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Synthesis of silver nanoparticles mediated by alpha amylase and their application in the photocatalytic degradation of organic dyes

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Abstract

Silver nanoparticles are nanoparticles of silver between 1 nm and 100 nm in size. Silver nanoparticles have an extensive range of applications in a variety of fields including biological research, medicine, chemical catalysis, textile industry etc. They are also used in the comprehensive treatment of environments containing infectious pathogens owing to its unique properties of high antimicrobial activity. Synthesis of silver nanoparticles is carried out by many methods including chemical, physical, photochemical and biological methods. Conventionally, chemical methods are used but due to their hazardous nature, other approaches are being investigated. Recently, green biosynthesis methods have garnered wide attention due to its relative simplicity and eco-friendliness. The present research work summarizes a green approach towards the synthesis of silver nanoparticles using alpha amylase. The color of the solution turned from colorless to light brown within 12 hours indicating silver nanoparticle formation. The preliminary characterization was done by using UV-Visible spectrophotometer. Photo catalytic degradation of organic dyes methylene blue and methyl orange were determined spectrophotometrically by using silver as nanocatalyst. The conclusion was drawn that the biosynthesized silver nanoparticles using alpha amylase were found to be notable in degrading the dyes.

Keywords: Alpha amylase, Silver nanoparticles, UV-Visible spectrophotometry, Methylene blue, Methyl orange.

Introduction

In the field of modern materials science, the nanotechnology branch is emerging into a prominent area of research. Nanoparticles show novel or enhanced features centered on specific attributes such as size, distribution and morphology. Nanoparticles are believed to be collection of atoms whose size is in the order of 1–100 nm[1].

Nanoparticles can be organized into four types namely carbon based, metal based, dendrimers and composites[2]. The carbon nanoparticles are also referred to as organic nanoparticles. Spherical and ellipsoidal carbon nanoparticles are called as fullerenes, while cylindrical ones are called nanotubes. Quantum dots, nanogold, nanosilver and metal oxides (titanium oxide) are examples of metal based nanoparticles. Dendrimers are nanosized polymers which can be synthsised to perform specific chemical functions. Composites are combinations of nanoparticles with other nanoparticles or with larger sized and heavier compounds. Silver nanoparticles are most commonly used in water filters and biosensors.

Dyes are defined as coloured, ionising and aromatic organic compounds which show affinity towards the substrate to which it is being applied and they are extensively utilized in the textile industry[3]. These non-biodegradable substances have to be removed from the environment and pose to be a dire environmental crisis. Many methods are regularly used for reducing dyes like activated carbon sorption, flocculation, electro coagulation, UV-light degradation and redox treatments[4]. However, due to the ineptitude of these methods in one way or the other, the present situation necessitates better and improved removal methods. Lately, studies have found that Agnano particles are good, highly efficient and stable photo catalysts under ambient temperature with visible light illumination for degrading organic compounds and dyes[5]. Hence, the purpose of the present study was to assess the degrading property of the synthesized silver nanoparticles from alpha amylase towards methylene blue and methyl orange.

Materials and methods

Materials: Silver nitrate, tris-HCl buffer, alpha amylase were procured from Hi Media Labs. All other chemicals used were of analytical grade and no further purification process was followed.

Method: Synthesis of silver nanoparticles: Synthesis of the silver nanoparticles was carried out by incubating 40 ml of alpha-amylase solution (2 mg/ml in Tris-HCl buffer, pH 8.0) and 60 ml of freshly prepared aqueous solution of Silver nitrate (1 mM). The solution was kept at 25°C and after regular intervals of time appropriate aliquots were withdrawn and the synthesis of silver nanoparticles was monitored by UV-VIS spectroscopy[6]. UV-VIS spectral analysis was performed on Elico double beam UV-VIS spectrophotometer operated at a resolution of 1 nm as a function of reaction time. A control was run in which only silver nitrate solution was taken without the enzyme alpha amylase and the spectra was recorded after regular intervals of time.

Purification of Silver Nanoparticles: Silver nanoparticles were separated from the solution by centrifugation at 9,000 rpm for 5 min at 4°C. The clear supernatant was discarded and the pellet was washed five times with distilled water, air dried and used for further studies.

Preliminary Characterization of Silver Nanoparticles: UV-VIS Spectroscopy: Metal nanoparticles would display distinctive absorption spectrum when suspended in aqueous solution. In theory, colloidal silver nanoparticles show an absorption peak, referred to as the surface plasmon resonance (SPR) which rests in the range of 400-460 nm[7]. The change from light-yellow to intense brown colour in the solution indicated the synthesis of silver nanoparticles. Bio reduction of aqueous Ag+ ions is measured by UV-Vis spectrophotometer and one significant characteristic in optical absorbance spectra of metallic nanoparticles is surface plasmon band which arises because of the combined electron oscillation about the surface mode of the particles. The concentration of color amplified as a function of time because of reduction of silver ions.

Photocatalytic activity of silver nanoparticles: The photocatalytic (in presence of sun light) activities of biogenic silver nanoparticles were examined for degradation of methylene blue and methyl orange. Preparation of suspension was done by addition of 20 mg of Ag nanoparticles to 50 ml of methylene blue/methyl orange solution[8]. Afterwards, the suspensions were kept in darkness and constantly stirred for 30 minutes to guarantee stable equilibrium of Ag nanoparticles in the organic solution. Next, the suspensions were held beneath sunlight confined in a Borosil glass container and agitated continuously. The mean temperature was observed to be 29°C with 10 hour mean shine period. The suspensions were finally centrifuged and absorption spectrum was measured at regular intervals using a UV–visible spectrophotometer to confirm the breakdown of methylene blue/methyl orange solution.

Results and discussion

Alpha amylase used for the *in vitro* synthesis of silver nanoparticles catalyzed silver nanoparticle formation by acting as the sole reducing and capping agent[6]. When alpha amylase solution (2 mg/ml in Tris-HCl buffer, pH 8.0) was incubated with silver nitrate solution (in distilled water) at 25°C, the solution turned light brown in 12 hrs.

UV-Visible spectrophotometer analysis: The visible spectrum of the solution (Figure-1) recorded at different time periods consisted of an increasingly intense absorption at 430 nm, characteristic of the surface plasmon resonance of silver nanoparticles. The absorption increased upto 36 hours and after that it was stable. Control sample did not show any color and consequently did not have any peak in the visible region indicating that alpha amylase is responsible for the synthesis of silver nanoparticles. Hence, the silver nanoparticles synthesis was verified by UV-Vis spectral analysis.

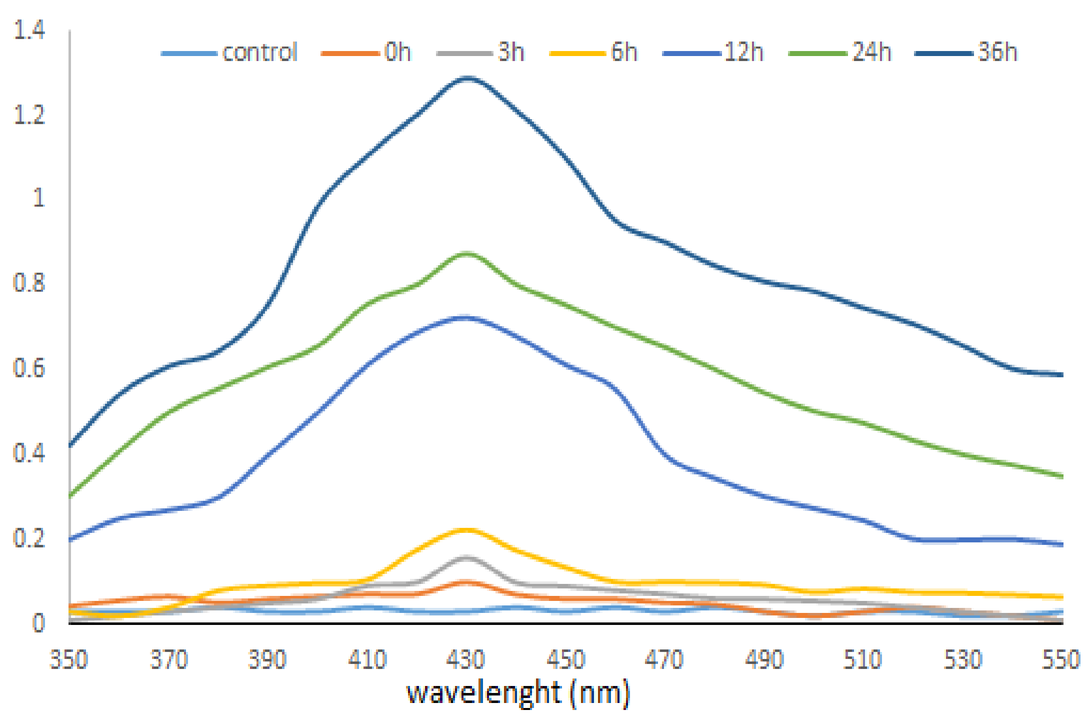

Figure-1: UV-visible spectra of synthesized silver nanoparticles recorded as a function of time.

Photocatalytic degradation of dyes: Photocatalytic activity of silver nanoparticles on degradation of dyes was demonstrated by using the dye methylene blue and methyl orange.

Methylene blue dye: The degradation of methylene blue was carried out in the presence of silver nanoparticles at different time in the visible region. The absorption spectrum showed the decreased peaks for methylene blue at different time intervals[9]. Initially, the absorption peaks at410 nm (Figure-2) for methylene blue dye decreases gradually with the increase of the exposure time and that indicates the photocatalytic degradation reaction of methylene blue. The completion of the photocatalytic degradation of the dyes is known from the gradual decrease of the absorbance value of dye approaching the base line. Dye degradation was initially identified by color change. Initially, the color of dye shows deep blue color changed into light blue after the 1 h of incubation with silver nanoparticles while exposed to solar light. Thereafter light blue was changed into light green. Finally, the degradation process was completed at 72 hand was identified by the change of reaction mixture color to colorless.

Methyl orange dye: At different time periods, the solar irradiated degradation of MO dye by Ag nanoparticles was studied. The typical absorption peak of MO solution was observed to be 410 nm (Figure-3). Breakdown of MO was seen by reduction in peak intensity within 75 mins of incubation time. The adsorption of silver nanoparticles on to the MO solution was initially low and further increased with constant increase in time. Dye degradation was initially identified by color change. Initially, the color of dye shows deep orange color changed into light orange after 1 h of incubation with silver nanoparticles while exposed to solar light. Finally, the degradation process was completed at 72 hand was identified by the change of reaction mixture color to colorless.

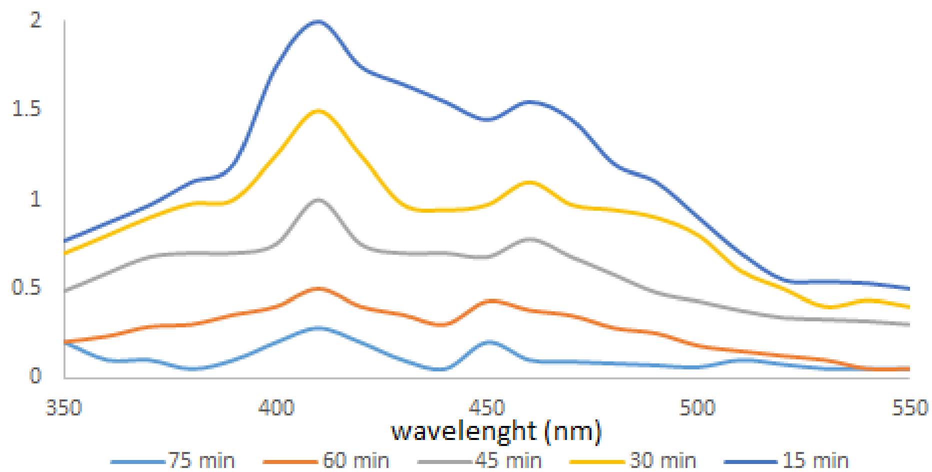

Figure-2: UV-Vis spectra for degradation of methylene blue.

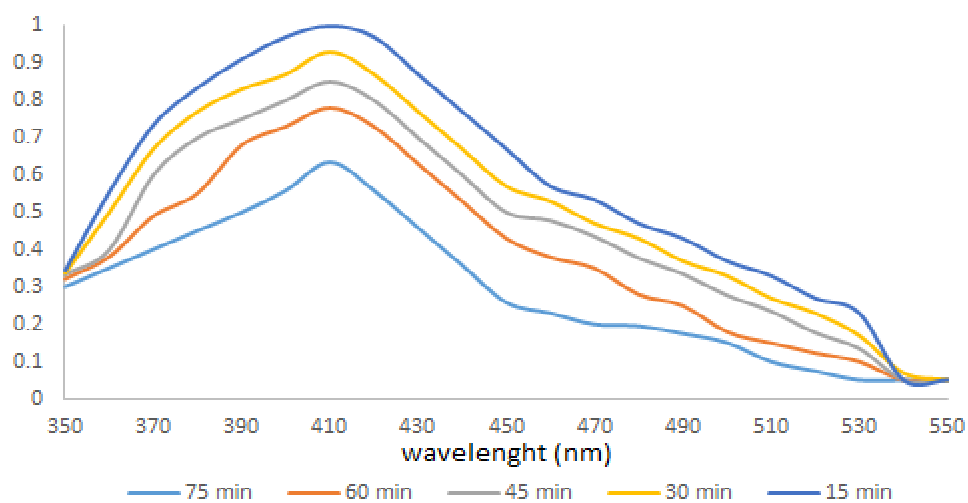

Figure-3: UV-Vis spectra for degradation of methyl orange.

Conclusion

In this work a bio friendly method has been used to synthesize silver nanoparticles. The process offers a simple and inexpensive method to generate large amounts of stable silver nanoparticles. Alpha amylase used for the *in vitro* synthesis of silver nanoparticles catalyzed silver nanoparticle formation by acting as the sole reducing and capping agent. The color of the solution turned from colorless to light brown within 12 hours indicating silver nanoparticle formation. The enzyme mediated synthesis could be the leading large-scale production method for nanoparticles in future. The synthesized silver nanoparticles exhibited excellent photocatalytic activity against dye molecules methylene blue and methyl orange. The results established that, the biosynthesized silver nanoparticles from alpha amylase were observed to be noteworthy in degrading the dyes and can be used in water purification systems and dye effluent treatment.

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