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Starch based biodegradable graft copolymer for the preparation of silver nanoparticles

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ABSTRACT

The synthesis and characterization of a novel biodegradable graft copolymer based on partially hydrolyzed polymethylacrylate (PMA) grafted amylopectin (AP) was reported which was developed for the synthesis of silver nanoparticles from silver nitrate solution by facile green technique. The prepared graft copolymer was biodegradable which was shown by fungal growth. Characterization of silver nanoparticles was carried out by UV–VIS spectroscopy (417 nm), HR-TEM, SAED and FESEM analysis. The TEM findings revealed that the silver nanoparticles are crystalline and globular shaped with average particle size ranging from 11 to 15 nm. The synthesized silver nanoparticles exhibit excellent antibacterial sensitivity towards both Gram negative and Gram positive bacteria namely *Vibrio parahaemolyticus* (ATCC-17802) and *Bacillus cereus* (ATCC-14579) respectively and were also shown a good catalytic activity towards 4-nitrophenol reduction.

1. Introduction

Over the past few years synthesis of metal nanoparticles is one of the upcoming area of research in the field of materials science owing to their wide variety of applications in the field of chemical and biosensing [1,2], catalysis [3,4], diagnostics and therapeutics [5,6], data storage [7], lithography [8], etc. Nano crystalline silver is well known for possessing an inhibitory effect towards many bacterial stains and microorganisms [9,10]. In medicines silver and silver nanoparticles have an ample application including skin ointments and creams containing silver, to prevent infection of burns and open wounds [11,12]. Silver nanoparticles were synthesized using various chemical and biological approaches [13–17]. Although chemical method requires short period of time for synthesis of large quantity of nanoparticles, this method requires reducing agents together with capping agents for size stabilization of the nanoparticles which are toxic and lead to non-ecofriendly by products. Therefore, there is an increasing demand of green procedure for synthesizing metal nanoparticles which are free from the use of toxic chemicals.

Many green methods and biological approaches for silver nanoparticles' synthesis using extracts of different plants [18–21], microorganisms, including bacteria [22] and fungi [23] are reported till date. Green synthesis of silver nanoparticles has also been studied considerably using natural polysaccharides, chitosan, sodium alginate, soluble starch, agar [24–27], etc. In recent years biodegradable graft copolymers based on natural polysaccharides have attracted more and more interest because they are eco-friendly and have wide applications as matrices for controlled drug release [28], flocculants [29–31] and drag reducing agents [32]. Though there are varieties of green routes for preparing metal nanoparticles with newer application, use of natural polysaccharides based graft copolymer is rare [33–36]. The present work is part of this new line of research.

In the present study, we have prepared silver nanoparticles by green procedure using a novel biodegradable graft copolymer partially hydrolyzed starch-g-polymethyl acrylate by the reduction of Ag(I) ions from silver nitrate solution with 20–30 min of reaction time at 70 °C with average particle size 11–15 nm. Furthermore, synthesized silver nanoparticles showed excellent antibacterial properties against one Gram positive and one Gram negative bacteria namely *Bacillus cereus* (ATCC-14579) and *Vibrio parahaemilyticus* (ATCC-17802) with a minimum inhibitory concentration (MIC) 42.5 mcg/mL and 71.25 mcg/mL, respectively and

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showed a good catalytic activity for the reduction of 4-nitrophenol to 4-aminophenol.

2. Materials and methods

2.1. Materials

Maize starch was procured from E-Merck, India and methyl acrylate (MA), potassium persulfate ($K_2S_2O_8$), acetone, p-nitrophenol, sodium hydroxide (NaOH), sodium borohydride ($NaBH_4$) and hydrochloric acid (HCl) were procured from Loba chemie, Mumbai, India. *Bacillus cereus* and *Vibrio parahaemolyticus* were collected from American Type Culture Collection (ATCC), USA. All the solutions were prepared by doubly distilled water.

2.2. Synthesis

2.2.1. Synthesis and purification of the graft copolymer starch-g-polymethylacrylate (St-g-PMA)

2 g of starch was dissolved in 100 mL of double distilled water in 250 mL stoppered Erlenmeyer flask. Then, 8 mL of methyl acrylate (0.088 mol) was added to it. A slow stream of nitrogen gas was purged through the mixture for 15 min to remove any dissolve oxygen. The reaction temperature was fixed at 70 ± 1 °C. At this stage, 0.05 g of $K_2S_2O_8$ was added to the solution. The reaction was allowed to continue for 6 h under nitrogen atmosphere. Then the reaction vessel was sealed and kept at 30 °C for 24 h, after which the reaction was terminated by adding saturated solution of hydroquinone. The polymer was precipitated in excess quantity of acetone. The homopolymer polymethylacrylate (PMA) was removed by solvent extraction using acetone [37]. The purified polymer was then dried in a vacuum oven at 80 °C for 24 h. The synthetic details are given in Table 1.

2.2.2. Preparation of starch-g-poly(methylacrylate-co-sodiumacrylate) [PHSt] by partial alkaline hydrolysis of St-g-PMA

In a 100 mL round bottom flask 0.5 g of powdered St-g-PAM was taken, then 50 cc of exact 1 (M) NaOH was added to it. The mixture was then refluxed for 6 h where the polymer was completely soluble in alkaline water. After cooling the polymer solution was titrated against exact 1 (M) HCl using phenolphthalein indicator. The volume of consumed NaOH was determined and the saponification equivalent (S.E.) parameter was calculated by the following equation [38].

$$\text{S.E.} = \frac{\text{Weight of the ester (mg)}}{\text{Volume (ml)} \times \text{strength (M) of NaOH} - \text{volume (ml)} \times \text{strength(M) of HCl}}$$

The partial hydrolyzed polymer was then precipitated in excess quantity acetone. The precipitated polymer was washed with a mixture of anhydrous methanol and ethanol (1:1 by volume). It was then pulverized and sieved after drying in a vacuum oven. The synthetic details are given in Table 2.

2.2.3. Synthesis of silver nanoparticles (AgNPs)

4 mL (2.264 mg) of silver nitrate (0.001 M) solution was taken in a 25 mL of stoppered conical flask. Then 2 mL (4.0 mg) of graft copolymer solution was added with stirring at 70 °C. After that 0.01 (M) NaOH was added in a dropwise fashion to reach the pH at 10.5. At this stage, the colour of the solution changes to deep brownish yellow. The colloidal solution was stirring up to 20 min, after that it was cooled at room temperature.

Similar procedure was followed to prepare silver nanoparticles by using starch instead of graft copolymers under exactly the same experimental conditions.

The concentration of graft copolymer, pH of the solution, reaction time and reaction temperature were varied for finding their effect onto the prepared AgNPs.

2.3. Characterization of the graft copolymer (PHSt)

2.3.1. FTIR spectroscopy

FTIR spectroscopy of the graft copolymer (PHSt) was taken in Perkin Elmer (L16000300 Spectrum Two LiTa, Llantrisant, UK) spectrophotometer and the potassium bromide (KBr) pellet method was applied for spectral analysis.

2.3.2. NMR spectroscopy

^{1}H NMR spectroscopy of the graft copolymer (PHSt) was carried out with a 500 MH_z NMR instrument (JEOL, Tokyo, Japan) in D_2O solvent at 25 °C.

2.3.3. SEM studies

A Cam Scan Series-2 (Cambridge Scanning Company, UK) was used for the study of St and the synthesized graft copolymer (PHSt). The small granules left after the pulverized graft copolymer were sieved and were subjected to SEM study. The samples were gold coated and the picture was taken by proper magnification.

2.4. Biodegradation studies

Biodegradation of the synthesized graft copolymer was performed in sterilized modified Czapex-Dox medium [39] in deionized water. The medium composed by 10 g/L graft copolymer, 3 g/L $NaNO_3$, 1.0 g/L K_2HPO_4, 0.05 g/L $MgSO_4$, and 0.00001 g/L of KCl at pH 5.0. Prepared semisolid plates of modified Czapex-Dox medium was inoculated with *Fusarium* sp. fungal mycelia and incubated at 28 °C. Plates were observed for fungal growth till 7 days.

Table 1
Synthetic details of the graft copolymer starch-g-polymethylacrylate (St-g-PMA).

Polymer	Polysaccharide St (g)	Methyl acrylate (mol)	Potassium persulfate $K_2S_2O_8$ (g)	Percentage grafting (% G)	Percentage grafting efficiency (%E)
St-g-PMA	2.0	0.088	0.05	415.2	448.3

Percentage grafting (%G) = (wt. of graft copolymer/wt. of polysaccharide) × 100.
Percentage grafting efficiency (%E) = (wt. of graft copolymer/wt. of homopolymer) × 100.

Table 2
Synthetic details of the partially hydrolyzed St-g-PMA (PHSt).

Polymer	Amount of St-g-PMA	Amount of NaOH 1.0 (M)	Amount of HCl 1.0 (M)	Saponification equivalent (S.E.) parameter
PHSt	0.5 g	50 ml	35.5 ml	34.48

$\text{S.E.} = \frac{\text{Weight of the ester (mg)}}{\text{Volume (ml)} \times \text{strength (M) of NaOH} - \text{volume (ml)} \times \text{strength(M) of HCl}}$.