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Ultrasound assisted green synthesis of poly(vinyl alcohol) capped silver nanoparticles for the study of its antifilarial efficacy

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ABSTRACT

Poly(vinyl alcohol) (PVA) capped stable silver nanoparticles (AgNP) have been synthesized sonochemically with the help of catalytic amount of a biomolecule (tyrosine). An attempt has been made to reduce the harmfull chemical additives (like sodium borohydride, hydrazine, dimethyl formamide, *etc.*) used in conventional methods. Tyrosine shows excellent reducing activity in presence of PVA stabilizer. Ultrasound increased the reaction rate and yield, and improved the quality of the AgNP in terms of regular size distribution. The synthetic route follows the principles of green chemistry. Bioactivity has been tested in the light of antifilarial efficacy through induction of apoptosis. The biocompatible polymer (PVA) capped AgNPs are suitable for the treatment of filarial nematode.

1. Introduction

The physical phenomena associated with the ultrasound assisted synthesis are cavitation (formation, growth and implosive collapse of bubbles in a liquid) and nebulization (the creation of heated micro-droplets like mist in a liquid) [1]. The micro-droplet reactors created by ultra-sound facilitate the formation of non-agglomerated nanocomposites. Compared to traditional methods, the main advantages of the sonochemical process are milder conditions, shorter reaction times and higher yields [2,3]. Thus ultrasonic irradiation method has gradually been introduced in synthesis as a green synthetic approach, which is safe for environment. Over the past decade, there has been an increased emphasis on the topic of "green" chemistry and chemical processes [4].

Green chemistry provides a number of advantages in process development and manufacturing as well as product design. The three main factors, which are very important in the perspective of "Green Nanoscience", are: (i) choice of the solvent medium, (ii) the choice of an environmentally benign reducing agent, and (iii) the choice of a nontoxic capping agent [4,5].

Very few researchers are engaged in the green synthesis of polymer capped silver nano particles [4,6]. Recently poly(vinyl alcohol) (PVA) (a water soluble and biocompatible polymer) is used as safer alternatives (reducing as well as stabilizing agents) to synthesize the silver nanoparticles [7–13]. Water solubility and extremely low cytotoxicity of PVA make it biologically friendly to allow a wide range of potential biomedical applications [6]. Ag nanoparticles embedded in PVA have been synthesized by Raneesh et al. [7] through polyol process to study its plasma effect. Chittee's group [8] prepared AgNP@PVA film by solution cast method using trisodium citrate as a reducing agent for studying electrical conductivity. Ananth et al. [9] prepared silver nanoparticle using PVA as stabilizer and sodium borohydride ($NaBH_4$) as reducing agent. They functionalized the polymer coated nanoparticles with Bovin Serum Albumin (BSA) for biosensing [9]. Pencheva and his co-workers reported PVA/AgNps as a model for testing biological activity [10]. Filippo et al. used PVA capped AgNP [11] as a colorimetric hydrogen peroxide sensor.

A large volume of work has been done on the topic of antibacterial and antifungal activity of AgNP [6,14–23]. In fact, AgNPs are used in various consumer products (toothpaste, fabrics, deodorants, filters, humidifiers, washing machines, and toys), food industry (packaging materials, nursing bottles and kitchen utensils) and cosmetics [24,25]. However, the antifilarial activity of polymer capped metal nanoparticles is a new field [26]. Lymphatic filariasis (LF), caused by the lymph-dwelling nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, is a major cause of global morbidity. It affects over 120 million people in 81 countries [27]. However, the WHO has now called for targeting "filariasis elimination" by 2020 [28]. Control programmes rely on sustained delivery of antifilarial drugs, such as ivermectin, albendazole and DEC, which have been the drugs of choice for filariasis control [29]. While effective on microfilariae (mf), these drugs are fairly ineffective at killing adult worms and provide only partial benefit to infected patients,

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and very often are associated with adverse reactions. Thus, there is a need to develop a cheap, non-toxic and novel drug that permanently sterilizes or kills adult worms. Now in this present study we have checked antifilarial efficacy by silver nano particle (AgNP). *Setaria cervi*, a filarial nematode (family Onchocercidae) parasite inhabits in the abdominal cavity of many species of the bovidae family. *S. cervi*, which resembles the human bancroftian parasite in its nocturnal periodicity and antigenic patterns, was used as a model parasite [30].

The present paper deals with the synthesis of PVA capped AgNP through new green approach, which minimizes the harmful reducing agents. Antifilarial efficacy of the synthesized material as well as mechanism of action was studied on filarial nematode *S. cervi* to have preliminary data before testing them as a potential application in nanomedicine.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and reagents

Poly(vinyl alcohol) (Air products of Burgoyne Burbidges company, India, having % hydrolysis = 98, DP = 1710 and viscosity = 30 CPS), sodium borohydride (Merck, Mumbai, India), silver nitrate (Merck, Mumbai, India), L-tyrosine hydrochloride (Sigma–Aldrich, St. Louis, USA), Sodium hydroxide (Merck, Mumbai, India).

2.1.2. Biological materials

FBS (Foetal Bovine Serum), HEPES buffer, streptomycin, penicillin, amphotericin-B and propidium iodide were purchased from Sigma–Aldrich, St. Louis, MO, USA. RPMI-1640, MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), agarose and trypan blue were obtained from Hi-Media Laboratories, Mumbai, India. TMB/H_2O_2 and Bradford reagent were purchased from Genei, India. Primary antibodies (EGL-1, CED-3, CED-4, CED-9 and β-tubulin) and horseradish peroxidase (HRP) conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA.

2.2. Instruments

The UV–vis spectrum was recorded by Optizen POP UV/VIS spectrophotometer. A sonicator (Branson-1510) was used to obtain ultrasound of 40 kHz. Beckman Coulter Ultracentrifuge machine was used for ultracentrifugation. Particle size and its distribution were recorded using Transmission Electron Microscope (TEM) (JEM-2100, 200 kV, Jeol). A light microscope (Dewinter, Victory, Italy) was used for observations and photographs. The fragmentation of DNA was visualized and photographed by UV transillumination (Bio-Rad, USA).

2.3. Synthesis of PVA capped silver nanoparticle (Ag@PVA)

The polymer PVA (0.6 g) was dissolved in 25 ml of Millipore water through sonication. Then, 10 ml of 2% $AgNO_3$ solution was added slowly from a micro-burette. The other ingredients were added according to the composition of the Table 1. The whole mixture was subjected to sonication at 60 °C at various time intervals (Table 1). All the four types of nanoparticles ($AgNP_{1–4}$) have been purified by ultra centrifugation and repeated washing with warm Millipore water to make the systems free from silver ion and impurities. All the samples were re-dispersed with Millipore water. No re-dispersible AgNP were found in the system 1 ($AgNP_1$) due to absence of reducing agent. The colour and stability of the AgNP solutions are given in the Table 1. Stability of the nanoparticles was tested by continuous UV–vis spectral monitoring. The three parameters (restoring of peak position, height and shape) indicates the stability of the nanoparticles. Ultra centrifugation of the colloidal solution has been made and the supernatant solution was subjected to estimation of silver ion by conventional method [31] to calculate percentage of conversion. The yield was calculated using the formula given below:

$$\text{Yield}\,(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

(W_1 = amount of Ag^+ taken; W_2 = amount of Ag^+ present in supernatant).

2.4. Studies of antifilarial properties

2.4.1. Movability assessment

The antifilarial activity of silver nano particle ($AgNP_2$ and $AgNP_3$) were assessed by relative movability (RM), followed by Zaridah et al. [32] with some modifications. RM was calculated using the following calculations:

$$\text{Relative Movability (RM)} = \left(\frac{\text{MI}_{\text{sample}}}{\text{MI}_{\text{control}}}\right) \times 100,$$

$$\text{whereas MI} = \frac{\sum(n\,Nn)}{\sum N}$$

where, n = score (0, 1, 2, 3 and 4); Nn = number of larvae with the score n; $\sum N$ = total number of parasites were used in the assay. Parasite motility was assessed under a microscope after 0, 6, 12, 18, 24, 30, 36, 42 and 48 h exposure to test substance ($AgNP_2$ and $AgNP_3$) at a concentration of 2.5, 5.0 and 10.0 μg/ml and scored as: 0 = dead; 1–4 = loss of motility (1 = 75%; 2 = 50%; 3 = 25% and 4 = no loss of motility). An RM value of 100 indicates no activity, while increasingly lower RM values approaching zero indicate stronger activity of the drugs against the adult worms. Strongest activity is obtained when RM is zero.

2.4.2. Parasite viability assessment by MTT assay

Viability of adult worms and mf was quantitatively assessed by the MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetra-zolium bromide] reduction assay as described by Comley et al. [33]. Twelve adult worms and mf ($n = 1.3 \times 10^3$) were used for each treatment group. The cultures for adult worms were carried out in duplicate and for mf in quadruplicate and repeated at least three times. This assay was performed after 48 h of incubation at concentrations

Table 1
Composition of silver nanoparticle synthesis.

Sample	$AgNO_3$ (g)	PVA (g)	$NaBH_4$ (mg)	Tyrosine (alkaline) (μg)	H_2O (g)	Sonication time (min)	Colour	Percentage of yield (%)	Stability
$AgNP_0$	0.20	0.0	0.0	0.0	50	100	Colourless	0.0	–
$AgNP_1$	0.20	0.6	0.0	0.0	50	100	Colourless	2.98	–
$AgNP_2$	0.20	0.6	0.5	0.0	50	40	Yellowish orange	74.3	25–30
$AgNP_3$	0.20	0.6	0.0	1.0	50	20	Deep orange	94.2	>45
$AgNP_4$	0.20	0.6	0.0	1.0	50	Without sonication	orange	76.4	32–35