Surface Functionalized Magnetic Fe_3O_4 Nanoparticles as a support for the Immobilization of Laccase and TEMPO Applied in Organic Synthesis

Mohammad Ali Faramarzi[1,*] and Mehdi Mogharabi-Manzari[1]

[1] *Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran*

INTRODUCTION: The immobilization of biological molecules and enzymes onto inorganic surfaces is critical to many modern techniques and underpins a wide range of emerging technologies from *in vitro* diagnostics and tissue engineering to direct self-assembly and biomolecular electronics. Magnetic nanoparticles are used extensively for the immobilization of enzymes because they show low toxicity and good biocompatibility, they have a large specific surface area, and they are easily separated from a reaction mixture simply by applying an external magnetic field. Heterogeneous bio-catalysts are prepared by physical and chemical enzyme immobilization techniques [1,2]. The development of green processes, such as employing safe catalysts, waste minimization, replacing toxic solvents with H_2O, and using O_2 as an environmentally benign oxidant, in the chemical industry has gained significant attention in recent years [3].

METHODS: Magnetic Fe_3O_4 nanoparticles were synthesized by ultrasound-assisted reverse co-precipitation. The synthesized Fe_3O_4 nanoparticles were coated by tetraethyl orthosilicate. The silica-coated iron oxide nanoparticles were separated with an external magnet, washed three times with ethanol, and dried under vacuum. Dry silica-coated magnetite nanoparticle powder (10 g) was mixed with 200 mL of toluene to produce a homogeneously mixed solution, followed by sonication of the mixture for 30 min. After addition of (3-aminopropyl)triethoxysilane with mechanical stirring. A mixture was prepared in a two-necked flask at 40°C by combining salicyl alcohol, an *o*-phenylendiamine, and the hybrid catalyst bearing immobilized laccase and immobilized TEMPO in citrate buffer.

RESULTS: Fe_3O_4 nanoparticles of 10.7 nm average diameter were prepared by co-precipitation from $FeCl_3/FeCl_2$ solution with NH_4OH. The nanoparticles were characterized by scanning electron microscopy, transmission electron microscopy, X-ray diffraction, and magnetic measurements were made by vibrating sample magnetometry. The surface morphology and size of the magnetic nanoparticles was determined by SEM and TEM. The results from Bradford assay showed that maximum enzymatic loading on the surface was 10 mg of enzyme per 80 mg of solid support. In addition it was found that 3.4 mg of TEMPO was attached per 40 mg of solid support (Fig. 1). The one-pot, two-step enzymatic aerobic oxidation reaction included the condensation of *in situ* produced salicylaldehyde derivatives with aromatic amines, followed by an enzymatic dehydrogenation process (Fig. 1). The hybrid catalyst retained more than 85 % of its initial activity after 10 runs.

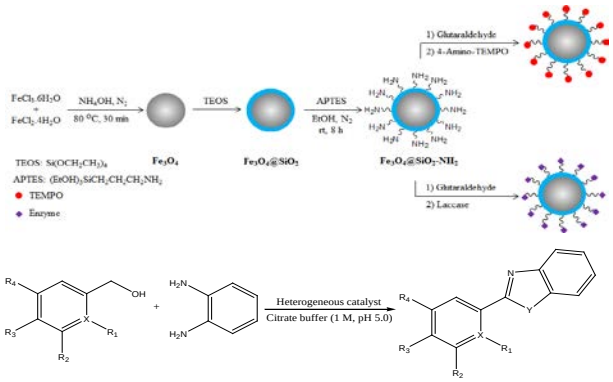

Fig. 1: Surface functionalization of magnetic Fe_3O_4 nanoparticles for immobilization of laccase and TEMPO in a one-pot and two-step enzymatic aerobic oxidation reaction.

DISCUSSION & CONCLUSIONS: Magnetic nanoparticle-supported catalytic systems have emerged as promising catalysts that can be easily separated from the reaction medium with the simple application of an external permanent magnet. Surface functionalized magnetic iron oxide nanoparticles are a type of novel functional materials that have been widely used in the biotechnology and catalysis. A highly efficient multifunctional hybrid catalyst was prepared based on heterogeneous materials. Future improvement of catalytic entities can be improved by applying surface functionalization strategies for efficient immobilization of biocatalysts.

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