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Synthesis of Silver Nanoparticles by Liquid Phase Pulsed Laser Ablation for Antibacterial Applications

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Abstract

The antibacterial property of silver makes it a versatile element in the applications related to clothing, medicine etc. The extremely small size of silver nanoparticles results in a large surface area relative to their volume which allows them to easily interact with micro organisms. The present work gives the analysis of antibacterial activity of laser ablated silver nanoparticles against Vibrio species. The silver nanoparticles of 6nm sizes were produced by liquid phase pulsed laser ablation (LP-PLA) of silver target in water media using second harmonics (532nm) of Nd: YAG laser operating at a repetition rate of 10Hz. The UV-visible absorption spectra and TEM confirms the presence of the nanoparticles. The antibacterial activity of the silver colloidal solution against Vibrio species is checked using pore plate method. It was found that the growth of the bacterial colony is very much reduced in the presence of silver nanoparticles. The antibacterial nature of the silver nanoparticles has a significant medicinal application, since all diseases like cholera caused by vibrio species are spread through water media. The usage of silver based paints on the surface of medical devices reduces the chance of spreading pathogens while surgery which is a severe problem while surgergical operations are performed.

INTRODUCTION

The use of antibacterial agents is widespread and can be seen in many products ranging from medicines, cosmetics up to household cleaners. All tend to rely on alcohol or triclosan based ingredients to eliminate pathogens; however, the use of triclosan in antibacterial products has been suspected of causing new antibiotic-

resistant bacteria. There is a need for a new agent that can be used in antibacterial applications that will be effective against antibiotic resistant bacteria. The antibacterial property of silver has been known for thousands of years with the ancient Greeks cook from silver pots and the old adage 'born to a silver spoon in his mouth' referring to more than just wealth. Silver nanoparticles are known as an effective germ fighter. Silver nanoparticles of sizes 1-10nm attached to HIV-1 and prevented the virus from bonding to host cells [1]. Silver is a versatile element with applications in the clothing, appliance and semiconductor industries. It can be used to destroy bacteria. The antimicrobial activity of silver nanoparticles against E. coli was investigated as a model for gram negative bacteria [2]. The extremely small size of nanoparticles results in a large surface area relative to their volume. In the case of silver nanoparticles, this allows them to easily interact with other particles and increases their antibacterial efficiency [3]. This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundred of square metres of substrate material.

Vibrios are gram-negative, curved, rod-shaped bacteria that are natural inhabitants of the marine environment and estuarine waters. Due to their flexible physiology, vibrios can persist in a variety of aquatic ecosystems where they interact with different biotic and abiotic surfaces. Vibrio Parahaemolyticus, V. Cholerae, and V. Vulnificus are the principal Vibrio species linked to seafood-borne infections. Some Vibrio bacteria are associated with diarrheal related diseases and other symptoms. Cholera is the majorly known disease spread by them. Among healthy people, ingestion of vibrio can cause vomiting, diarrhea and abdominal pain, decreased blood pressure (septic shock) and blistering skin lesions. Patients with liver disease are at particularly high risk for significant morbidity and mortality associated with these infections.

A number of methods have been proposed to control the size and shape of nano clusters including chemical reduction, photolysis of silver salts and ultrasonic reduction. In contrast to the usual chemical reduction methods [4-6] of producing metal nanoparticles, which involve the use of toxic solvents and highly reactive chemical reducing agents, the metal nanoparticle are produced in a simple, cost-effective and environmentally safe manner based on liquid phase pulsed laser ablation (LP-PLA) of a solid target in liquid media[7]. The resulting solution would consist only the colloids and solvent, no by products of silver would be present. Solutions of silver nanoparticles prepared by laser ablation do not require the addition of stabilizers for maintaining homogeneity of the solution. In addition, for medicinal applications, the nanoparticles in water media without any foreign materials is a must as far as the health is concerned. The work gives the analysis of antibacterial activity of extremely pure silver nanoparticles in water media produced by LP-PLA against Vibrio species checked using pore plate method.

EXPERIMENTAL

Second harmonics of Nd: YAG laser (532nm) operating at a repetition rate of 10Hz was focused on to a high purity silver target immersed in 15 ml deionised water for a

duration of 1 hour. The incident laser fluence was maintained to be at 1.2J/cm^2. The UV-visible absorption spectra of the as prepared solutions were recorded using JASCO V570 Spectrophotometer in the wavelength region 300-800nm. The size of the nano particles were analyzed by Transmission electron microscopy (TEM). The antibacterial activity of the silver colloidal solution against Vibrio species is checked.

ANTI-BACTERIAL ANALYSIS

The bacterial culture is done by the pore plate method. 1ml of brackish water sample is taken and is poured in to a petri plate and is incubated for 48 hours at a temperature 37^{0}c using the Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar as the selective medium [8]. One loopful of bacterial innoculam for culture is transferred in to 10ml of nutrient broth and is again incubated for 24 hours at 37^{0}c and the resultant is spread over dried nutrient agar plates using cotton swab. Filter paper dipped in silver nanoparticles were placed over it and is kept for 48 hrs at 37^{0}c in BOD incubator. The presence of yellow colored colonies indicates the presence of Vibrio species [9]. The Petri plates with and without silver nanoparticles are compared in order to analyze the anti bacterial efficacy of the silver nanoparticles.

RESULTS AND DISCUSSIONS

Figure 1 shows the UV-visible absorption spectra of the silver nanoparticles in water media. The absorption spectra shows strong surface plasmon resonance bands peaked around 400nm indicating the generation of the nanoparticles [10]. The plasmon band around 400nm is attributed to the longitudinal surface plasmon resonance of the free electrons in the silver nanoparticles.

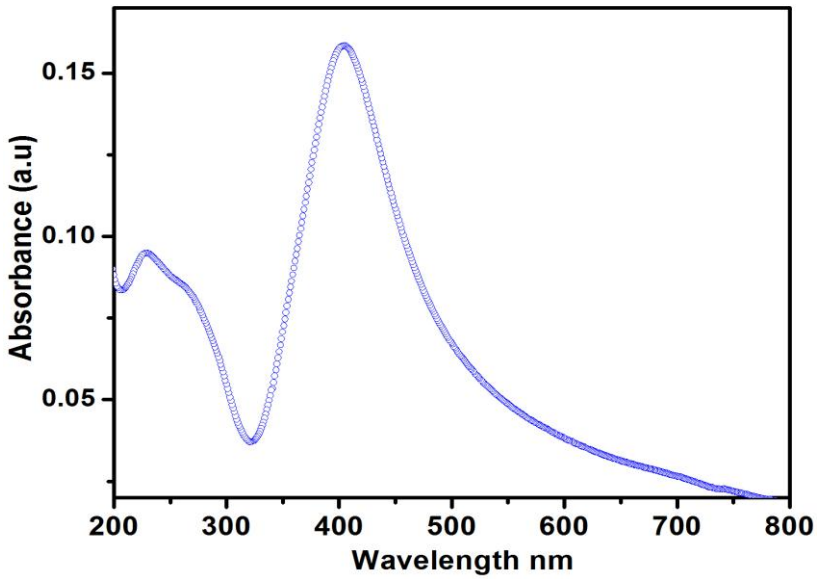

Figure 1. UV-visible absorption spectra of silver nanoparticles prepared by LP-PLA.

Figure 2(a) and figure 2(b) show the TEM and HRTEM images of the silver nanoparticles prepared, the size of the silver nanoparticles is found to be 4nm. Inset of Figure 2(b) gives the selected area electron diffraction (SAED) pattern of silver nanoparticles showing the (100) and (200) planes of silver. The HRTEM image (Figure 2b) of the silver nanoparticles shows the parallel lines of atoms. And the calculated inter planar spacing $2.35A^0$ is in well agreement with (111) plane of silver.

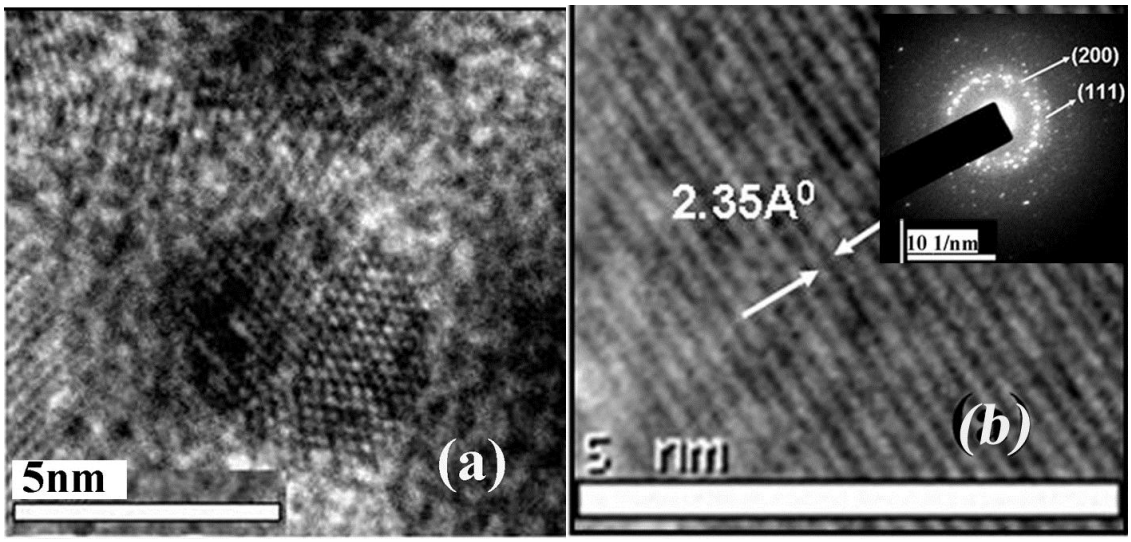

Figure 2 (a) The TEM image (b) HRTEM images of silver nanoparticles (Inset shows SAED pattern of nanoparticles).

Figure 3 shows the results of the antibacterial analysis. Figure 3(a) refers to the petri plate with agar medium in which bacterial innoculam of the Vibrio is not added and hence free of the bacterial colony. Figure 3(b) is the petri plate to which no silver nanoparticles is added and therefore the bacterial colony is grown completely on the surface of agar medium. Figure 3(c) represents the petri plate to which silver nanoparticles dipped filter paper pieces are added, the white circles represent the filter paper pieces. From figure 3(c) it is observed that the growth of the colony is very much reduced and even a halo (a region with out the bacteria) appearing around the filter paper [11]. The appearance of the halo is clearly shown in the magnified image (figure 3(d)).

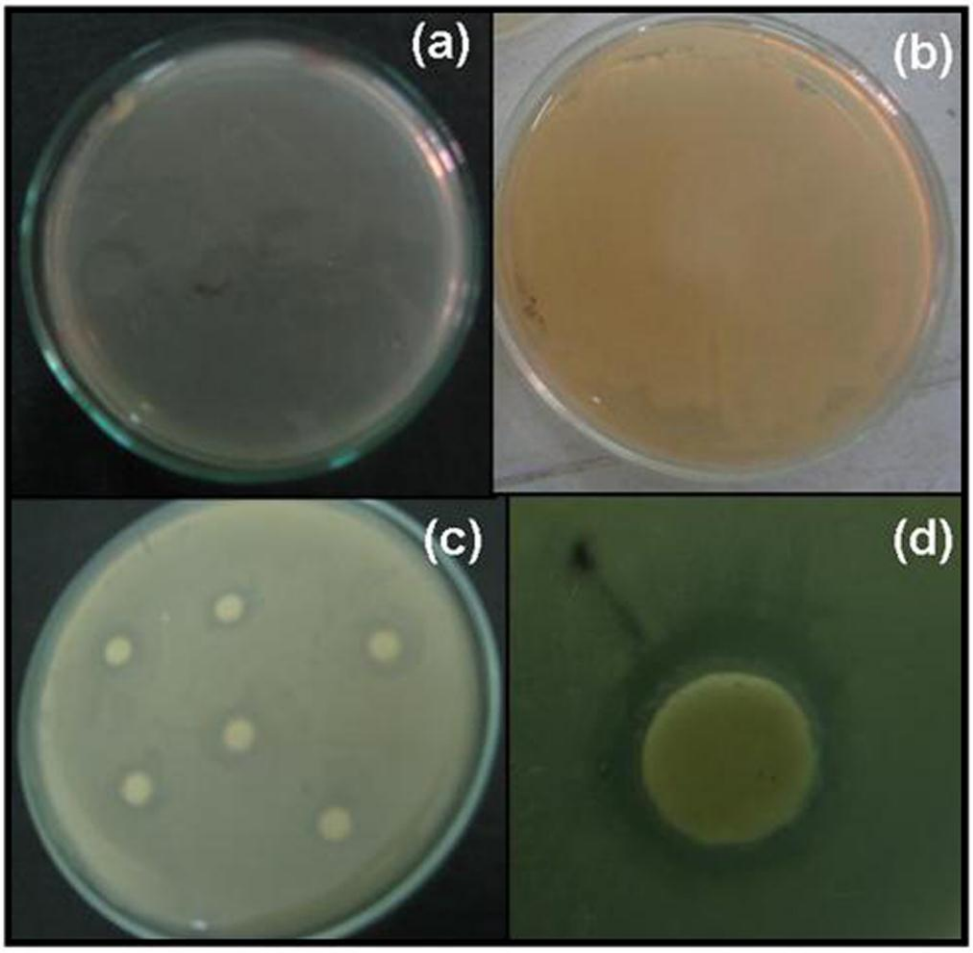

Figure 3 (a). Petri plate with out bacterial colony (b) Petri plate with bacterial colony and with out silver nanoparticles(c) Petri plate containing bacterial colony after the addition of silver nanoparticles (d) halo appearing around the filter paper dipped with silver nanoparticles.

The antibacterial efficacy of the silver nanoparticles can be explained as follows. All bacteria use an enzyme as a form of 'chemical lung' in order to metabolise oxygen. Silver ions cripple the enzyme and stop the take up of oxygen. This effectively suffocates any bacteria, killing it within 6 minutes and leaving surrounding tissue or material unaffected. Hence the bacterial colony will not grow into a region containing silver nanoparticles [12].

The antibacterial nature of the silver nanoparticles against the entire vibrio species has a significant medicinal application, since all diseases like cholera caused by vibrio species are spread through water media and the bacterial growth is in the intestinal region. Also usage of silver based paints on the surface of medical devices reduces the chance of spreading pathogens while surgery which is a severe problem while surgergical operations are done.

CONCLUSIONS

Silver nanoparticles in water media are prepared by pulsed laser ablation of silver target in deionised water using second harmonics of Nd: YAG laser at a wavelength of 532nm. The absorption spectrum showed strong surface plasmon resonance bands around 400nm indicating the presence of silver nanoparticles. The TEM analysis shows a homogenous distribution of particles with an average size of 4nm. Antibacterial activity of silver nanoparticles against the Vibrios species is analysed which showed that silver is very effective in killing the Vibrio bacterial colony. Thus silver nanoparticles in water media can be used against Vibrio spreading diseases.

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