Biosurfactant Facilitated Synthesis and Stabilization of Silver Nanoparticles

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Abstract: A biosurfactant produced by Pseudomonas aeruginosa PBSC1 cultivated in a low-cost medium formulated with Cashew Apple Juice as such was employed to synthesize and stabilize silver nanoparticles in the liquid phase. The particles were initially synthesized using NaBH4 as reducing agent in biosurfactant reverse micelles and were extracted from the micellar solution to disperse in heptane. A silver particle size in the range of 11 nm was observed. The UV–vis absorption spectra proposed that silver nanoparticles could be formed in the reverse micelles and relatively stabilized for at least 3 months without passivator addition. The Transmission Electron Microscope (TEM) shows that the silver nanoparticles are of spherical form and relatively uniform. This method provided a simpler way for nanoparticle synthesis compared to existing systems using whole organisms or partially purified biological extracts, showing that the low-cost biosurfactant can be used for nanoparticle synthesis as a non-toxic and biodegradable stabilizing agent.

Keywords: Pseudomonas aeruginosa PBSC1, Cashew Apple Juice, Silver nanoparticles, Reverse micelles, TEM.

1. Introduction

Presently, there is a growing concern at global level to have nontoxic, nonhazardous surface-active agents; contrary to synthetic surfactants, their biological counterparts or biosurfactants play a primary function, facilitating microbial presence in environments dominated by hydrophilic-hydrophobic interfaces. Microbial biosurfactants/bio-emulsifiers from marine and deep-sea environments are attracting major interest due to their structural and functional diversity as molecules actives of surface and an alternative biomass to replace fossil forms of carbon. Microbial surfactants are lipid in nature and classified as glycolipids, phospholipids, lipopeptides, natural lipids, fatty acids, and lipopolysaccharides. These metabolic bioactive products are applicable in a number of industries and processes, viz., food processing, pharmacology, and bioremediation of oil-polluted environments (Sarafin et al., 2014).

Currently, many techniques have been devoted to synthesizing nanosize silver particles, such as chemical reduction (Siegel, 1993), photochemical reduction, reverse micelle based and lamellar liquid crystals approaches (Limin et al., 1999), aerosol techniques and an electrostatic spraying technique. Since reverse micelles system was used to form metal nanoparticles by Boutonnet et al. (1982), these methods have been paid more and more attention (Hiroyuki et al., 2001). However, most surfactants we used were chemical surfactants, which are toxic and will pollute the environment. Biosurfactant (Desai and Banat 1997), as a natural surfactant, which derived from microbial origin have bulky and complicated structures, higher biodegradability, lower toxicity, and excellent antiviral activities. So biosurfactant as a “green” stabilizer is one of the best candidates. It is believed
that biosurfactant will be increasingly attraction as multifunctional materials for the new century (Kitamoto et al., 2002).

In the present study the possibility of synthesizing silver nanoparticles in water-in-oil microemulsion stabilized biosurfactant is studied. The silver nanoparticles obtained are characterized by UV–vis absorption spectrum, Dynamic Light Scattering (DLS) and, Transmission Electron Microscope (TEM).

2. Materials and Methods

2.1. Microorganisms

\textit{Pseudomonas aeruginosa} PBSC1 was isolated from Pithavarm Mangrove soil sediments, Tamilnadu, India found to produce rhamnolipid biosurfactant in a cheap source of Cashew Apple Juice medium evaluated and validated in our earlier studies is used in the current study.

2.2. Synthesis Of Silver Nanoparticles

For the synthesis of silver nanoparticles \textit{in situ} in the water-in-oil micro emulsion phase, a 0.05 mol/l aqueous AgNO$_3$ solution and a 0.1 mol/l aqueous NaBH$_4$ solution were separately used instead of water to form reverse micelles with the biosurfactant. NaBH$_4$ was used here to act as reducing agent. The first synthesis involved mixing 1.0 ml of 0.05 mol/l aqueous AgNO$_3$ solution, 0.1 g/l biosurfactant and 25 ml n-heptane together and stirred vigorously at room temperature until homogeneous reverse micelles formed and the same bulk of 0.1 mol/l aqueous NaBH$_4$ solution was used to replace aqueous AgNO$_3$ to form the other reverse micelles. The two samples were mixed under stirring for 60 min. Then, the particles were precipitated from the solution and isolated by centrifugation at 14,000 × g. Then, 0.5 ml ethanol was added for each 1 ml reverse micelles. Ethanol was added to the complete removal of the surfactant and n-heptane. The prepared silver nanoparticles could be readily redispersed to obtain a suspension in 10 ml n-butanol aided by sonication. The second microemulsion was prepared by dissolving 0.1 g/l of the biosurfactant in 6.25 ml of n-heptane and 1 ml AgNO$_3$. Solution was added to the mixture with continuous stirring for 10 min at room temperature. Then, 1 ml NaBH$_4$ was added to the mixture which was agitated for 30 min. After agitation, 10 ml ethanol was added to break the reverse micelles, thus forming two phases. The precipitate was separated by centrifugation at a speed of 14,000 × g for 30 min and 10 ml of n-butanol was added to obtain a suspension.

2.3. Characterization Of Silver Nanoparticles

The optical characterizations of the synthesized silver nanoparticles were analyzed through absorption spectra measured in room temperature in a UV Visible absorption spectrometer (ELICO SL 244) at the wavelength of 200 to 800nm under dispersion mode. In the present study, prepared silver nanoparticles was taken in disposable sizing cuvette, at 25°C temperature, 109.7 kcps Count Rate and 4.65 mm Measurement Position to measure the average particle size and Zeta potential using computer controlled particle size analyzer Zetasizer Ver. 6.01 (Malvern Instruments Ltd..). The dispersant used for dispersing the nanoparticles was double distilled water. Further High Resolution Transmission Electron Micrograph of Silver nanoparticles was taken according El-Shanshoury et al., (2011).

3. Result and Discussion

3.1. Production Of Silver Nanoparticles From Biosurfactant

Micro-emulsion techniques using oil–water–surfactant mixture were shown to be a promising approach for nanoparticle synthesis.

The optical absorption spectrum of the silver nanoparticles synthesis using biosurfactant from \textit{P. aeruginosa} PBSC1 was shown in the Figure 1. From the figure, the optical absorption of the silver nanoparticles possess narrow band edge (432 nm), which originated from the uniform sized particle distribution of the sample. The absorption edge was shifted to the lower wavelength region confirming nano-sized formation of the final product, which was caused by the quantum confinement effect. The optical band gap of the material was calculated using effective mass approximation was found to be.
3.7 eV (432 nm). Light below this wavelength holds sufficient energy to excite electrons and hence absorbed by silver nitrate. On the other hand, light with longer wavelength which was higher than the band gap energy (towards the visible light) will not be absorbed.

![UV-Visible Absorption spectrum of silver nanoparticles synthesized using biosurfactant from P. aeruginosa PBSC 1](image)

UV–visible absorption spectrum is sensitive to the formation of silver nanoparticles because silver particles can show an intense absorption peak around 400 nm originating from the surface plasmon absorption of nanosized silver particles (Ji et al., 1999). Decrease in the intensity is due to a change in the free electron density. Particle aggregation was studied with change in yields, a variation of the width and the red-shift of the maximum in the absorption spectrum (Limin et al., 1999). Metal nanoparticles have a surface Plasmon resonance absorption in the UV–visible region. This result evidenced that the Nano-scale silver can be synthesized in reverse micelles using glycolipid as stabilizer (Kitamoto et al., 2002). This result indicates that the nano-scale silver can be synthesized in reverse micelles using the low-cost biosurfactant as stabilizer. Decrease in the intensity is due to a change in the free electron density.

Xu et al. (2006) studied that the UV–visible absorption spectrum of silver nanoparticles in n-heptane. A strong absorption peak at approximately 406 nm originates from the surface plasmon absorption of nanosized silver particles. Similar results were recorded in our study with the absorption spectrum of 432 nm for the SNPs synthesized using biosurfactant from PBSC1. The good symmetric absorption peak implies that the size distribution of the nanoparticles is narrow (He et al., 2001). No obvious variation in the shape, position and symmetry of the absorption peak is observed, which indicates that the as prepared silver nanoparticles can remain stable for at least 2 months.

**High Resolution Transmission Electron Micrograph Of Silver Nanoparticles**

HR-TEM analysis was carried out on the silver Nano particles to observe the individual size and shape of it. HR-TEM micrograph of samples synthesized was shown in Figure 3a. A large number of smaller particles were distributed on the films in the size range of 15-32 nm of silver nanoparticles from PBSC1. This indicates that the distribution of silver nanoparticles stabilized by the biosurfactant was rather uniform.

The Selected Area Electron Diffraction (SAED) analysis showed those continuous ring patterns which originate from polycrystalline state or by the more crystallites attached to the surface of the single particles (Figure 3b). Bright ring pattern showed the high density of crystallites in the materials in the silver nanoparticle samples (PBSC1).

The typical TEM micrographs of the silver nanoparticles (Limin et al., 1999) were obtained in this study. This indicates that the distribution of silver nanoparticles stabilized by rhamnolipid is rather uniform. However, some larger particles on the films are observed. Two possibilities are concerned. One is that the nanometer-sized water layers limit the packing of the particles in the direction perpendicular to the water layers when the particles are growing in reverse micelles, the absorption of surfactant molecules cannot totally prevent particles from aggregating and the thickness of the water layers cannot absolutely restrict the particle size due to the flexibility of the surfactant bilayers (Limin et al., 1999). The other is
that during the extraction and redispersion process a part of particles impact each other and aggregation.

**Fig 3a and 3b: HR-Transmission Electron Micrograph and SEAD of SNPs synthesized using biosurfactant from *P. aeruginosa* PBSC1**

The structure of the biosurfactant plays an important role in determining the morphology of the synthesized nanoparticles. These micelles are spherical in shape and favoured the formation of spherical nanoparticles during synthesis. As biosurfactants are natural surfactants derived from microbial origin composed mostly of sugars and fatty acids moieties, they have higher biodegradability, lower toxicity and excellent biological activities. Since the biosurfactants reduce the formation of aggregates due to electrostatic force of attraction they facilitate uniform morphology and stability of nanoparticles (Xie *et al.*, 2006)

Some larger particles on the films are also observed. Two possibilities are of concern. One is that the nanometer-sized water layers limit the packing of the particles in the direction perpendicular to the water layers when the particles are growing in reverse micelles, the absorption of surfactant molecules cannot totally prevent
particles from aggregating and the thickness of the water layers cannot absolutely restrict the particle size due to the flexibility of the surfactant bilayers (Kiran et al., 2010). The other is that during the extraction and re-dispersion process many particles impact each other promoting aggregation between them. The stability of silver nanoparticles synthesized through the biosurfactant, were stable for 3 months. The biosurfactant would have acted as stabilizing agent and prevented the formation of aggregates and favoured the production and stability of the nanoparticles under the experimental conditions (Farias et al., 2014).

3.7. Stability Study Of Silver Nanoparticles

The silver nanoparticles synthesized using biosurfactant from \textit{P. aeruginosa} PBSC1 after a day showed a relatively intense absorption peak around 432nm UV spectroscopy.

Fig 5: UV–Vis absorption spectra of silver nanoparticles synthesized from biosurfactant from \textit{P. aeruginosa} PBSC1 in the n-heptane solution at room temperature for 1(A), 30 (B) and 60 (C) days.

On increasing the time from 1, 30 to 60 days the Plasmon absorption bands of three samples are quite similar for both silver nanoparticles synthesized using biosurfactant from PBSC1 (Figure 5). No obvious changes in the position and symmetry of the absorption peak except for the decrease of the absorbance, indicating a little aggregation of silver nanoparticles. During the entire chemistry process, no passivator was added into the system. It proves that the silver nanoparticles solution prepared in such proportional reverse
micelles can remain relatively stable for at least 2 months. The remnant rhamnolipid and lipopeptide in the solution was regarded as the stabilizer, which form a steric hindrance around the particles to preventing them aggregation greatly by electrostatic interactions.

Xie et al. (2006) reported that on increasing the time from 1 to 60 days, the Plasmon absorption bands are quite similar. They have no obvious changes in the position and symmetry of the absorption peak except for the decrease of the absorbance, indicating a little aggregation of silver nanoparticles upon storage. The silver nanoparticles solution prepared in reverse micelles can remain relatively stable for at least 2 months. The remnant rhamnolipid in the solution is regarded as the stabilizer, which form a steric hindrance around the particles to preventing them aggregation greatly by electrostatic interactions.

Kiran et al. (2010) used a glycolipid biosurfactant produced from sponge-associated marine Brevibacterium casei MSA19 synthesized silver nanoparticles were uniform and stable for 2 months. Farias et al. (2014) reported that the silver nanoparticles solution prepared in such proportional reverse micelles can remain relatively stable for at least three months. Similar results were obtained in the present study that the silver nanoparticles were stable for 2 months in the solution, hence it was proved that the biosurfactant act as a stabilizing agent and prevented the formation of aggregates.

Considering the need of greener bioprocess and novel enhancers for the synthesis using microbial processes, biosurfactants and/or biosurfactant producing microbes are emerging as an alternate source of rapid synthesis of nanoparticles (Xie et al., 2006). A micro-emulsion technique using oil–water–surfactant mixture was shown to be a promising approach for nanoparticles synthesis (Xie et al., 2006). Although chemical surfactants are highly promising, these chemicals could be toxic to the environment. Recently, the focus on biosurfactant-mediated processes is steeply increasing due to their potential implications on the synthesis of silver nanoparticles (Palanisamy and Raichur, 2009). Xie et al., (2006) reported that rhamnolipid biosurfactant could be used as a stabilizing agent for silver nanoparticles. In the present study revealed the possibilities’ of using glycolipid and lipopeptide mediated synthesis of silver nanoparticles would be effective and advantageous over chemical surfactants.

The green synthesis of silver nanoparticles involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) selection of solvent medium, (2) selection of environmentally benign reducing agent and (3) selection of nontoxic substances for the silver nanoparticle stability (Barnickel et al., 1992).

Micro-emulsion techniques using oil-water surfactant mixtures were shown to be a promising approach for nanoparticle synthesis, as described by Xie et al. (2006); Kasture et al. (2008) and Reddy et al. (2009). According to these literatures, in the present study the silver nanoparticles were synthesized and stabilized.

Kiran et al. (2010) studied glycolipid biosurfactant produced from sponge-associated marine Brevibacterium casei MSA19 using the agro-industrial and industrial waste as substrate to synthesize silver nanoparticles. In our present study the agro industrial wastes such as Cashew Apple Juice was used to synthesize biosurfactant from P. aeruginosa PBSC1. The recovered biosurfactant was used to synthesize the silver nanoparticles by reverse micelles method.

Farias et al.(2014) reported that the synthesis of silver nanoparticles from a laboratory biosurfactant produced from agro-industrial waste are promising since the majority of reports describing the use of biosurfactants in the synthesis of silver nanoparticles are already published in the literature used commercial rhamnolipids.

4. Conclusion

The present work demonstrates a simple eco-friendly method for synthesizing spherical silver nanoparticles by microemulsion technique. Silver nanoparticles were successfully synthesized using the biosurfactant from P. aeruginosa PBSC1. The synthesized nanoparticles were found to be spherical in shape with uniform distribution. The silver nanoparticles can be stabilized correspondingly for at least 3 months without passivator addition.
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6. References


