Preparation of Chitosan-Coated Magnetite Nanoparticles and Application for Immobilization of Laccase

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Statement of Purpose: In the last decades, applications of nanosized magnetic particles (maghemite, γ-Fe_3O_4 or magnetite, Fe_3O_4) in biology and medicine have been extensively studied in the areas of magnetic resonance imaging, hyperthermia generation, magnetically controlled transport of anticancer drugs, RNA and DNA purification, magnetic cell separation and purification, enzyme and protein immobilization. Magnetic nanoparticles as carriers for the enzymes provide rapid and easy recovery of the biocatalyst from the reaction medium in an external magnetic field and so has advantages of higher specific surface area for the binding of higher amounts of enzymes. The hypothesis of this study is that, laccase enzyme which is a commercialized catalysts used in bioremediation, would have high catalytic activity and efficient reuse capacity upon immobilization on functionalized magnetic nanoparticles. The objectives of the study are, to functionalize Fe_3O_4 nanoparticles by coating with a functional polymer; to immobilize laccase on these magnetic nanosystems; to measure and compare the activity and reusability of the enzyme containing systems at different conditions.

Methods: Fe_3O_4 nanoparticles were coated and functionalized with chitosan (CS) and laccase from *Trametes versicolor* was immobilized onto chitosan coated magnetic nanoparticles (Fe_3O_4-CS) by adsorption or covalent binding after activating the hydroxyl groups of chitosan with carbodiimide (EDAC) or cyanuric chloride (CC) (Figure 1).

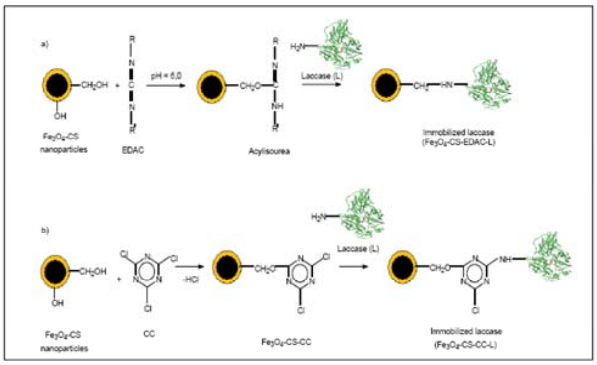

Figure 1. Scheme of laccase immobilization onto Fe3O4-CS nanoparticles (a) by EDAC and (b) by CC.

The presence and effects of chitosan on Fe_3O_4 was characterized by TEM, FTIR, zeta potential, VSM, and TGA. For free laccase and immobilized laccase systems, the optimum pH, temperature, and kinetic parameters were investigated; and the change of the activity against repeated use of the immobilized systems were examined.

Results: For chitosan-coated magnetic nanoparticles, the thickness of CS layer was estimated as 1.0–4.8 nm by TEM (Figure 2).

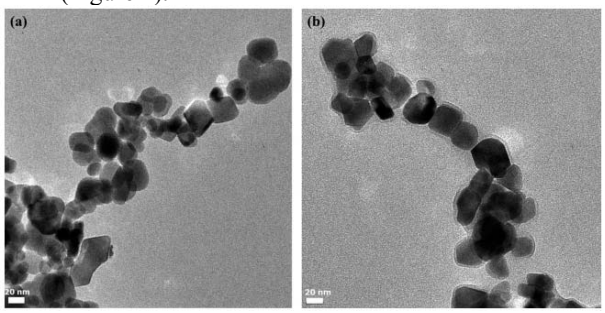

Figure 2. TEM images of nanoparticles. (a) Fe_3O_4, (b) Fe_3O_4-CS (scales are 20 nm).

Isoelectric point of these nanoparticles determined by zeta-potential measurements were found to be 6.86 and the saturation magnetization of chitosan-coated magnetite nanoparticles was determined as 25.2 emu g^{-1} by VSM analysis indicating that these nanoparticles were almost superparamagnetic.

Conclusions: Chitosan-coated magnetite nanoparticles with a high magnetic property were prepared by reversed phase suspension technique, and the structural and magnetic properties of these nanoparticles were characterized. Laccase from *Trametes versicolor* was immobilized on these magnetically separable chitosan-coated magnetite nano particles through adsorption and covalent binding methods. The dependence of immobilized laccase activities against pH and temperature demonstrated that enzyme became more stable upon immobilization compared with the free form. Kinetic studies showed that; catalytic efficiencies of the immobilized enzymes were also quite close to the free enzyme. All the immobilized systems prepared either by adsorption or covalent binding exhibited more than 71% activity at the end of 30th repeated use. Therefore, it can be concluded that laccase immobilized magnetic nanosystems prepared in this study can be good candidates to be used in bio industrial applications in terms of catalytic activity and reuse capacity.

References: Kalkan NA., Aksoy SA., Aksoy EA., Hasirci N. J Appl Polym Sci 2012;123: 707–716.