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Bio synthesis of silver nanoparticles and its application in microbial treatment of drinking water

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ABSTRACT

The microbial contamination of water is a world-wide environmental problem. Nanotechnology is one of the efficient tools which can be applied to solve this problem. Silver nanoparticles are known to be highly toxic to microorganisms, showing strong biocidal effect and are nontoxic to the human body at low concentration. The aim of this work was to determine the antimicrobial potential of bio synthesized Ag nanoparticles for the treatment of water. Silver nanoparticles were synthesized using the microorganisms; *Escherichia coli* and *Klebsiella sp.* and the *Carica papaya* plant extract. The presence was confirmed by UV-Visible spectroscopy, Scanning Electron Microscopy (SEM) and Energy dispersive X-ray analysis (EDS). The silver nanoparticles were adsorbed on granular activated carbon and were used as a bacterial filter for treating contaminated water. Antibacterial action of Ag nanomaterials was studied using Colony Forming Unit (CFU) and Most Probable Number (MPN). The effects of Ag nanoparticles on microbial strains were calculated. Time and dose dependent study of Ag nanoparticles showed that the effectiveness of particles increases with increasing particle dose and treatment time. Also the effect of *Ocimum tenuiflorum* (Tulasi) and *Caesalpinia sappan* (Pathimugam) extract on the antimicrobial activity of silver nanoparticles in water treatment was studied. The obtained results showed that the *Ocimum tenuiflorum* was more efficient compared to *Caesalpinia sappan* in the treatment of water. Our work suggests that silver-adsorbed granular activated carbon can be used as an excellent antibacterial media and would have several applications in water treatment system. Biosynthetic method is an economical, efficient, eco-friendly simple process and can be used as an alternative to chemical and physical one.

KEYWORDS

Silver nanoparticles;
Drinking water;
Microorganisms.

INTRODUCTION

Water can support the growth of many types of microorganisms. The presence of disease causing microbes in water is unhealthy and even life threatening. The WHO estimates that 94% of these diarrheal cases are preventable through modifications to the environment, including access to safe water andto improve

sanitation and hygiene[1]. The primary motive for wastewater treatment is that less than 1 percent of the world's water is suitable for drinking while the remaining is brackish. As a result, there is a growing need for fresh and clean water especially for drinking purposes. The use of metal nanoparticles for water disinfection is relatively new[2]. Nanoscale particles are promising in this area because of their unique properties such as small particle sizes, large surface to volume ratio and the ease with which they can be anchored onto the solid matrices for enhanced treatment of water, waste water, and gaseous process streams[3]. The study of bactericidal nanomaterials is particularly timely considering the recent increase of new resistant strains of bacteria to the most potent antibiotics. This has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles[4]. Silver has been described as being 'oligodynamic' because of its ability to exert a bactericidal effect at minute concentrations[5]

Ag-nanoparticles are attractive as these are non-toxic to human body at low concentration and having broad-spectrum antibacterial nature. It is well known that silver ion and silver-based compounds are highly toxic to microorganisms, showing strong biocidal effect against as many as 16 species of bacteria, including *Escherichia coli*.[6] Ag nanoparticle inhibits the bacterial growth at very low concentration than antibiotics and as of now no side effects are reported[7]. It is believed that silver nanoparticles after penetration into the bacteria would inactivate their enzymes, generating hydrogen peroxide and results in bacterial cell death[8]. A variety of techniques have been developed to synthesize metal nanoparticles, including chemical reduction using a number of reducing agents including $NaBH_4$, N_2H_4, NH_2OH, ethanol, ethylene glycol and *N*,*N*-dimethyformamide (DMF), aerosol technique, electrochemical or sonochemical deposition, photochemical reduction, and laser irradiation technique[9]. Biosynthetic methods employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods[10]. Biosynthetic method is an economical, efficient, eco-friendly simple process and can be used as an alternative to chemical and physical one.

EXPERIMENTAL

Silver nanoparticles were synthesized using the microorganisms; *Escherichia coli* and *Klebsiella sp.* and the *Carica papaya* plant extract. The silver nanoparticles were synthesised by the reduction of aqueous Ag+ ions by simultaneous reduction of aqueous Ag+ with the culture broth of some tested bacteria[11]. The enzyme involved in the synthesis of nanoparticles may be the nitrate reductase present in the microbe. This enzyme induced by nitrate ions reduces silver ions to metallic silver. The possible mechanism that may involve the reduction of silver ions is the electron shuttle enzymatic metal reduction process, which was proposed for gold nanoparticles[12]. NADH and NADH-dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles. The cofactor NADH and NADH-dependent enzymes especially nitrate reductase, which might be responsible for the bio reduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles[13]

Prereration of cell free microbial extract

In the case of microbial biosynthesis of silver nanoparticles, 350 ml of nutrient broth was prepared, sterilized and inoculated with fresh culture of test strains of bacteria such as *Escherichia coli, Klebsiella sp*. The cultured flasks were incubated at 37°C for 24h for bacterial species. After incubation time, the cultures were centrifuged at 12000 rpm for 15 minutes and their supernatants were used for further experiments.

Preparation of the carica papaya extract

Fully ripped *Carica papaya* fruit weighing 25g was thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 ml sterile distilled water and filtered through Whatmann No.1 filter paper (pore size 25 μm). The filtrate was further filtered through 0.6 μm sized filters. This filtrate was used for reduction of silver nitrate.

Biosynthesis of silver nanoparticles

Silver nitrate having a concentration of 10^{-3} M was separately added to the reaction vessels containing different supernatants (1% v/v). The reaction between different supernatants and Ag+ ions was carried out in the sunlight. Aliquots of the reaction solution were removed

and the absorptions were measured using a UV-Vis spectrophotometer. Furthermore, the silver nanoparticles synthesized were characterized by Scanning Electron Microscopy.In the case of biosynthesis of silver nanoparticles from *Carica papaya* fruit extract, 10 ml of *Carica papaya* fruit extract was added into 90 ml of aqueous solution of 1mM Silver nitrate and was kept at room temperature for 5 hours.

Adsorbtion of silver nanoparticle on activated charcoal

100 g of activated charcoal was added to 250 ml of solution containing nanoparticles and allowed to adsorb by keeping it in orbital shaker for 3 days. After that activated charcoal was filtered, washed and dried in oven at 105 °C.

Characterization

The synthesized silver nanoparticle solutions and silver-coated activated charcoal were characterized by analytical techniques such as UV-VIS Spectroscopy, SEM and EDX analyzer. Hitachi U-2800 UV-Visible Double Beam Spectrophotometer was used for the characterisation of silver nano particles. This instrument has a Scan Speeds to 3000 nm/min and exceptional reliability. The absorption in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.UV-Visible spectra indicate the presence of metal nanoparticles by the surface plasmon resonance absorption peak at ~400 nm for Ag.

The size and shape of the nano- and submicro-particles was examined with a Field Emission scanning electron microscope equipped with Horiba EDX analyser, made by Hitachi (SU-6600). It utilizes advanced Variable Pressure (VP) technology and an improved Schottky field emission electron source that provides exceptional imaging and high probe current with great stability in both high vacuum and variable pressure operation.SEM images were observed with a magnification of 2.00μm with an accelerating voltage of 15.0kV. The presence of Ag nanoparticle was confirmed by EDX spectra

Microbiological tests

Antibacterial action of Ag nanomaterials was studied using Colony Forming Unit (CFU) and Most Probable Number (MPN).In CFU detection method samples treated with silver nanoparticles were spread on nutrient agar plates and after incubation at 37± °C for 24 h, and the number of CFU/ml was counted.

In MPN method 10 ml, 1 ml, 0.1ml of the sample was transferred to Mac Conkey fermentation tubes, incubated at 37±0.5°C. After 48 hours, swirled each tube and examined for gas production. Recorded the presence or absence of growth. Presence of both gas and growth constitutes a positive presumption test. Noted the number of positive tubes for each dilution and compared with the MPN index chart to get the Most Probable Number (MPN)/100ml of total coliforms.

Effect of ocimum tenuiflorum/ceasalpinia sappan on the antimicrobial activity of silver nanoparticles

To the silver nanoparticle adsorbed activated charcoal taken in different conical flasks, 5 ml, 10 ml and 15 ml of *Ocimum tenuiflorum/Ceasalpinia sappan* extract were adsorbed. To the media so prepared, 5 ml of bacteriologically contaminated water sample was added. Then the sample was kept in orbital shaker for 2 hours. Then it was then filtered and spread plating was done and incubated. A control was also kept. Then the before treatment and after treatment results were compared.

RESULTS AND DISCUSSION

Formation of brown coloured solution after addition of silver nitrate indicates the formation of silver nanoparticles[14]. These reactions occurred in the light and the nanoparticles were not produced in darkness. The silver nanoparticles were characterized by UV-visible spectroscopy. The obtained result showed a strong, but broad peak located at a range 380-420 nm (Figure 5). This peak is assigned to a surface plasmon, phenomenon that is well-documented for various metal nanoparticles with sizes ranging from 2 *nm* to 100 *nm*[15]. The silver nanoparticles were further characterised using Scanning Electron Microscopy (Figure 3) and EDS analysis (Figure 6). Dose dependent study of Ag nanoparticles showed that the effectiveness of particles increases with increasing particle dose. (TABLE 1) Also as the retention time increases antimicrobial activity increases. (TABLE 2) The obtained results showed that the per-

centage of reduction of total coliform bacteria was about 95 % and *E.coli* was 100%. The antimicrobial activity of silver nanoparticles was further improved in the presence of *Ocimum tenuiflorum* and *Caesalpinia sappan*. *Ocimum tenuiflorum* showed high efficency compared to *Caesalpinia sappan*. (TABLE 3)

TABLE 1 : Percentage reduction of coliforms by varying weight of silver nanoparticles adsorbed on activated carbon.

Weight of Ag adsorbed Charcoal (gm)	0.5	1.0	1.5	2.0
Initial CFU	360	360	360	360
Final CFU	200	75	12	0
Percentage of reduction (%) after 1 hour	44.4	79.1	96.7	100

TABLE 2 : Percentage reduction of coliforms by varying contact time

Time in Minuts	Weight of Ag adsorbed Charcoal 2g					
	30	60	90	120	150	180
Initial CFU	360	360	360	360	360	360
Final CFU	200	155	140	20	12	9
Percentage of reduction (%)	44.4	56.9	61.1	94.4	96.7	97.5

TABLE 3 : Effect of plant extracts on the antimicrobial activity of Ag nanoparticles

Contents	Ag adsorbed Charcoal (1gm)	*Ocimum tenuiflorum* extract (10ml)	*Caesalpinia sappan* extract (10ml)	1g Ag adsorbed Charcoal and10ml *Caesalpinia sappan* Extract	1g Ag adsorbed Charcoal and10ml *Ocimum tenuiflorum* extract
Initial CFU	380	380	380	380	380
Final CFU	75	140	200	20	7
Percentage of reduction (%)	80.3	63.2	47.4	94.7	98.2

Figure 1 : Microbially synthesized silver nanoparticles

Figure 2 : Silver nanoparticles synthesize from carica papaya extract

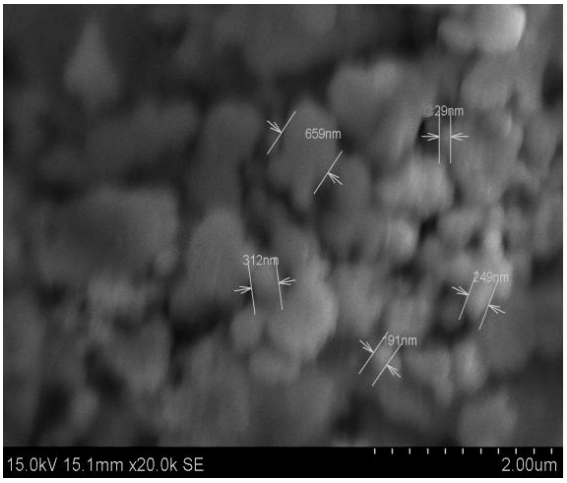

Figure 3 : SEM image of silver nanoparticle in solution

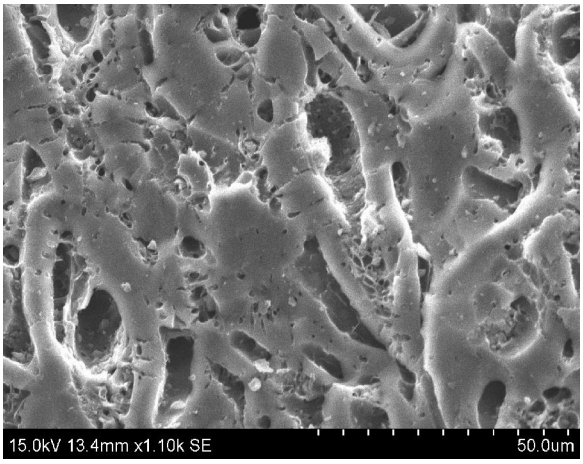

Figure 4 : SEM image of silver nanoparticle adsorbed on GAC

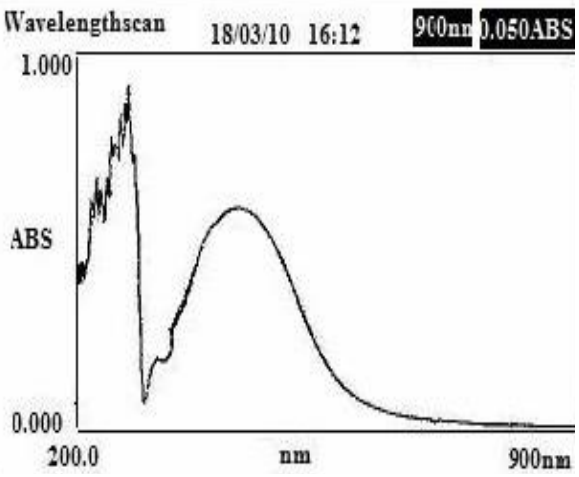

Figure 5 : UV-visible spectra of Ag nanoparticles

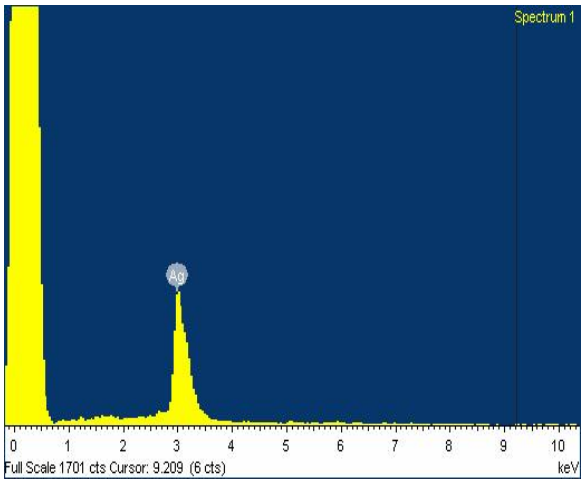

Figure 6 : EDS analysis of Ag nanoparticles

CONCLUSION

From this study it can be concluded that silver nanoparticles can be synthesized using the microorganisms such as *Escherichia coli* and *Klebsiella sp*. and also using *Carica papaya* plant extract. The silver nanoparticles were characterized using UV-Visible spectroscopy and Scanning Electron Microscopy. Time and dose dependent study of Ag nanoparticles showed that the effectiveness of particles increases with increasing particle dose and treatment time. The silver ions can deactivate the bacterial DNA and thus disinfect the contaminated water. Various antibacterial plants extracts such as *Ocimum tenuiflorum* and *Caesalpinia sappan* can be used to improve the antimicrobial activity of silver nanoparticles in water treatment. *Ocimum tenuiflorum* showed high efficiency in bacterial removal from contaminated water.

Our work suggests that silver-adsorbed granular activated carbon can be used as an excellent antibacterial media and would have several applications in water treatment system. Biosynthetic method is an economical, efficient, eco-friendly simple process and can be used as an alternative to chemical and physical one.

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