Journal of Sciences, Islamic Republic of Iran 33(2): 133 - 138 (2022) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Photo-Mediated Preparation of Silver Nanoparticles Dry Concentrate in an Organic Solvent System, Using Culture Supernatant of *Klebsiella pneumonia*: A Preliminary Study

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Received: 19 April 2022 / Revised: 6 July 2022 / Accepted: 20 July 2022

Abstract

Silver nanoparticles (AgNPs) are well-known nanomaterials that have been mainly used as antimicrobial agents Hundreds of chemical or biological methods have been reported and described for the preparation of AgNPs in water so far. Aqueous colloids of AgNPs normally have a limited capacity to safely change to the concentrated form using conventional methods such as different evaporation methods. Organic solvents which could be easily evaporated using conventional evaporation vacuum methods are good candidates for the preparation of highly concentrated AgNPs formulations or dried concentrates. In this study, we used a previously described biological method for preparing AgNPs in an acetone-water solvent mixture. The nanoparticles were synthesized using culture supernatant of *Klebsiella pneumonia* in the above organic solvent mixture supplemented with polyethylene glycol 6000 in a bright condition and subsequently dried by a conventional evaporation method. In the next step, dried residues were re-dispersed in water under ultra-sound treatment and characterized with different instrumentation methods. The results showed spherical AgNPs with a size range of ≤ 200 nm. This is the first report on the biological synthesis of AgNPs in an organic solvent mixture which could be easily converted to a dried form. This dried AgNPs concentrate is a good candidate for the preparation of very thick formulations of AgNPs such as solid or semi-solid pastes.

Keywords: Silver nanoparticles; Organic solvents; Biological synthesis; *Klebsiella pneumonia*; Dry concentrate.

Introduction

In the metallic particle preparation process, decreasing the particle size to nano-scale improves the particle size distribution, morphology, and specific surface area compared to larger particles [1]. Recently, researchers have paid particular attention to metallic

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nanoparticles because of their unique chemical, optical, electrical, and magnetic properties [2-4]. Nanomedicine is another noticeable field in which metal nanoparticles can improve medical equipment and procedures such as diagnostic kits, magnetic resonance imaging (MRI), radiotherapy, gene, and drug delivery [3-5]. Among metallic nanoparticles, silver nanoparticles (AgNPs) are extensively used in antimicrobial, antitumoral, drug/gene delivery, catalytic, sensing, and electronic applications [6-8]. Hundreds of chemical or biological methods have been reported and described for the preparation of AgNPs in water in previous years [9-11]. Aqueous colloids of AgNPs normally have a limited capacity to safely change to the concentrated form using conventional methods such as different evaporation methods. Organic solvents which could be easily evaporated using conventional vacuum methods are good candidates for the preparation of highly concentrated AgNPs formulations or dried concentrate. Some reports have been published on the synthesis of AgNPs in organic solvents in recent years which can be applied for example for the conductive ink formulation in printed electronics [12, 13]. To the best of our knowledge, based on a literature survey, there is no report on the biological synthesis of AgNPs in organic solvents using whole cells or culture supernatants of microorganisms.

In the previous study, we described the rapid photo-aimed synthesis of AgNPs in an aqueous medium by culture supernatant of *Klebsiella pneumoniae* [14, 15]. Here, we primarily tested whether by this method the synthesis of AgNPs in organic solvents or organic solvent mixtures is possible or not. Fortunately, the culture supernatant of *Klebsiella pneumoniae* could successfully convert silver ions dissolved in an organic solvent mixture to AgNPs in reaction vessels that were irradiated by visible light emission. The AgNPs dispersed in such an organic solvent mixture could be easily changed to dry concentrate using conventional vacuum evaporation procedures and used for the preparation of very thick formulations of AgNPs such as solid or semi-solid pastes.

Materials and Methods

1- Characterization equipment descriptions

Ultraviolet-visible (UV-Vis) absorption spectra of Ag-NP aqueous colloids prepared from AgNPs dry concentrate were recorded by a Cecil model 9200 UV-Vis spectrophotometer. All particle size distribution and zeta potential studies were carried out using the dynamic light scattering (DLS) method (Malvern Zetasizer nano-ZS equipment) [16, 17]. Also, field emission scanning electron microscopy (FESEM) images were recorded on a Tescan Mira3 microscope operating at 15 kV [18].

2- Synthesis of AgNPs in an organic medium: selection of an appropriate culture medium and an organic solvent reaction mixture

In this part, different solvent systems were screened for this purpose and a solvent system composed of acetone-water (95:5) was selected for further experiments. No reaction was taken place in other solvent systems which were used during this study such as ethyl acetate, chloroform, butanol, or pure acetone (data not shown).

For the synthesis of AgNPs, silver nitrate (1 mM) was dissolved in an acetone-water (95:5) solvent system. *K. pneumoniae* from our culture collection was inoculated in different culture media (Merck™ Mueller-Hinton Broth (MHB), Merck™ Nutrient Broth (NB), Oxoid™ Tryptic Soy Broth (TSB), and starch supplemented TSB (STSB) and incubated at 37 °C for 24 hours. It should be noticed that the selection of these culture media was based on a random screening program which have been carried out previously [14, 15]. In the next step all bacterial culture media were centrifuged (12,000 rpm) and all supernatants were collected. Then, these collected supernatants were separately added to the above silver nitrate organic solution (1% v/v) at static condition and irradiated under a halogen lamp at a light intensity of 1000 μmol/m^2s. After 5 minutes, all the reaction mixtures (MHB-AgNPs, NB-AgNPs, TSB-AgNPs and STSB-AgNPs) were subjected to ultraviolet-visible (UV-Vis) spectroscopy and their plasmon resonance properties were recorded and compared.

For the preparation of AgNPs dry concentrate from the best reaction mixture (STSB-AgNPs), different amounts of polyethylene glycol 6000 (PEG6000) were added (100, 200, and 500 mg/1000 ml) to the selected organic solvent reaction mixture containing AgNPs (STSB-AgNPs) to stabilize particles during vacuum evaporation procedure. In the next step, mentioned PEG 6000 supplemented organic reaction mixtures were evaporated and concentrated under vacuum conditions by a BUCHI model rotary evaporator. Finally, all prepared dried concentrates samples prepared from the selected organic solvent mixture (STSB-AgNPs) were re-dispersed in distilled water (bringing the sample concentration to 1 mM) and subjected to physiochemical characterizations using the characterization methods described in the section above, before and after ultra-sound treatment for 6 min in an ultra-sound sonicator bath.

Results and Discussion

1- Synthesis of Ag-NPs in acetone-water mixture using culture supernatants of K. pneumoniae

Among four culture media that were tested during this study a culture medium (5 % starch supplemented TSB) was best to be used for the synthesis of AgNPs using culture supernatant of *K. pneumonia* in an acetone-water mixture (95:5). The left-hand illustration of Figure 1 shows the recorded UV-Vis spectra of AgNPs prepared using different culture supernatants of *K. pneumonia* under visible-light irradiation (1000 μmol/m^2s). As can be observed in Figure 1, the largest plasmon resonance peak was observed for the silver nitrate solution which was treated by culture supernatants of *K. pneumonia* grown in TSB which was supplemented by 5% starch (STSB-AgNPs). In contrast, no surface plasmon's resonance peak was detected in the reaction vessel in which culture supernatant of *K. pneumonia* cultivated in NB as a bio-reducing agent. Also, the right-hand illustration of Figure 1 shows the petri-dishes containing brownish AgNPs colloids prepared in acetone-water mixture using different culture supernatants of *K. pneumonia* grown in mentioned tested culture media. We described previously a rapid biological method for synthesis of AgNPs in aqueous medium [14, 15] but in current study we could successfully use the mentioned described biological method for fabrication of AgNPs in an organic reaction mixture for the first time.

2- Preparation of AgNPs dry concentrate

Different STSB-AgNPs organic colloids were prepared again in an acetone-water mixture (95-5 v/v) using culture supernatant of *K. pneumonia* which was

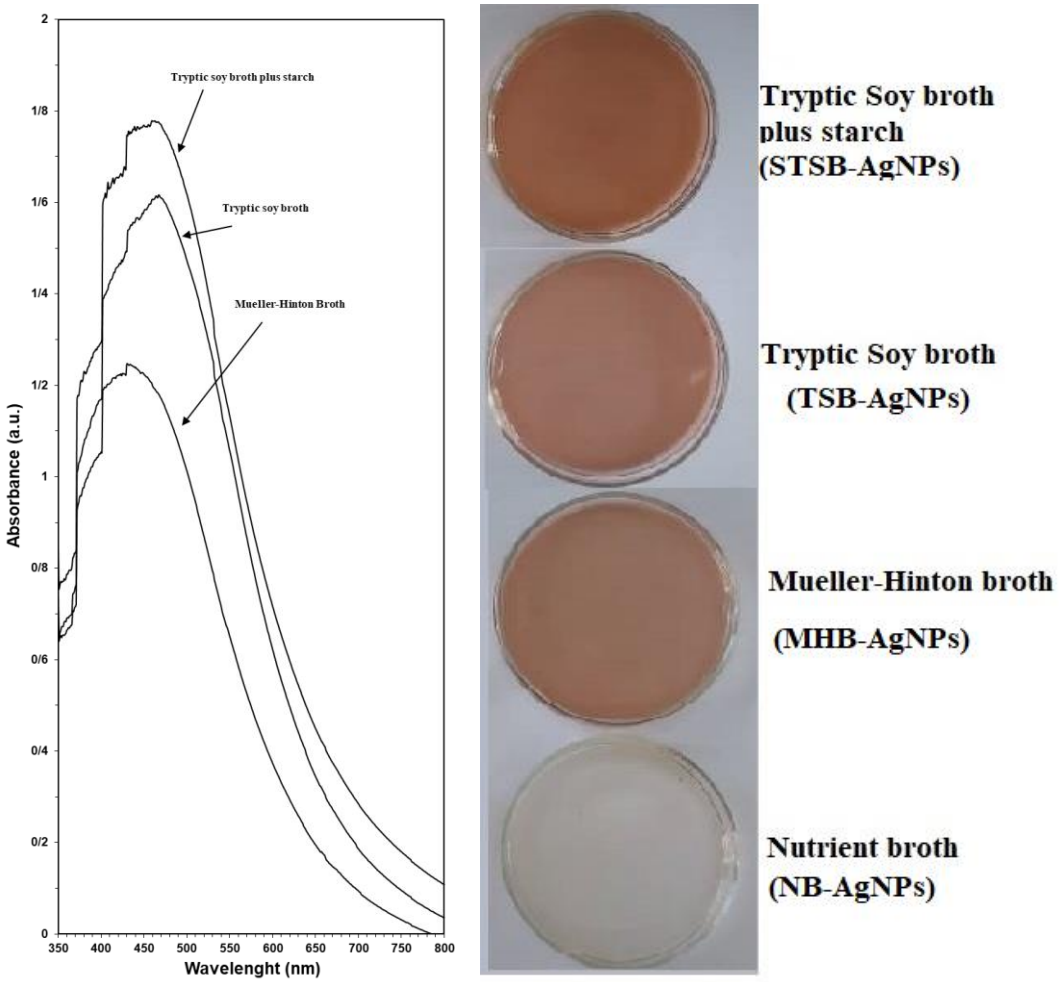

Figure 1. Left illustration demonstrates UV–vis spectra recorded after the addition of different culture supernatants of *K. pneumonia* grown in tested culture media (1 ml) to silver nitrate organic solution (1 mM; 100 ml) and allowed to stand for 5 min in bright condition under visible light irradiation (1000 mmol/m^2 s). Right illustration shows the test petri-dish containing the AgNPs samples prepared in acetone-water mixtures.

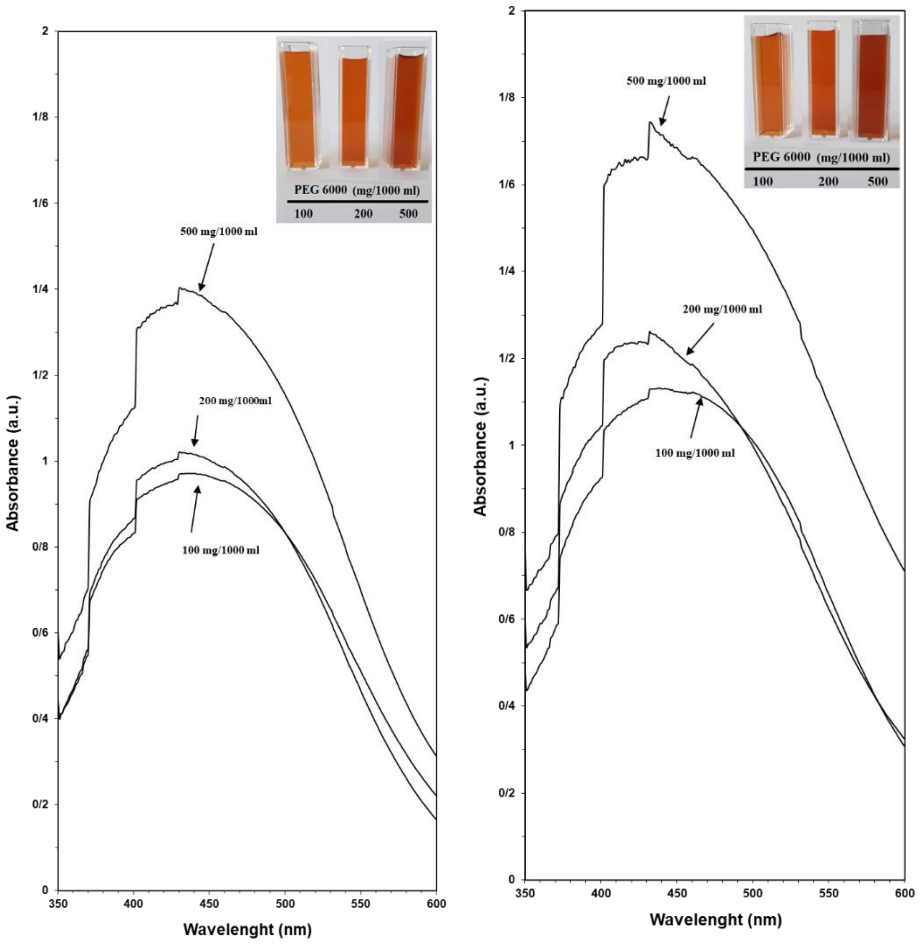

Figure 2. UV–vis spectra of STSB-AgNPs colloids. Spectra recorded after re-dispersion of different tested dry AgNPs concentrates in distilled water before (left illustration) and after (right illustration) treatment in ultra-sound sonicator bath for 6 min. The insets in the left and right illustrations show the cuvettes containing the different STSB-AgNPs colloids before and after sonication procedure.

Table 1. DLS size distribution and zeta potential of STSB-AgNPs colloids prepared from different dried concentrates of AgNPs using a re-dispersive process with and without ultra-sound treatment

DLS Data	**Before sonication**			**After sonication**		
	100	200	500	100	200	500
Z-Average (d.nm)	276	162	146	186	149	126
Zeta potential (mV)	ND	ND	ND	-23.0	-25.4	-27.3
PDI	0.581	0.480	0.363	0.541	0.384	0.379

ND: Not determined
PDI: Polydispersity Index

cultivated in a selected culture medium (starch supplemented TSB) at 37 ° C for 24 hours. All the prepared samples (STSB-AgNPs) were subsequently supplemented with different concentrations of PEG 6000 as a conventional stabilizer and thickener [19, 20]. These samples were evaporated under vacuum condition and re-dispersed again in distilled water with and without ultra-sound treatment. The left and right-hand illustrations in Figure 2 have been respectively demonstrated the UV-Vis spectra of the different aqueous colloids prepared from mentioned STSB-AgNPs dried concentrates which treated in an ultra-sonic sonication bath for 6 minutes (right illustration) or not treated (left illustration). The insets in these illustrations also show the cuvettes containing mentioned AgNPs aqueous colloids prepared

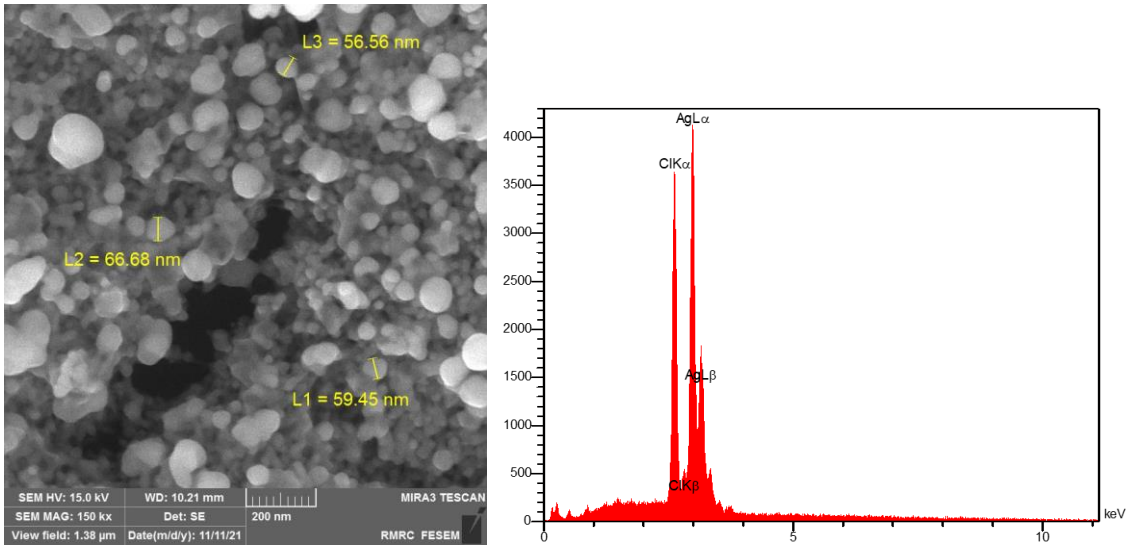

Figure 3. Left illustration: FESEM image of STSB-AgNPs prepared from best dry concentrate after re-dispersive process in water and treated in by ultra-sound sonicator bath for 6 min. The dried concentrate for this purpose was prepared from STSB-AgNPs resulted mixture, which was fabricated in bright condition (1000 mmol/m^2 s) by culture supernatant of *K. pneumonia* (grown in starch supplemented TSB) in an acetone-water mixture using a conventional rotary vacuum evaporator apparatus. Right illustration demonstrates EDS spectrum of the above AgNPs sample (STSB-AgNPs). Different X-ray emission peaks are labeled. Strong signals from the atoms in the nanoparticles are observed, while weaker signals from Na, Ca and Fe atoms are also visible. Also the chlorine signal is contributed to TSB culture medium which contains considerable amounts of sodium chloride.

throughout the re-dispersing process of dried concentrate of STSB-AgNPs containing different concentrations of PEG 6000, treated or not treated in an ultra-sonic sonication bath. The particle sizes and zeta potential of mentioned fabricated nanoparticles were further studied by the dynamic light scattering (DLS) method. Table 1 summarized all comparative data recording by DLS. The results show that less particles' mean size and the best size distribution are contributed to aqueous STSB-AgNPs colloid containing %5 w/v PEG6000 and solicited for 6 min. This colloid was subjected to further characterization. The left and right illustrations in Figure 3 demonstrate the FESME image and EDS pattern of this fabricated AgNPs sample (STSB-AgNPs), respectively. This aqueous STSB-AgNPs colloidal sample as mentioned above has been prepared by the re-dispersive process of a dried concentrate which is categorized as the best sample for further characterization. This selected sample was biologically fabricated in a bright condition and dried from silver ions organic solution treated by culture supernatant of *K. pneumonia* grown in TSB supplemented with starch (5% w/w) and abbreviated as STSB-AgNPs throughout of this manuscript. FESME image (Fig. 3, left-hand illustration) shows spherical particles in size rage mainly between 50 to 100 and in overall ≤200 nm, and in the analysis of the AgNPs by EDS, the presence of elemental silver signal was confirmed (Fig. 3, right-hand illustration).

Conclusion

This work report a photo-aimed synthesis of AgNPs in an acetone-water mixture (95-5) for the preparation of dried AgNPs concentrate using a conventional vacuum evaporation method. AgNPs were successfully synthesized by using culture supernatant of *K. pneumonia* grown in a starch supplemented TSB in the above organic water mixture under bright condition (1000 µmol/m^{2}s). This prepared organic reaction mixture containing AgNPs could be easily dried by a rotary vacuum evaporator and re-dispersed in distilled water. Size distribution analysis of re-dispersive sample, which was treated in ultra-sound sonicator bath showed the particle size range was mainly between 50 to 100 nm and in overall ≤200 nm. Furthermore, UV-visible, SESEM, and EDX analyses confirmed the presence of elemental silver with a spherical structure. AgNPs is a well-known nanomaterial which are widely used as an antimicrobial agent in different fields [13, 15]. This is the first report on the fabrication of AgNPs using a

biological method in an organic solvent mixture which could be easily converted to a dried form. This dried AgNPs concentrate is a good candidate for the preparation of solid or semi-solid antimicrobial pastes which could be used for making of working solutions at site. However this study is still preliminary and further investigations using appropriate additives or excipients cocktails should be performed on the formulation of this dried nanomaterial as an antimicrobial paste.

Acknowledgment

Research reported in this publication was supported by Elite Researcher Grant Committee under award number [971034] from the National Institute for Medical Research Development (NIMAD), Tehran, Iran.

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