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Microwave-assisted facile synthesis of silver nanoparticles in aqueous medium and investigation of their catalytic and antibacterial activities

Siby Joseph [a], Beena Mathew [b,*]

[a] Department of Chemistry, St.George's College, Aruvithura, Kottayam-686 122, Kerala, India
[b] School of Chemical Sciences, Mahatma Gandhi University, Kottayam- 686 560, Kerala, India

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ABSTRACT

A simple, fast, efficient and cost effective synthetic strategy based on microwave irradiation is described for the preparation of silver nanoparticles in aqueous medium using hexamine as the reducing agent and agar as the stabilizer. The formation of nanoparticles is confirmed using UV-vis., XRD, EDX, and HR-TEM analysis. TEM images suggest that the nanoparticles are of spherical shape with an average diameter of 10.16 nm. The agar stabilized silver nanoparticles show excellent catalytic activity for the reduction of methyl orange in the presence of $NaBH_4$ in aqueous medium. The reaction follows pseudo-first order kinetics, and the reaction rate increases with increase in amount of the catalyst. The synthesized silver nanoparticles are expected to be promising material for the application in environmental protection. They also show very good antibacterial activity against *Staphylococcus aureus* (Gram positive) and *Salmonella typhi* (Gram negative).

1. Introduction

In recent years, metal nanoparticles have received immense interest because of their unique properties compared to their macro scaled counterparts. Among metal nanoparticles, silver nanoparticles (AgNPs) continue to be interesting nanomaterials in nanoscience and nanotechnology due to their excellent optical and electronic properties as well as their wide applications in various fields such as catalysis [1], surface-enhanced Raman scattering [2], degradation of environmental pollutants [3], biosensors [4] and in cancer therapy [5]. Several synthetic strategies have been developed for the synthesis of silver nanoparticles including chemical [6], photochemical [7], sonochemical [8], radiochemical [9] and biological methods [10]. Among these, chemical reduction of a silver salt in presence of a stabilizer is the most frequently applied method for the generation of silver nanoparticles as stable colloidal dispersions in water or organic solvents [11]. The major drawback of chemical method is that the highly reactive chemical reductants as well as the stabilizers such as synthetic polymers, surfactants, dendrimers etc. used in this method cause chemical toxicity and serious environmental problems, thus limiting their utility. In recent years, an unprecedented research has occurred in this field by using eco-friendly and easily available materials with the aim to reduce environmental hazards. The selection of a non-toxic reducing agent, a cost-effective and easily renewable stabilizing agent and an environmentally benign solvent system are the three main criteria for a greener nanoparticles synthesis.

Microwave-assisted nanoparticle synthesis using biopolymers as capping agents is getting more attention recently because of their eco-friendly nature. Furthermore, it affords smaller nanoparticles with narrow particle size distribution in much lesser reaction time. The microwave-assisted synthesis of nanoparticles is characterized by rapid and homogeneous heating in contrast to a conventional thermal synthesis, even though the thermal effects are similar to those of other heating methods [12]. Many successful reports based on microwave-assisted synthesis have been published in recent years. In a green process that used microwave radiation, Chen and co-workers synthesized silver nanoparticles employing sodium salt of carboxymethyl cellulose both as reducing and stabilizing agents [13]. Using starch as the template, Nair et al. prepared silver nanoparticles with the aid of microwave heating [14]. In one study, nanoparticles were constructed using microwave and carboxymethyl chitosan solution [15]. Another greener approach synthesized Ag nanoparticles in aqueous medium using bamboo hemicellulose and glucose [16].

Synthetic azo dyes are widely used in various industrial processes. The waste discharges containing these dyes pose huge environmental threats. Studies have shown that many of the dyes are carcinogenic, mutagenic and detrimental to the environment. These dye pollutants are chemically stable, and so traditional water treatment methods are usually ineffective. Wastewaters containing water soluble dyes are generally not decolorized effectively by the aerobic biological treatment. Recently, nanotechnology has been extended to the area of waste

* Corresponding author. Tel.: +91 9447145412; fax: +91 481 2731036.
E-mail address: beenamscs@gmail.com (B. Mathew).

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water treatment. Several groups reported the use of nanomaterials for the efficient removal of dye stuffs from waste water [17–19].

The present work reports for the first time the use of hexamethylenetetramine ($(CH_2)_6 N_4$) commonly known as hexamine as reducing agent for the synthesis of silver nanoparticles. In this study, silver nanoparticles (AgNPs) were generated in aqueous medium by a microwave-assisted method using hexamine as reducing agent and agar as the stabilizer. Hexamine is a low cost and easily available material which is widely used for the treatment of urinary tract infection. It also finds application in food industry as a preservative. Usually, when a strong reducing agent like $NaBH_4$ is used, the reaction is very fast and has to be cooled to control the rate of reduction in many cases. Moreover, it is highly toxic, and synthesis of larger nanoparticles has been found to be difficult [20]. On the other hand, when a mild reducing agent like sodium citrate or ascorbic acid is used, the reduction reaction is slow and has to be carried out at elevated temperatures to enhance the rate of reduction. In addition, they usually yield relatively larger nanoparticles of varying size and shape [21]. In contrast, hexamine is non-toxic, easy to handle, and yields spherical nanoparticles with narrow size distribution at a moderately fast rate. Agar is a natural polysaccharide derived from red algae. It is a mixture of the linear polysaccharide agarose and a heterogeneous mixture of smaller molecules called agaropectin. Its main structure is chemically characterized by repetitive units of D-galactose and 3, 6-anhydro-L-galactose. Since all materials used in this method are non-toxic, this is a greener approach for nanoparticle synthesis. This synthetic strategy is simple, fast, economic and environment friendly. The catalytic activity of the synthesized agar stabilized silver nanoparticles (AgNP-agar) was examined using the reduction of methyl orange by $NaBH_4$. We also investigated the antibacterial activity of AgNP-agar against two human pathogenic bacteria.

2. Materials and methods

2.1. Materials

All the materials purchased were of analytical grade and used as received. Silver nitrate ($AgNO_3$), hexamine, methyl orange and sodium borohydride ($NaBH_4$) were purchased from Merck India Ltd. Agar, nutrient broth, and Mueller Hinton agar (MHA) were obtained from Himedia Chemicals (Mumbai, India). Clinical isolates of microorganisms were used for antibacterial study. All aqueous solutions were prepared by using double distilled water.

2.2. Methods

2.2.1. Synthesis of silver nanoparticles (AgNPs)

In a typical procedure, 0.1 g of agar was dissolved in 90 mL of double distilled water. To this, 10 mL of 0.05 M $AgNO_3$ solution was added drop wise and stirred for 15 minutes. This was followed by the addition of 0.07 g (0.005 M) hexamine, and the mixture was placed in a domestic microwave oven (Sharp R-219 T (W)) operating at a power of 800 W and frequency 2450 MHz. The solution was subjected to microwave irradiation for five minutes. The colorless solution turned into yellowish-brown, clearly indicating the formation of silver hydrosol. The formation of AgNPs was monitored using UV-vis. spectrophotometer by analyzing the reaction mixture after different irradiation time.

2.2.2. Catalytic reduction of methyl orange

The reduction of methyl orange by $NaBH_4$ was selected as a model reaction to investigate the catalytic efficiency of AgNP-agar hydrosol. In order to follow this reaction, 0.5 mL freshly prepared $NaBH_4$ solution (0.06 M) was added to 2 mL of methyl orange solution (0.01×10^{-2} M) taken in a quartz cell (1 cm path length). Then 0.5 mL of AgNP-agar solution was added to start the reaction. The variation in the concentration of methyl orange with time was monitored by UV-vis. spectrophotometry. The absorption spectra were recorded in one minute intervals in the range of 200–600 nm at ambient temperature (26 °C).

2.2.3. Antibacterial study

The antibacterial activity of the AgNPs was tested by disc diffusion method and broth macrodilution method. Commonly observed human pathogenic bacteria strains of *S. aureus* and *S. typhi* were used in this study. To study the antibacterial efficacy by disc diffusion method, the bacteria were grown in liquid nutrient broth for 24 h prior to the experiment. Sterilized Mueller Hinton agar (MHA) medium was then transferred into autoclaved Petri dishes in a laminar air flow. When the medium was solidified, the culture of each bacterium was uniformly spread on the surface using a cotton swab. To each of this inoculated Petri dish, sterile paper discs impregnated with 20 μL each of hexamine, $AgNO_3$ and AgNP-agar hydrosol of different concentration were placed, and the plates were incubated at 37 °C for 24 h. Finally, the zone of inhibition was measured in mm.

The minimum inhibitory concentration (MIC) of the synthesized AgNPs was also evaluated. The MIC was determined by broth macrodilution method. Microbial inoculums were prepared by subculturing strains of *S. aureus* and *S. typhi* in nutrient broth at 37 °C for 18 h. The sterile test tubes were arranged in rows for both bacterial strains. The colloidal solutions of AgNP-agar of different concentration were prepared in sterile water, and 1 ml each of these solutions was transferred to the test tubes. The test tubes were then inoculated with 4 ml of test strains (final cell density of 10^5 CFU/mL). The contents in the test tubes were mixed thoroughly and incubated at 37 °C for overnight. The lowest concentration of silver nanoparticles showing growth inhibition as seen visually was taken as the minimum inhibitory concentration.

2.2.4. Characterization

UV-vis. spectral studies were carried out on a Shimadzu UV-2450 spectrophotometer. EDX measurement was done using JEOL JSM-6390 scanning electron microscope equipped with EDX attachment. The sample for XRD measurement was prepared by depositing a thin film of the sample on a microscopic glass slide and the diffraction pattern was recorded on a PANalytic X'PERT-PRO X-ray spectrometer. High resolution-transmission electron microscopic (HR-TEM) images were obtained using a JEOL JEM-2100 microscope.

3. Results and discussion

3.1. Formation of silver nanoparticles (AgNPs)

The formation of silver nanoparticles (AgNPs) is evident from the change in color of the reaction mixture during the irradiation process and also from UV-vis. absorption spectral studies. Upon microwave heating, the color of the solution changes from colorless to yellowish brown. Fig. 1 shows the UV-vis. absorption spectra of the solution recorded at different irradiation time. The absorption spectra show one peak around 429 nm which is due to the characteristic surface plasmon resonance (SPR) of spherical silver nanoparticles. The peak is almost symmetrical, and there are no peaks in the range of 450–700 nm indicating the absence of silver nanoparticle aggregation [22]. Initially, there was no absorption peak in the range of 250–700 nm, but after irradiation for 2 min, a peak was observed at about 429 nm. The intensity of this SPR band increased with increasing reaction time due to the formation of more nanoparticles. When $AgNO_3$ is added to agar solution, Ag^+ ions get bounded to the polysaccharide chain through electrostatic interaction of Ag^+ ions with the oxygen atoms of polar -OH and -C-O-C- groups. Upon microwave irradiation in presence of the reducing agent hexamine, the Ag^+ ions are reduced to Ag which combines to form uniformly distributed monodispersed spherical nanoparticles. During microwave heating, the reaction mixture is warmed up uniformly and instantaneously thus preventing the aggregation of the particles. The