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Synthesis of silver nanoparticles using a biosurfactant produced in low-cost medium as stabilizing agent

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ABSTRACT

Background: A biosurfactant produced by *Pseudomonas aeruginosa* cultivated in a low-cost medium formulated with 2.5% vegetable oil refinery residue and 2.5% corn steep liquor and distilled water was employed to stabilize silver nanoparticles in the liquid phase. The particles were initially synthesized using $NaBH_4$ as reducing agent in biosurfactant reverse micelles and were extracted from the micellar solution to disperse in heptane.
Results: A silver particle size in the range of 1.13 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.
Conclusions: This process provided a simpler route 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.

1. Introduction

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment [1,2]. This increasing demand must be accompanied by "green" synthesis methods. In the global efforts to reduce generated hazardous waste, "green" chemistry and chemical processes are progressively integrating with modern developments in science and industry. Implementation of these sustainable processes should adopt the 12 fundamental principles of green chemistry [3,4,5,6]. These principles are geared to guide in minimizing the use of unsafe products and maximizing the efficiency of chemical processes. Hence, any synthetic route or chemical process should address these principles by using environmentally benign solvents and nontoxic chemicals [7].

Silver nanocrystallites exhibit an enhancement of some potential properties including catalysis [8], magnetic and optical polarizability [9], electrical conductivity [10], and antimicrobial activity in surface-enhanced Raman scattering (SERS) [11]. Currently, many techniques have been devoted to synthesizing nanosized silver particles, such as chemical reduction [12,13,14], photochemical reduction, reverse micelle based and lamellar liquid crystal approaches [15], aerosol techniques and an electrostatic spraying technique [16]. Since the reverse micelle system has been used to form metal nanoparticles, this method has been paid more and more attention [17]. However, most surfactants we used were chemical surfactants, which are toxic and will pollute the environment. Biosurfactants, which derived from microbial origin, have bulky and complicated structures, higher biodegradability, lower toxicity, and excellent antiviral activities [18]. So biosurfactant as a "green" stabilizer is one of the best candidates. It is believed that biosurfactants will be increasingly attractive as multifunctional materials for the new century [14].

In the present paper the possibility of synthesizing silver nanoparticles in water-in-oil microemulsion stabilized by a low-cost biosurfactant is studied. The silver nanoparticles obtained are characterized by UV–vis absorption spectrum and a Transmission Electron Microscope (TEM).

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2. Materials and methods

2.1. Materials

n-Heptane and *n*-butanol were purchased from Sigma-Aldrich Chemical Corporation, USA. Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich Chemical Corporation, USA. Sodium borohydride ($NaBH_4$, 97%) was purchased from Sigma-Aldrich Chemical Corporation, USA. All of the components are of reagent grade and used without further purification. Milli-Q grade water was used in all the experiments.

2.2. Bacterial strain and preparation of seed culture

A strain of *Pseudomonas aeruginosa* UCP0992 was provided from the culture collection of the Centre of Research in Environmental Sciences, Universidade Católica de Pernambuco, Brazil, registered with the World Federation of Culture Collections (WFCC). The cultures were maintained in nutrient agar slants at 4°C. For pre-culture, the strain from a 24 h culture on nutrient agar was transferred into 50 ml nutrient broth to prepare the seed culture. The cultivation condition for the seed culture was 28°C, 150 rpm, and 10–14 h of incubation time.

2.3. Biosurfactant production

For liquid fermentation, a 1% cell suspension of 0.7 OD (optical density) at 600 nm, corresponding to an inoculum of 10^7 C.F.U./ml, was inoculated into a 500 ml flask containing 100 ml medium consisting of distilled water amended with 5% vegetable oil refinery residue and 2.5% corn steep liquor. The pH of the medium was initially adjusted to 7.0 by 1.0 M HCl. The culture temperature and agitation rate were 37°C and 150 rpm, respectively. At the end of fermentation (120 h), samples were taken from the liquid culture to determine the surface tension and surfactant concentration.

2.4. Biosurfactant isolation

The biosurfactant was extracted from culture media after cell removal by centrifugation at 5,000 × *g* for 30 min. The supernatant pH was adjusted to 2.0 with 6.0 M HCl, and an equal volume of $CHCl_3/CH_3OH$ (2:1) was added. The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice again. The biosurfactant was concentrated from the pooled organic phases using a rotary evaporator. The viscous yellowish product obtained was dissolved in methanol and concentrated again by evaporation of the solvent at 45°C [19].

2.5. Surface tension

Surface tension and interfacial tension (against hexadecane) changes were carried out on the cell-free broth obtained by centrifuging the cultures at 5,000 × *g* for 20 min by the ring method using a Sigma 70 Tensiometer (KSV Instruments Ltd. — Finland) at room temperature. Tensiometers determine the surface tension with the help of an optimally wettable ring suspended from a precision balance. In the ring method the liquid is raised until contact with the surface is registered. The sample is then lowered again so that the liquid film produced beneath the liquid is stretched. As the film is stretched a maximum force is experienced, the force is measured and used to calculate the surface tension. The instrument was calibrated against Milli-Q-4 ultrapure distilled water (Millipore, Illinois, USA). Prior to use the platinum plate and all the glassware were sequentially washed with chromic acid, deionized water, acetone and finally flamed with a Bunsen burner.

2.6. Preparation method

The nanoparticles were synthesized using two modified approaches, according to Xie et al. [20] and Palanisamy and Raichur [21].

For the synthesis of silver nanoparticles in situ in the water-in-oil microemulsion 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.

2.7. Characterization

The resulting samples were used for absorption spectroscopy characterization on a Shimadzu UV-2450 spectrophotometer from 300 to 800 nm. A FEI Tecnai™ (USA) TEM operated at 200 kV was used for studying the shape and size of the nanoparticles. The sample for TEM study was prepared by dispersing the powder particles in acetone solution followed by sonication for 5 min. A droplet of the solution mixture was taken on a carbon coated copper grid.

3. Results and discussion

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 [22].

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 [19,23]. Micro-emulsion techniques using oil–water–surfactant mixture were shown to be a promising approach for nanoparticle synthesis, as described by Xie et al. [20].

Based on this approach, we have produced and tested a low-cost biosurfactant as a stabilizing agent of silver nanoparticles synthesized in microemulsion.

The biosurfactant from *P. aeruginosa* cultivated in a low-cost medium formulated only with two agro-industrial substrates was produced during 120 h at 37°C. The medium surface tension was reduced to 29.0 mN/m at the end of fermentation and the isolated biosurfactant corresponded to a concentration of 7.4 g/l.

3.1. Absorption spectra characterization

The result showed that a silver particle size in the range of 1.13 nm was obtained by the use of the biosurfactant from *P. aeruginosa*.

The UV–vis absorption band of nanoparticles at 400 nm is a typical characteristic of silver nanoparticles (Fig. 1). Metal nanoparticles have a surface plasmon resonance absorption in the UV–vis region. This result evidenced that the nano-scale silver can be synthesized in reverse micelles using glycolipid as stabilizer [24,25,26,27]. The particles are extracted from micelles by adding ethanol to the microemulsion. 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.

To monitor the stability of the final prepared silver particles, we measured the absorption spectra of the solution on different days. During the entire chemistry process, no passivator was added into the system. It proves that the silver nanoparticle solution prepared in such proportional reverse micelles can remain relatively stable for at least 3 months. The remnant biosurfactant in the solution is regarded as the stabilizer, which forms a steric hindrance around the particles to prevent them from aggregating greatly by electrostatic interactions.

The particle size found in this work is similar to the one obtained by Xie et al. [20], who found sizes in the range of 2–8 nm for the silver nanoparticles stabilized by the biosurfactant-rhamnolipid purchased from Jeneil Biosurfactant Co., LLC (USA) in heptane. On the other hand, Palanisamy and Raichur [21] showed nickel oxide particles of about 47 nm when the same commercial Jeneil Biosurfactant was employed under optimized pH conditions. Research involving synthetic surfactants demonstrated the synthesis of silver nanoparticles on the order of 59–70, 43–53 and 57–76 nm with the cationic cetyltrimethylammonium chloride (CTAC), the anionic sodium dodecyl sulfate (SDS) and the non-ionic Tween 80, respectively, according to the variation of concentration of solutions of these surfactants [28]. Kiran et al. [29] used a glycolipid biosurfactant produced from sponge-associated marine *Brevibacterium casei* MSA19 using the agro-industrial and industrial waste as substrate to synthesize silver nanoparticles. The silver nanoparticles synthesized in this study by the glycolipid were uniform and stable for 2 months.

3.2. TEM analysis

TEM analysis was carried out on the silver particles to observe the individual size and shape of the nanoparticles. TEM micrograph of sample synthesized is shown in Fig. 2, which is consistent with the absorption spectra results. A large number of smaller particles are distributed on the films, the size range of which is 1–2 nm. This indicates that the distribution of silver nanoparticles stabilized by the biosurfactant is rather uniform.

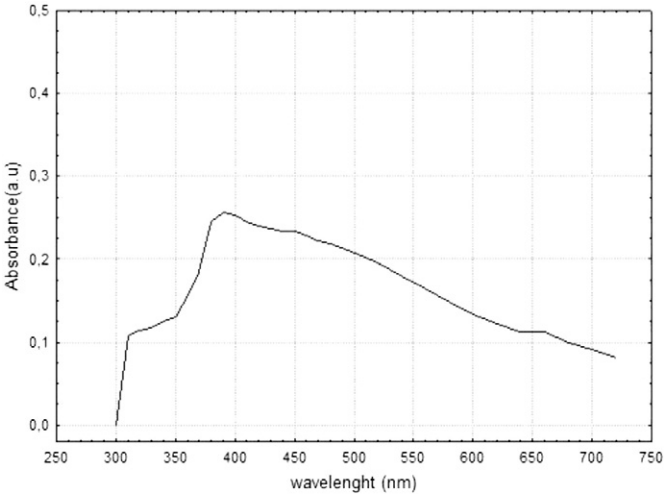

Fig. 1. UV–vis absorption spectra of silver nanoparticles after synthesis in reverse micelles at room temperature.

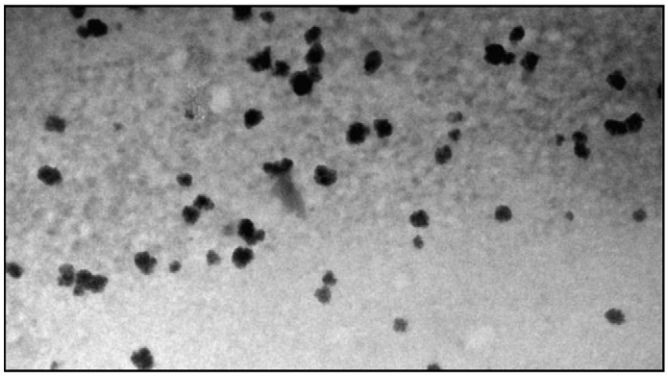

Fig. 2. Transmission Electron Micrograph of silver nanoparticles in heptane (scale = 200 nm).

The structure of the biosurfactant plays an important role in determining the morphology of the synthesized nanoparticles. These micelles are spherical in shape and favored the formation of spherical nanoparticles during synthesis.

As biosurfactants are natural surfactants derived from microbial origin composed mostly of sugar and fatty acid 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.

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 [29]. The other is that during the extraction and redispersion process many particles impact each other promoting aggregation between them.

To determine the stability of silver nanoparticles synthesized through the biosurfactant, the prepared silver particles were kept at room temperature for different day intervals. The results evidenced that nanoparticles were stable for 3 months. Control experiments without biosurfactant were included in the experimental set-up and it was observed that instead of nanoparticles, aggregated clumps were observed after 7 d. The biosurfactant would have acted as stabilization agent and prevented the formation of aggregates and favored the production and stability of the nanoparticles under the experimental conditions.

The results described here for 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 nanoparticles already published in the literature make use only of commercial rhamnolipids, *i.e.* with a high degree of purification.

4. Concluding remarks

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*. The synthesized nanoparticles were found to be spherical in shape with uniform distribution. The experimental observation was supported by TEM analysis. The silver nanoparticles can be stabilized correspondingly for at least 3 months without passivator addition. The use of low-cost, renewable and

biodegradable biosurfactants in replacement to toxic synthetic surfactants is a promising alternative for the synthesis of inorganic nanoparticles for industrial application.

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Author contribution

Proposed the theoretical frame: LAS, JEGS; Conceived and designed experiments: LAS, JEGS; Contributed reagents/materials/analysis tools: RDR, JML; Wrote the paper: LAS; Performed the experiments: CBBF, AFS, RDR, JML; Analyzed the data: LAS, JEGS.

Conflict of interest

There is no conflict of interest.

References

[1] Dahl JA, Maddux BLS, Hutchison JE. Toward greener nanosynthesis. Chem Rev 2007;107:2228–69. http://dx.doi.org/10.1021/cr050943k.
[2] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 2009;145:83–96. http://dx.doi.org/10.1016/j.cis.2008.09.002.
[3] Poliakoff M, Anastas P. A principled stance. Nature 2001;413:257. http://dx.doi.org/10.1038/35095133.
[4] Desimone JM. Practical approaches to green solvents. Science 2002;297:799–803. http://dx.doi.org/10.1126/science.1069622.
[5] Gross RA, Kalra B. Biodegradable polymers for the environment. Science 2002;297:803–7. http://dx.doi.org/10.1126/science.297.5582.803.
[6] Raveendran P, Fu J, Wallen SL. Completely "green" synthesis and stabilization of metal nanoparticles. J Am Chem Soc 2003;125:13940–1. http://dx.doi.org/10.1021/ja029267j.
[7] Anstas PT, Warner JC. Green chemistry: Theory and practice. New York: Oxford University Press Inc.; 1998 30.
[8] Shiraishi Y, Toshima N. Oxidation of ethylene catalyzed by colloidal dispersions of poly(sodium acrylate)-protected silver nanoclusters. Colloids Surf A 2000;169:59–66. http://dx.doi.org/10.1016/S0927-7757(00)00417-9.
[9] Bloemer MJ, Haus JW, Ashley PRJ. Degenerate four-wave mixing in colloidal gold as a function of particle size. J Opt Soc Am B 1990;7:790–5. http://dx.doi.org/10.1364/JOSAB.7.000790.
[10] Chang LT, Yen CCJ. Studies on the preparation and properties of conductive polymers. VIII. Use of heat treatment to prepare metallized films from silver chelate of PVA and PAN. Appl Polym Sci 1995;55:371–4. http://dx.doi.org/10.1002/app.1995.070550219.
[11] Matejka P, Vlckova B, Vohidal J, Pancoska P, Baumrunk VJ. The role of triton X-100 as an adsorbate and a molecular spacer on the surface of silver colloid: A surface-enhanced Raman scattering study. J Phys Chem 1992;96:1361–6. http://dx.doi.org/10.1021/j100182a063.
[12] Murray BC, Noris DJ, Bawendi MG. Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites. J Am Chem Soc 1993;115:8706–15. http://dx.doi.org/10.1021/ja00072a025.
[13] Taleb A, Petit C, Pileni MP. Synthesis of highly monodisperse silver nanoparticles from AOT reverse micelles: A way to 2D and 3D self-organization. Chem Mater 1997;9:950–9. http://dx.doi.org/10.1021/cm960513y.
[14] Li XL, Zhang JH, Xu WQ, Jia HY, Wang X, Yang B, et al. Mercaptoacetic acid-capped silver nanoparticles colloid: Formation, morphology, and SERS activity. Langmuir 2003;19:4285–90. http://dx.doi.org/10.1021/la0341815.
[15] Limin Q, Yueying G, Jiming M. Synthesis of ribbons of silver nanoparticles in lamellar liquid crystals. Colloids Surf A 1999;157:285–94. http://dx.doi.org/10.1016/S0927-7757(99)00053-9.
[16] Jiang LP, Wang AN, Zhao Y, Zhang JR, Zhu JJ. A novel route for the preparation of monodisperse silver nanoparticles via a pulsed sonoelectrochemical technique. Inorg Chem Commun 2004;7:506–9. http://dx.doi.org/10.1016/j.inoche.2004.02.003.
[17] Lin J, Zhou W, O'Connor CJ. Formation of ordered arrays of gold nanoparticles from CTAB reverse micelles. Mater Lett 2001;49:282–6. http://dx.doi.org/10.1016/S0167-577X(00)00385-2.
[18] Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, et al. Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 2010;87:427–44. http://dx.doi.org/10.1007/s00253-010-2589-0.
[19] Kasture MB, Patel P, Prabhune AA, Ramana CV, Kulkarni AA, Prasad BLV. Synthesis of silver nanoparticles by sophorolipids: Effect of temperature and sophorolipid structure on the size of particles. J Chem Sci 2008;120:515–20. http://dx.doi.org/10.1007/s12039-008-0080-6.
[20] Xie Y, Ye R, Liu H. Synthesis of silver nanoparticles in reverse micelles stabilized by natural biosurfactant. Colloids Surf A 2006;279:175–8. http://dx.doi.org/10.1016/j.colsurfa.2005.12.056.
[21] Palanisamy P, Raichur AM. Synthesis of spherical NiO nanoparticles through a novel biosurfactant mediated emulsion technique. Mater Sci Eng C 2009;29:199–204.
[22] Barnickel P, Wokaun A, Sager W, Eicke HF. Size tailoring of silver colloids by reduction in WO microemulsions. J Colloid Interface Sci 1992;148:80–90. http://dx.doi.org/10.1016/0021-9797(92)90116-4.
[23] Reddy AS, Chen CY, Baker SC, Chen CC, Jean JS, Fan CW, et al. Synthesis of silver nanoparticles using surfactin: A biosurfactant as stabilizing agent. Mater Lett 2009;63:1227–30. http://dx.doi.org/10.1016/j.matlet.2009.02.028.
[24] Petit C, Lixon P, Pileni MP. Structural change in AOT reverse micelles induced by changing the counterions. Prog Colloid Polym Sci 1992;89:328–31. http://dx.doi.org/10.1007/BFb0116340.
[25] Huang HH, Ni XP, Loy GL, Chew CH, Tan KL, Loh FC, et al. Photochemical formation of silver nanoparticles in poly(N-vinylpyrrolidone). Langmuir 1996;12:909–12. http://dx.doi.org/10.1021/la950435d.
[26] Ji M, Chen XY, Wai CM, Fulton JL. Synthesizing and dispersing silver nanoparticles in a water-in-supercritical carbon dioxide microemulsion. J Am Chem Soc 1999;121:2631–2. http://dx.doi.org/10.1021/ja9840403.
[27] Kitamoto D, Isoda H, Nakahara T. Functions and potential applications of glycolipid biosurfactants — From energy-saving materials to gene delivery carriers. J Biosci Bioeng 2002;94:187–201. http://dx.doi.org/10.1263/jbb.94.187.
[28] Soukupová J, Kvítek L, Panácek A, Nevecná T, Zboril R. Comprehensive study on surfactant role on silver nanoparticles (NPs) prepared via modified Tollens process. Mater Chem Phys 2008;111:7–81. http://dx.doi.org/10.1016/j.matchemphys.2008.03.018.
[29] Kiran GS, Sabu A, Selvin J. Synthesis of silver nanoparticles by glycolipid biosurfactant produced from marine *Brevibacterium casei* MSA19. J Biotechnol 2010;148:221–5. http://dx.doi.org/10.1016/j.jbiotec.2010.06.012.