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Reusable magnetic nanobiocatalyst for synthesis of silver and gold nanoparticles

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ABSTRACT

In the present work, we describe a simple procedure for the biosynthesis of nanosilver and gold by the reduction of silver nitrate and auric chloride respectively using a nanobiocatalyst. The nanobiocatalyst was prepared by covalent coupling of alpha amylase on (3-aminopropyl)triethoxysilane (APTES) modified iron oxide magnetic nanoparticles. The nanobiocatalyst retains 77% of its activity as compared to free alpha amylase. The nanobiocatalyst can be used up to three consecutive cycles for the synthesis of nano silver and gold. The biosynthesized nanoparticles after each cycle were characterized by UV–vis spectrophotometer, Dynamic Light Spectroscopy (DLS), Transmission Electron Microscope (TEM), X-ray powder diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Silver and gold nanoparticles of same morphology and dimensions were formed in each cycle. The procedure for synthesis of nanoparticles using an immobilized enzyme is eco-friendly and can be used repeatedly.

1. Introduction

Metal nanoparticles are certain to be the building blocks of the next generation of electronic, optoelectronic, biomedical and chemical sensing devices [1]. Among several metal nanomaterials silver and gold nanoparticles are one of the most commercialized because of their unique optical, electrical, and photo thermal properties [2]. They have wide applications in bio-sensing, diagnostic imaging, waste water treatment, chemo-catalyst, cancer diagnosis and therapy [3].

Therefore, development of protocols for the synthesis of metal nanoparticles (NPs) has been an important area of research. Broadly, these nanoparticles can be synthesized by physical, chemical and biological means [4–6]. Biological methods are considered green and economical as compared to physical and chemical methods [7]. Although lot of work has been done in this area still, the mechanism of biosynthesis is not clear. Different types of microorganisms and plants/plant extracts have been exploited for the synthesis of metal nanoparticles [8]. The proteins/enzymes, amino acids, carbohydrates, phytochemicals present in the biomaterial are believed to be responsible for the reduction of metal ions into the nano form. Recent advancement to the biological methods is the use of pure enzymes for the synthesis of metal nanoparticles [9]. Advantages of using enzymes are that they are available in pure form and are highly specific. Enzymes itself can act as reducing as well as stabilizing agent thus eliminating an additional step generally employed to stabilize the nanoparticles. Moreover, there is strong interaction of various amino acid residues of protein with nanoparticles [10–13]. The use of in vitro enzymatic reactions for synthesis of silver and gold nanoparticles is still an unexplored area. There is a need to design a rational enzymatic strategy for the synthesis of silver and gold nanoparticles on large scale. But the commercial viability of industrial biosynthesis of nanoparticles using pure enzymes is not fulfilled due to the high cost of the enzymes. The use of pure enzymes in soluble form leads to the loss of enzyme activity and also cannot be reused. The reusability issue can be solved using immobilized enzyme systems [14–17].

In the recent past, nanosized materials have been widely employed for enzyme immobilization. Due to the large surface area of nanosized materials, they provide superior loading capacity and low mass transfer resistance [17,18]. Magnetic metal nanoparticles have been used for protein/enzyme immobilization owing to their unique properties such as super paramagnetism, high surface area, large surface-to-volume ratio and easy separation under external magnetic fields [19–22]. Coupling agents such as glutaraldehyde (GA) [23] and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) [24] are often utilized to covalently cross-link the modified magnetic nanoparticles and proteins because their functional groups (e.g., aldehyde group) can interact with both functional groups of the modified magnetic nanoparticle and pro-

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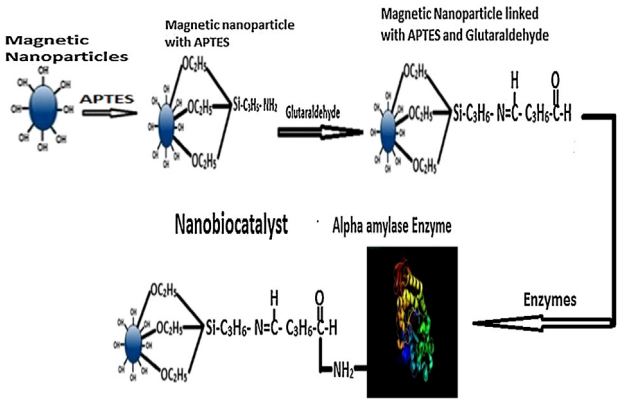

Fig. 1. Schematic representation of preparation of nanobiocatalyst.

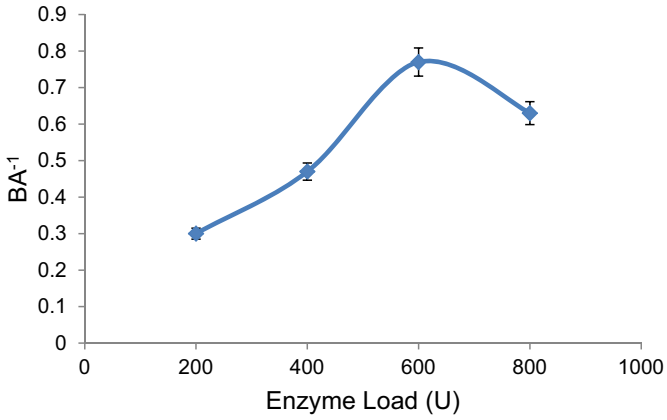

Fig. 2. Immobilization of Alpha amylase. Various aliquots of the free enzyme were adsorbed on the matrix as described in experimental part. Load refers to the initial enzyme activity units in the solution. A represents the amount of enzyme bound to the matrix. B represents the expressed activity of the particular immobilized preparation, measured after incubating the immobilized enzyme (after dissolving) with the substrate. BA^{-1} refers to the immobilization efficiency of the preparation. Experiments were run in triplicate and the difference in individual sets of readings was less than 5%.

teins. Alvaro Cruz [25] prepared Cross-Linked Enzyme Aggregates (CLEAs) of *Candida antarctica* lipase B (CALB) which were covalently bound to magnetic nanoparticles. The magnetic biocatalyst can be easily recovered from the reaction mixture and can be reused for at least ten consecutive cycles of 24 h without apparent loss of enzyme activity. Talekar et al. reported the preparation of stable cross-linked enzyme aggregates (CLEAs) of NADH-dependent nitrate reductase and they use it for the synthesis of silver nanoparticle from silver nitrate [26].

Thus, in the present study, alpha amylase enzyme from *Aspergillus oryzae* was immobilized on APTES modified magnetic nanoparticles. The resultant nanobiocatalyst was used for the synthesis of silver and gold nanoparticles.

2. Materials and methods

Iron (II, III) Oxide, Glutaraldehyde, $HAuCl_4$ (49% w/v) and (3-aminopropyl)triethoxysilane (APTES) were obtained from Sigma-Aldrich. Alpha-amylase enzyme was obtained from HiMedia. Starch and other salts for the preparation of buffer were obtained from Merck. All other chemicals and solvents used were of analytical grade.

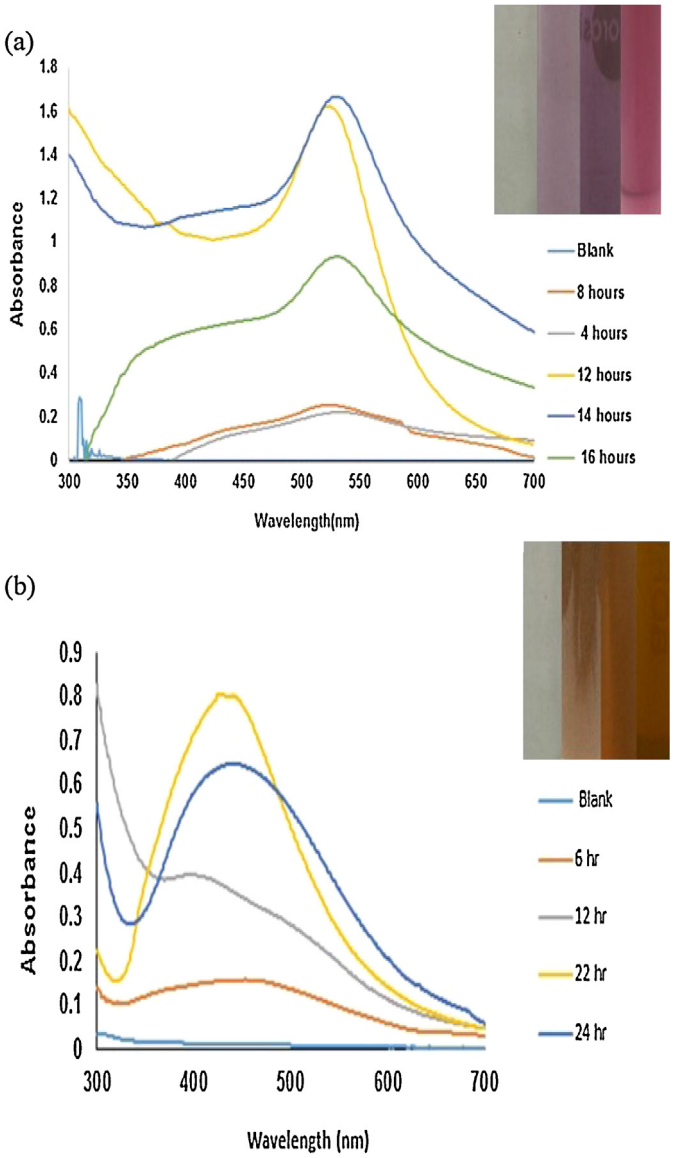

Fig. 3. Optimization of nanoparticles synthesis by alpha amylase (first cycle); (a) UV Visible spectra of AuNP after regular interval of time; (b) UV Visible spectra of AgNP after regular interval of time.

2.1. Activity measurement of α-amylase

Activity of α-amylase was estimated using starch as the substrate [27]. The activity of the nanobiocatalyst was determined in the similar way. The nanobiocatalyst was continuously shaken for the entire duration of the assay. One enzyme unit is defined as the amount of the enzyme that catalyzes the conversion of 1 μmol of substrate per minute.

2.2. Protein estimation

Protein was estimated by the dye binding method using bovine serum albumin as standard [28].

2.3. Surface modification of iron oxide nanoparticles with (3-aminopropyl)triethoxysilane (APTES)

Surface modification of the Iron oxide nanoparticles with APTES was carried out as described by Campo et al. [29]. Briefly, 0.612 ml of APTES was added to 0.03 gm of iron oxide nanoparticles pre-