, 5 g/L in 500 ml flasks. Then, the flasks placed in a shaker-incubator (Jaltajhiz, Iran) for 48 h, rotational speed of 150 rpm, and temperature of 35 °C. Finally, the bacterial cells were isolated from liquid culture medium by centrifuging for 15 min at 5000 rpm using Hettich (Universal, 320R) and then washed three times with distilled water. The washed bacterial cells were placed in 100 ml distilled water in 500 ml flask for bio-surfactant production. Then the flask was placed in shaker-incubator for 96 h incubation. Afterward, cells were isolated by centrifuging for 20 min at 5000 rpm and 4 °C temperature and the remained liquor was kept for the green synthesis of nanoparticles.

C. Synthesis of Nanoparticles

Silver nitrate ($AgNO_3$) was dissolved (15 mM) in the obtained liquor and then placed on a magnet stirrer under intense mixing condition for 2 h in environment temperature. Progressively, the solution color turned to brown during these 2 h. Afterward, the solution was placed in the shaker-incubator (35 °C, 150 rpm) for 48h to complete the reaction (Fig. 1). Finally, the brown colloid solution was centrifuged at 12000 rpm for 25 min, the solution was still brown after centrifugation. The obtained brown color powder was dried in an oven (Azar furnace, Iran) for 3 h at 70 °C, and then used for further analysis and studies.

D. Caracterization of Nanoparticles

The formation of SPNPs was checked by XRD technique using an X-ray diffractometer (Asenware AW/XDM 300. China) with Cu Kα radiation k = 1.5405 Å over a wide range of Bragg angles ($10 < 2\theta < 80$). Also, FTIR spectra were recorded using Bruker Tensor 27 (USA). UV-Vis adsorption of the produced colloidal suspension was registered using Unico 2100 (USA) spectrophotometer. The elemental composition of the prepared sample was studied by using Energy Dispersive X-ray analysis (EDS), (Seron AIS 2300, Korea). In addition, TEM images were obtained by Philips CM200 (Netherland) microscope with an accelerating voltage of 200 kV.

III. Results and Discussion

After the completion of the synthesis procedure, the obtained powder was undergone different analysis for nanoparticles characterization. As the produced solution had not settled after months, it was conjectured that the size of particles should be in nanometer ranges. It was also observed that the remained liquor is still brown in color after centrifugation at 12000 which is another evidence of nanometer sized particles. These observations attest that the nanoparticles were instantly covered and stabilized by bio-surfactants secreted by the bacterium after their generation, and this organic shell prevented the joining of nanoparticles and increasing the particle size.

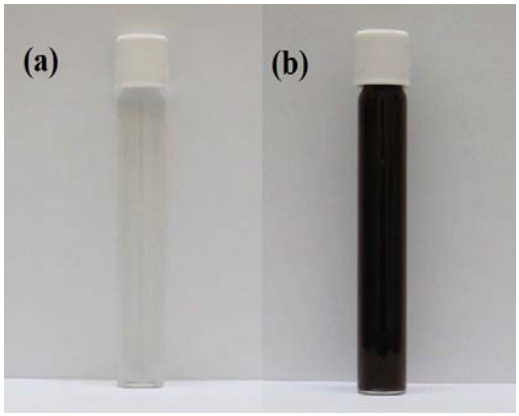

Fig. 1 Silver-phosphate solutions (a) before and (b) after synthesis

XRD analysis was used in contemplation of the crystal structure of the synthesized nanoparticles. Fig. 2 which is the result of XRD analysis exhibits prominent broad peaks at 2θ values of 20.907, 29.736, 33.343, 36.633, 47.872, 52.774, 55.108, 57.377, 61.753, and 72.021. This pattern could be indexed to the (110), (200), (210), (211), (310), (222), (320), (321), (400), and (421) facets of the cubic phase Ag_3PO_4 which is in consistent with the reference code of 01-070-0702.

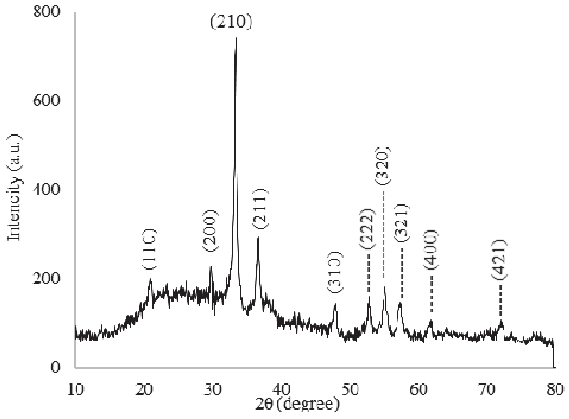

Fig. 2 XRD pattern of SPNPs

The peak list of this reference pattern was compared to the peak list acquired for Ag_3PO_4 nanoparticles and is revealed in Fig. 3. The associated PDF card denotes that the nanoparticles were in line with the face-centered cubic crystal lattice having unit cell parameters of a = b = c = 6.0040 belonging to space group P-43n with the number of 218. Also, using Williamson-Hall Equation (1) [19], the crystal size of Ag_3PO_4 calculated as 22.4 nm.

$$\beta \cos\theta = \left(\frac{0.9\lambda}{d}\right) + 2A\varepsilon \sin\theta \quad (1)$$

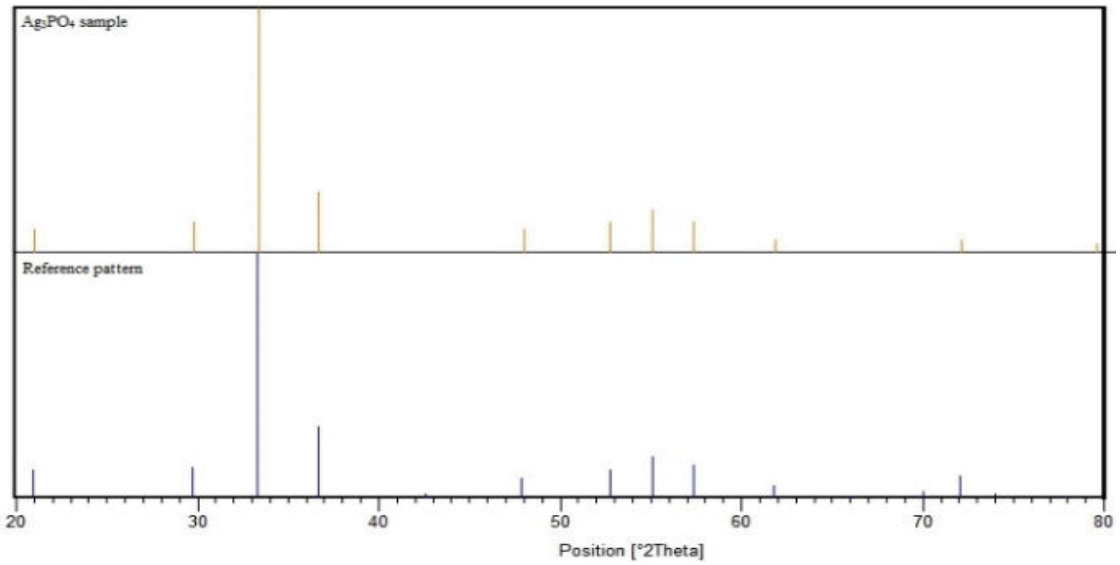

Fig. 3 Comparison of Ag_3PO_4 reference pattern with the pattern of synthesized nanoparticles

Regarding the FTIR spectra of the synthesized nanoparticles using extracellular polymeric substance (EPS) of *S. pasteurii* as bio-surfactant, many different absorption peaks were detected which can be associated with several functional groups present in the bacterial EPS (Fig. 4). This spectrum shows the presence of band at 1384(1), 1535(2), 1645(3), 2855(4), 2925(5), and 3446(6) cm^{-1}. The band at 1384 cm^{-1} corresponds to the free symmetric stretch of COO^- group [20]. Peaks around 1535 cm^{-1} are associated to the bending vibration of amide II (N–H) of the polypeptides or proteins [21]. It can also be seen that the bands near 1645 cm^{-1} are due to the presence of primary amine N-H bond [21], [22]. The peaks observed at 2855 cm^{-1} and 2925 cm^{-1} could be assigned to the antisymmetric and symmetric vibrations of the $–CH_2$ groups of the hydrocarbons present in bacterial proteins [23]. In addition, a broad peak at around 3446 cm^{-1} indicates a large amount of water adsorbed on the surface and/or hydroxyl groups of SPNPs [24].

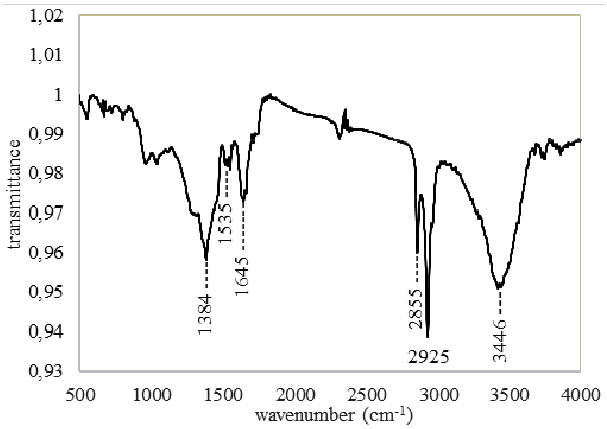

Fig. 4 FTIR of SPNPs

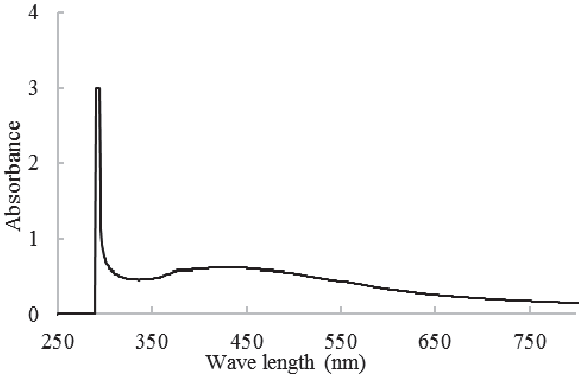

Fig. 5 UV-Vis absorbance spectrum of SPNPs

UV-Vis adsorption of the produced colloidal suspension is illustrated in Fig. 5. The sharp peak at 290 nm shows the presence of bio-surfactants in the solution and confirms the FTIR results [25]. It also indicates that the produced SPNPs can absorb visible light with a wavelength almost shorter than 650 nm [26].

Elemental analysis of the synthesized particles was performed by Energy Dispersive X-ray spectroscopy (EDS). This analysis was utilized for gaining a better understanding of the reaction process and to achieve further knowledge about the features of the Ag_3PO_4 nanoparticles. The obtained spectrum shows different peaks related to silver and phosphorus elements (Fig. 6) which approves the XRD analysis. Moreover, TEM image of SPNCs (Fig. 7) exhibits a specific spherical and an ellipsoidal morphology. According to this image, it is obvious that biologically stabilized particles are dispersed perfectly. From TEM micrograph, the size range of the Ag_3PO_4 particles was almost found to be 20 nm which conforms the crystal size calculated by using the XRD data.

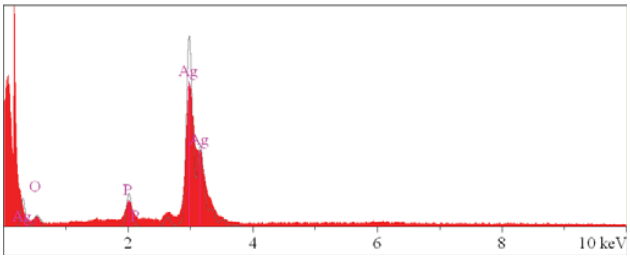

Fig. 6 EDS analysis of Ag_3PO_4 nanoparticles

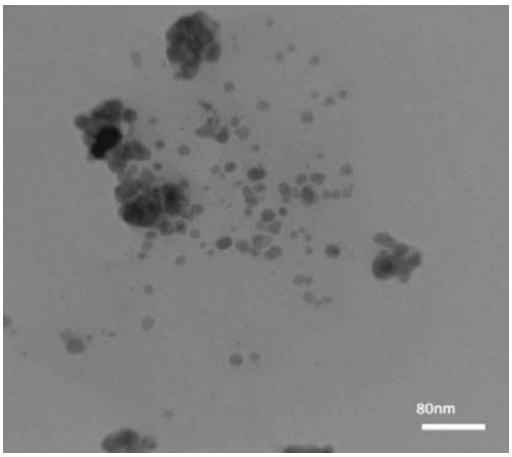

Fig. 7 TEM image of the green synthesized Ag_3PO_4 nanoparticles

IV. Conclusion

The present study demonstrates the first time synthesis of SPNPs by *Sporosarcina pasteurii* species. XRD and EDS analysis were proved the presence of phosphorus and formation of silver-phosphate crystals. Also, the approximate size of nanoparticles was calculated based on the XRD results using Williamson-Hall Equation, and according to the TEM micrographs. Furthermore, FTIR analysis showed the important role of proteins and amino acids in the formation of Ag_3PO_4 nano-sized particles. Considering the results of this study, the low-cost, and environment-friendly biosynthesis of SPNPs is possible and can replace the conventional chemical approaches.

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