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Hydrogen peroxide sensing and cytotoxicity activity of *Acacia* lignin stabilized silver nanoparticles

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ABSTRACT

The study is aimed at detection of hydrogen peroxide (H_2O_2) using *Acacia* lignin mediated silver nanoparticles (AGNPs). The synthesis of AGNPs was achieved at conditions optimized as, 3 ml of 0.02% lignin and 1 mM silver nitrate incubated for 30 min at 80 °C and pH 9. Initial screening of AGNPs was performed by measuring the surface plasmon resonance peak at 410–430 nm using UV–vis spectrophotometer. Transmission electron microscopy, atomic force microscopy, X-ray diffraction and particle size analysis confirmed the spherical shaped face centered cubic structure and 10–50 nm size of AGNPs. The infrared spectroscopy study further revealed that the active functional groups present in lignin were responsible for the reduction of silver ions (Ag^+) to metallic silver (Ag^0). Lignin stabilized silver nanoparticles showed good sensitivity and a linear response over wide concentrations of H_2O_2 (10^{-1} to 10^{-6} M). Further, the *in vitro*cytotoxicity activity of the lignin mediated AGNPs (5–500 μg/ml) demonstrated toxicity effects in MCF-7 and A375 cell lines. Thus, lignin stabilized silver nanoparticles based optical sensor for H_2O_2 could be potentially applied in the determination of reactive oxygen species and toxic chemicals which further expands the importance of lignin stabilized silver nanoparticles.

1. Introduction

Nanoparticles are important due to their small size and large surface to volume ratio, which improves their physical and chemical properties [1]. Metal nanoparticles have potential applications in the fields of electronics, environment, catalytic and in biosensing [2–4]. One crucial aspects of nanotechnology concerns the improvement of quick and consistent experimental protocols for the synthesis of nanomaterials. The existing chemical and physical approaches are usually expensive and involve the use of toxic chemicals. Hence, green or biological originated compound based synthesis of silver nanoparticles (AGNPs) has become a major focus of research. Green chemistry involves the development and application of bio-originated products based method to diminish the generation and consumption of hazardous materials [5–8].

Green synthesis of silver nanoparticles by using plant extract such as mango peel extract [6], microorganism [9,10] and some biopolymer such as chitosan [11], starch [12], pullulan [13] have been reported previously. However, the literature reports limited synthesis of silver nanoparticles using agriculture or forest wastes such as banana, *Annona squamosa* and mango peel extract [6,14,15]. The property to act as both stabilizing and reducing agent marks the advantage of using biological compounds over organic chemicals for the synthesis of nanoparticle. Traditionally, for the synthesis of silver nanoparticles, several reducing agents like sodium borohydride ($NaBH_4$), formaldehyde, sodium citrate, hydrazine, ascorbic acid, glucose and UV irradiation are utilized to reduce the silver cations [16,17]. Some polymeric materials such as poly(vinyl pyrrolidone), poly(ethylene glycol), and surfactants are also used as stabilizers to prevent nanoparticles agglomeration and precipitation [18,19].

Lignin is the second most abundant biopolymer on earth. It confers mechanical support to the plant and also provides rigidity, mediates internal transport of water and nutrients and protect against attack by microorganisms. The chemical structure of lignin is challenging to define because its structure and properties are largely influenced by its isolation process and the type of sources used for its extraction. Generally, it is an amorphous polyphenolic macromolecule which is composed of a large number of polar functional groups [20]. Polymeric natures and wide variation in the functional groups of lignin make it flexible for varied industrial applications. Literature shows that the polyphenols like poly-eugenol and kraft lignin serve as an effective stabilizing agent for gold nanoparticles and dispersing agent for carbon nanotubes,

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respectively. *Acacia* lignins are potent antioxidant and could be possibly used to reduce metal ions that display a relatively high redox potential [16,21].

Hydrogen peroxide (H_2O_2) is widely used as strong oxidant in many fields, including agriculture and food products [22], pharmaceutical and cosmetic formulations [23], environmental protection [24], chemical and biochemical industries [25]. Hydrogen peroxide is also used for various medical applications and paper bleaching [26,27]. The wide application of H_2O_2 demands a fast, precise and easily accessible based sensor for its determination. In recent years, a significant interest have evolved in the design and development of new methods for the determination of hydrogen peroxide [28]. Particularly, owing to its low cost, high catalytic activity and noteworthy optical properties, silver nanoparticles (AGNPs) becomes a promising materials in the recognition of ultrasensitive chemical and biological molecules. AGNPs has been successfully applied for the detection of some chemicals and biomolecules such as glucose, folic acid, H_2O_2 and nitroaromatic compounds [29–31]. *Acacia* lignins are effective antioxidant, for instance, they are used as a reducing and stabilizing agent for the preparation of colloidal silver nanoparticles.

In the present work, a single step green spectroscopic method for the rapid detection of hydrogen peroxide using *Acacia* lignin mediated silver nanoparticles has been studied. Various operational parameters viz. influence of pH, incubation time, temperature, concentration of lignin and silver nitrate has been evaluated for the green synthesis of lignin based silver nanoparticles (AGNPs). AGNPs was characterized by ultraviolet–visible (UV–vis) spectrophotometer, particle size analyzer, X-ray diffraction (XRD), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The catalytic and sensing application of AGNPs in their aqueous colloidal solutions for hydrogen peroxide sensing has been investigated. To the best of our knowledge, this is the first study describing the use of *Acacia* alkali lignin mediated silver nanoparticles for the detection hydrogen peroxide. In addition, cytotoxicity activity of lignin stabilized AGNPs was also evaluated.

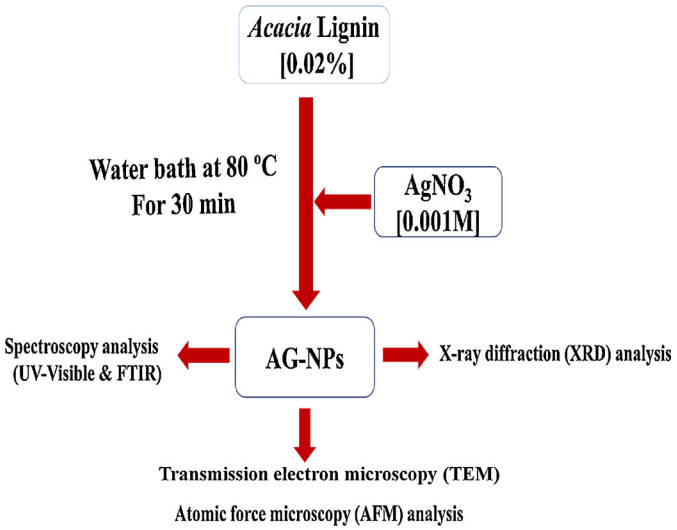
2. Materials and methods

2.1. Materials

Purified *Acacia* lignin was used as a starting material in the experiment, extracted from *Acacia* wood powder using alkali method. Silver nitrate ($AgNO_3$), 3-[4,5-dimethylthiazol-2-yl]-2-5-diphenyltetrazolium (MTT), dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. Sodium hydroxide and hydrogen peroxide were purchased from Hi-Media Chemical Laboratory Ltd., Mumbai, India. Ultra filtered high purity deionized water (Millipore) was used throughout the experiment for the preparation of solutions.

2.2. Extraction and purification of lignin

Lignin was extracted from the pre dried *Acacia* wood powder by the alkali method as described in previous study [21]. Briefly, the sample was de-waxed using toluene-ethanol (2:1, v/v) in a soxhlet extractor. De-waxed *Acacia* wood powder was treated with 0.2 N NaOH at 120 °C for 45 min. The solid/liquid ratio was kept at 1:10 (w/v). The dark brown liquor was filtered out and concentrated to reduce the volume to 50 ml. The hemicellulose fraction was removed through precipitation by decreasing the pH of the filtrate up to 5.5 with 5 N HCl followed by adding 3 volumes of 95% ethanol. After the subsequent elimination of hemicellulose, soluble alkali lignin fractions were recovered by re-precipitation of lignin at pH 1.5–2.0. Purified lignin (0.02%) was freshly prepared by dissolving in deionized water and used as reducing agent for the synthesis of silver nanoparticles.



Scheme 1. Schematic diagram of the green synthesis procedure of lignin mediated silver nanoparticles.

2.3. Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, silver nitrate solution (1 mM) was prepared in deionized water. Typical reaction mixture contained 3 ml of lignin solution (0.02%) in 27 ml of $AgNO_3$ solution (1 mM). The reaction mixture was incubated at 80 °C in a water bath for 30 min. The effect of pH on nanoparticle synthesis was determined by adjusting the pH of the reaction mixtures to 2.0, 3.0, 5.0, 7.0, 9.0, and 11.0 using 1 N HCl and 1 N NaOH, respectively. The effect of the silver salt was determined by using varying concentrations of $AgNO_3$ (0.5, 1.0, 2.0, 4.0 and 8.0 mM). The influence of lignin concentration on the synthesis of silver nanoparticles was also determined by adding different volumes of lignin (1, 2, 4, 6 and 8 ml). To study the effect of temperature and incubation time on nanoparticle synthesis, reaction mixtures were incubated at different temperatures (°C) (25, 40, 60, 80 and 100) and incubation time (min) (15, 30, 45, 60, 75 and 90), respectively. The schematic diagram of the lignin mediated silver nanoparticles synthesis process is summarized in Scheme 1.

2.4. Characterization of silver nanoparticles

2.4.1. UV–vis spectrophotometer

Lignin mediated reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) was monitored by measuring the UV–vis absorbance spectra of the reaction mixture using UV–vis spectrophotometer (UV-1800, Shimadzu, Japan) in the range of 200–600 nm.

2.4.2. Particles size and zeta potential analysis

The particles size and zeta potential of the lignin stabilized silver nanoparticle was determined using particle size analyzer (Malvern Zeta sizernanosizer) after one week of incubation.

2.4.3. FTIR analysis

FTIR analysis was carried out to evaluate the functional groups of lignin responsible for providing reducing and stabilizing property to the resultant AGNPs. Samples were dried overnight in oven at 60 °C. Approximately 1 mg dried samples were mixed with 100 mg of KBr before analysis. FTIR spectra of purified and dried lignin stabilized AGNPs were recorded in the range of 4000–400 cm^{-1} with