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Synthesis of fibrinolytic active silver nanoparticle using wheat bran xylan as a reducing and stabilizing agent

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ABSTRACT

A facile synthesis of highly stable silver nanoparticles (AgNPs) was reported using a biopolymer, xylan as both a reducing and stabilizing agent. Xylan was isolated from waste biomass, wheat bran (WB) by alkaline treatment and was characterized by Fehling's test, dinitrosalicylic acid assay, FTIR, ^{1}H NMR and ^{13}C NMR. The synthesized nanoparticles were characterized by UV–Vis spectroscopy and transmission electron microscopy. The nanoparticles were polydispersed with the size ranging from 20 to 45 nm. The synthesized WB-xylan AgNPs showed excellent free radical scavenging activity. In addition, WB-xylan AgNPs showed fibrinolytic activity as evidenced by the zone of clearance in fibrin plate assay. The biomedical potential of the WB-xylan AgNPs was demonstrated by dissolution of preformed blood clots. These results suggest that the development of xylan-metal nanoparticle composite would be feasible to treat thrombus related diseases.

1. Introduction

In recent years, nanoparticles (NPs) have become an integral part of science and technology development and have found application in diverse fields including biomedical sciences. NPs with size range between 1 and 100 nm are effectively employed as sources for drug carriers, biosensing, bioimaging, phototherapy, immunoassay and so forth. Inorganic metal NPs possess antimicrobial and healing properties, especially silver nanoparticles (AgNPs) shows antimicrobial properties against a wide range of microorganisms like bacteria, fungi, protozoa and recently virus. The growing demand for NPs in numerous applications has led to the development of novel synthetic strategies including chemical, photochemical and biological methods. Among many methods, it is believed that the NPs prepared by using biomolecules are simple, safe, cost-effective and eco-friendly. Biopolymers such as cellulose, chitosan, starch, lignin and xylan are effectively utilized in the synthesis of nanoparticles (Luo, Pan, Ling, Wang, & Sun, 2014; Luo, Shen, Luo, Wang, & Sun, 2015; Shen, Luo, Wang, Wang, & Sun, 2014). Our team has recently reported number of biomolecules, for example, pectin (Venkatakrishnan, Veerappan, Elamparuthi, & Veerappan, 2014), levan (Ahmed, Kalla, Uppuluri, & Anbazhagan, 2014), β-glycoside (Ahmed, Subramaniam, et al., 2014), modified lipids (Anbazhagan, Ahmed, & Janani, 2014) and casein (Chakrapaniet al., 2014), for the preparation of coinage metal nanoparticles. In the present work, for the first time, we isolated xylan biopolymer from the waste biomass, wheat bran (WB) and demonstrated its ability to synthesis stable nanoparticles.

Xylans are found in wide variety of agricultural crops such as straw, sorghum, sugar cane, corn stalks, cobs, grasses, cereals, herbs as well as forest and pulping waste products from hardwood. The biosynthesis, constitution, fine structure and the application potential of xylan polymers isolated from industrial and field crops have paid much attention during the last decade (Ebringerova & Heinze, 2000; Panthapulakkal, Kirk, & Sain, 2015). Xylan, a hemicellulose exists predominantly as a cell wall material in annual plants and cereals and is one of the major constituent of lignocellulosic materials (Ebringerova & Heinze, 2000). Xylan is a heteropolysaccaride composed of β(1–4) and/or β(1–3) xylose residues. Depending upon the method of extraction and the botanical source, they may contain substitutions such as galactopyranosyl, glucuronosyl, arabinosyl and acetyl residues (Ebringerova & Heinze, 2000). Xylan has been used in paper making, wrapping films, textile printing and has also been used as an adhesive, thickener and additive to plastics. It has been used in the food industry as an emulsifier and protein foam stabilizer during heating (Ebringerova, Hromadkova & Hřibalová, 1995). Xylan can be degraded by the colonic micro flora and exhibit prebiotic effect (Grootaert et al., 2007). Hence,

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it has been used in the preparation of colon specific drug carrier to deliver anti-inflammatory and toxic drugs, such as sodium diclofenac, 5-aminosalycilic acid and usnic acid (Nagashima et al., 2008; Oliveira et al., 2010). The presence of reducing sugar xylose and free hydroxyl groups makes it a promising biopolymer to be used in nanotechnology, as similar to chitosan, cellulose, starch, etc. (Mochochoko, Oluwafemi, Jumbam, & Songca, 2013; Oluwafemi et al., 2013). In this paper, we extended the application of xylan to synthesize fibrinolytic active silver nanoparticles.

The formation of blood clot (thrombus) and dissolution of blood clot (thrombolysis or fibrinolysis) are well balanced in the healthy biological system. Any failure in the fibrinolysis process may lead to serious consequences including stroke, pulmonary embolism, deep vein thrombosis and acute myocardial infarction (Meissner, Zierler, Bergelin, Chandler, & Strandness Jr, 2002; Meyer et al., 2014; Millán, Dorado, & Dávalos, 2010). Currently, recombinant tissue plasminogen activator, urokinase, streptokinase and heparin are used clinically to inhibit blood clot formation. However, these agents are not safe and may cause serious bleeding complications associated with reocclusion and reinfarction. Therefore, developing a safer fibrinolytic agent is highly warranted. Recent development in nanotechnology showed the potential of silver and gold nanoparticles as an anticoagulant (Croes, Stobberingh, Stevens, Knetsch, & Koole, 2011; Ragaseema, Unnikrishnan, Krishnan, & Krishnan, 2012; Shrivastava et al., 2009; Stevens, Crespo-Biel, et al., 2009; Stevens, Knetsch, Sen, Sambhy, & Koole, 2009). Kemp et al. (2009) demonstrated an effective anticoagulant activity of the heparin stabilized silver nanoparticles. Kim et al. (2013) showed the anticoagulant activity of gold nanoparticle synthesized with earth worm extract. Shrivastava et al. (2009) reported that the AgNPs do not show any cytolytic effect on platelets and proposed as a antiplatelet/antithrombotic agents. Ragaseema et al. (2012) showed that the PEG-stablized AgNPs inhibit the platelet adhesion. In the present study, using fibrin plate assay we show that xylan silver nanocomposite has the ability to induce fibrinolyis in a concentration dependent manner. The effect of WB-xylan AgNPs in the dissolution of thrombus was demonstrated using *in vitro* blood clot. In addition, the synthesized nanocomposites exhibit excellent free radical scavenging activity. In short, our findings suggest that the synthesized WB-xylan silver nanocomposite has the potential to be promoted as an antioxidant and fibrinolytic agent after careful evaluation.

2. Materials and methods

2.1. Materials

Wheat bran was purchased from the local market in Thanjavur. Sodium hydroxide, silver nitrate, ethanol, hydrochloric acid and sodium dihydrogen phosphate were obtained from Merck, India. Disodium hydrogen phosphate, agarose and fibrinogen from bovine plasma were obtained from Himedia, India. Beechwood xylan, DPPH and thrombin from bovine plasma were obtained from Sigma India. Double distilled water was used throughout this work.

2.2. Extraction of xylan from wheat bran

Xylan present in the wheat bran was extracted by alkaline treatment as reported in the reference (Hauli, Sarkar, Mukherjee, Chattopadhyay, & Mukhopadhyay, 2013). Briefly, 20 g of the milled wheat bran powder was soaked in 200 ml of 10% sodium hydroxide. The mixture was kept at 60 °C in a shaker with constant agitation for 12 h. The alkaline treated wheat bran was then steamed at 100 °C for 3 h. After that the supernatant was recovered by centrifugation (10,000 rpm for 15 min) and pH was adjusted to 5.0 with 12 N HCL. To this solution, 1.5 vol. of 95% ethanol was added to precipitate the xylan. After centrifugation, the xylan was allowed to air dry before drying in hot air oven at 55 °C for 4 h. The pellets were powdered in a mixer. The impurities associated with extracted xylan were further purified by repeatedly dissolving it in water, and by centrifugation and precipitating the soluble xylan from the supernatant using 95% absolute ethanol. The pellets were then dried, powdered and stored at room temperature for further analyses.

1 g of the extracted xylan was hydrolyzed with 10 ml of 10% NaOH and steamed at 100 °C for 1 h. The hydrolyzed xylan was characterized by Fourier transform infrared spectroscopy, ^{1}H NMR and HPLC. The commercial beechwood xylan was used as a reference. FTIR spectroscopic measurements were carried out on a Perkin Elmer spectrum-one instrument in the diffuse reflectance mode at a resolution of 1 cm^{-1} in KBr pellet. ^{1}H NMR were measured in a Bruker 300 MHz instrument.

2.3. Synthesis of silver nanoparticles

Typically, 25 mg of xylan was dissolved in 49 ml of 0.2% sodium hydroxide solution. A 1 ml of silver nitrate (1 mM final concentration) aqueous solution was added to the alkaline xylan solution. The mixture solution was allowed to stir at room temperature for 5 min and then heated to 100 °C for 30 min. The appearance of brown color indicated the reduction of silver ion into AgNPs.

Optical absorbance of the synthesized AgNPs was monitored by UV–Vis spectrophotometer (Thermo Scientific Evolution 201) between the wavelength 350 and 800 nm at a resolution of 1 nm. The size and morphology were examined using high-resolution transmission electron microscopy (HR-TEM) (JEOL-JEM 1011, Japan) operating at an accelerated voltage of 200 kV. The samples were prepared by placing a drop of NPs solution on a graphite grid and drying it in vacuum. The average particle size and Zeta potential of the silver nanoparticles were measured using Malvern Zeta sizer nano-series (version 6.20).

2.4. DPPH free radical scavenging assay

The scavenging activity of DPPH free radical by WB-xylan AgNPs was performed according to the method previously reported (Mani, Seetha Lakshmi, & Gopal, 2012). Briefly, 0.1 mM of DPPH solution was prepared in 40% buffered methanol (40 ml methanol and 60 ml of 0.1 M acetate buffer, pH 5.5). The synthesized silver nanoparticles was added (100–500 μl) to 2 ml of freshly prepared DPPH solution and the final volume was made upto 3 ml by adding buffered methanol. The reaction mixture was shaken and incubated in the dark for 30 min. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. Ascorbic acid (0.01 mg/ml) was used as the positive control. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity. The scavenging activity was calculated from the following equation:

$$\%\,\text{scavenging} = \left[\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right] \times 100$$

2.5. Fibrinolytic assay of silver nanoparticles

Fibrinolytic activity of the WB-xylan AgNPs was carried out by fibrin plate method (Astrup & Müllertz, 1952). Typically, fibrin was prepared by mixing 6 ml of 0.6% bovine plasma fibrinogen and 6 ml of 1.5% agarose containing 10 NIH units of bovine plasma thrombin. 0.1 M phosphate buffer, pH 7.4 was used throughout the experiment. The prepared solution was quickly poured into a 90 mm diameter Petri plate and allowed to stand for 1 h in room temperature and labeled as fibrin plate. The fibrinolytic activity of