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# Evaluation of different saccharides and chitin as eco-friendly additive to improve the magnetic cross-linked enzyme aggregates (CLEAs) activities

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ABSTRACT

The cross-linked enzyme aggregates (CLEAs) involves formation of a number of covalent bonds between enzyme and the matrix using glutaraldehyde. In general, amino groups of lysine, sulfhydryl groups of cysteine, phenolic OH groups of tyrosine, or imidazol group of histidine are used for enzyme binding under mild conditions. The main advantage of this method is its simplicity, economic advantages in the industrial bio catalysis. The $Fe_3O_4$ magnetic nanoparticles were synthesized by coprecipitating $Fe^{2+}$and $Fe^{3+}$in alkaline solution. Tannic acid was used to functionalize the $Fe_3O_4$ magnetic nanoparticles. After functionalization process, tannic acid magnetic cross-linked enzyme aggregates of enzyme (TA-MNPs-CLEAs) were prepared by cross-linking of enzyme aggregates with different saccharides as additive. The present result reported high stability, simplicity, low cost and recyclability of a saccharide-TA-MNPs-CLEAs-enzyme make it efficient as a highly active biocatalyst in biotechnological applications. The obtained results suggest that disaccharides (maltose, sucrose and lactose) and polysaccharide such as starch are eco-friendly additives to TA-MNPs-lipase and TA-MNPs-CLEAs-peroxidase and can become a powerful biocatalyst in industry applications.



## 1. Introduction

Carbohydrates are probably the most abundant and widespread organic substances in nature, a number of classification schemes have been devised for carbohydrates, the division into four major groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides used here is among the most common. Two molecules of a simple sugar that are linked to each other form a disaccharide. The disaccharide sucrose, consists of one molecule of glucose and one molecule of fructose [1]. The lactose, and maltose are also disaccharides. Before the energy in disaccharides can be utilized by living things, the molecules must be broken down into their respective monosaccharides. Oligosaccharides, which consist of three to six monosaccharide units, are rather infrequently found in natural sources, although a few plant derivatives have been identified [2]. Starch is the major dietary polysaccharides in plant [1].

Chitin is a derivative of glucose. The structure of chitin is comparable to another polysaccharide - cellulose, forming crystalline Nano fibrils or whiskers. In terms of function, it may be compared to the protein keratin. It has useful for several industrial and biotechnological purposes [3,4].

Cross-linked enzyme aggregates (CLEAs) are prepared by aggregating the enzymes in precipitants such as ammonium sulfate, ethanol and acetone, followed by a cross-linker, Usually, glutaraldehyde has been used for CLEAs [5]. However, glutaraldehyde was found to modify necessary ε-amino groups, which resulted in CLEA with significant loss of biological activity [6]. Cross-linking of enzymes to electrospun nanofibers has shown better residual activity due to increased surface area and porosity. Lysozyme-immobilized electrospun Chitosan (CS) nanofibers via CLEAs have also been reported to be effective in continuous antibacterial applications [7]. The addition of polymers containing amino groups could increase cross-link effectiveness. On the other hand, the microenvironment that surrounds the enzyme was altered. As a result, the prepared CLEAs did not exhibit more conformational steady than the normal CLEA. The BSA addition is known to support CLEAs preparation in cases in which the enzyme activity is weak to high concentrations of cross-linker or low protein concentration of the enzyme [8], but to our knowledge, there is no systematic work in the previous literature about the immobilization of enzymes as CLEAs or magnetic CLEAs by addition of different saccharide. Therefore, in this study, magnetic CLEAs of two enzymes lipase and peroxidase were prepared by co-aggregation of lipase and different saccharides to improve the activity of enzymes, increase cross-linking efficiency.

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## 2. Materials and methods

### 2.1. Materials

Lipase enzyme (9 U/mg), peroxidase from horseradish (~150 U/mg) sodium dodecyl sulfate (SDS), potassium phosphate dibasic, glucose, chitin, and glutaraldehyde (25%, w/v solution) were purchased from Sigma Aldrich Company, USA. Tris(hydroxymethyl)-amino methane, ammonium sulfate, maltose, ferrous sulfate heptahydrate, starch, sucrose, acetone, tannic acid, triton X, Ferric chloride hexahydrate, sodium hydroxide, *p*-nitrophenol, *p*-nitrophenyl palmitate, lactose, ammonium hydroxide (25%, w/w), nitric acid ($HNO_3$) and ethanol were provided by Merck (Germany).

### 2.2. Synthesis and modification of $Fe_3O_4$ MNPs

The $Fe_3O_4$ nanoparticles (MNPs) were synthesized by dissolving 4.4483 g of $FeSO_4 \cdot 7H_2O$ and 7.5684 g of $FeCl_3 \cdot 6H_2O$ in deionized water. The mixture heated at 80 °C under $N_2$ for 1 h (at 200 rpm). Then, 40 mL of the $NH_3 \cdot H_2O$ was add to the mixture with stirring under $N_2$ for 1 h and then cooled to room temperature. The precipitated were washed several times with deionized water and one times ethanol. Lastly, $Fe_3O_4$ were dried at 70 °C in oven overnight under vacuum [9].

In order to modify $Fe_3O_4$ MNPs, 1.0551 g MNPs were sonicated in deionized water for 15 min to get regular dispersal and mixed at 200 rpm under $N_2$ atmosphere at 40 °C for 1 h. After that 0.5 g of tannic acid added to 20 mL deionized water and mixed at 200 rpm under $N_2$ (40 °C,2 h). After cooling the mixture, the tannic acid modified $Fe_3O_4$ MNPs (TA-MNPs) washed with ethanol, followed with deionized water for times. The TA-MNPs were dried at 70 °C in a vacuum oven overnight.

### 2.3. Preparation of TA-MNPs-CLEAs-enzymes

The CLEAs enzymes were prepared according to Lopez-Serrano et al.'s method [10]. One milliliter of enzymes were added into a Falcon tubes, different saccharides as additives were used such as glucose, maltose, sucrose, lactose, starch and chitin (4–20 mg). Two hundred milligrams of TA-MNPs were also added. The samples in all case were mixed at 200 rpm for 30 min. The ammonium sulfate (30–70%) were added and mixed for 30 min at 200 rpm. Glutaraldehyde solution (50–150 mM) were added. The mixture was left for 17 h at 200 rpm. After adding 5 mL of deionized water, the mixture was centrifuged at 4000 rpm (4 °C) for 25 min. The precipitate was washed with water seven times.

To prepare of saccharide-TA-MNPs-CLEAs-enzyme in organic solvent, 1 mL of enzymes were added to falcon tube contain 3 mL acetone or ethanol. After that 0.113 μL of glutaraldehyde (25% w/v) were added. The samples were left at 4 °C for 17 h. One milliliter of acetone was added to the mixture and centrifuged. The deposit washed 5 more times with the corresponding ethanol or acetone (5 mL each time).

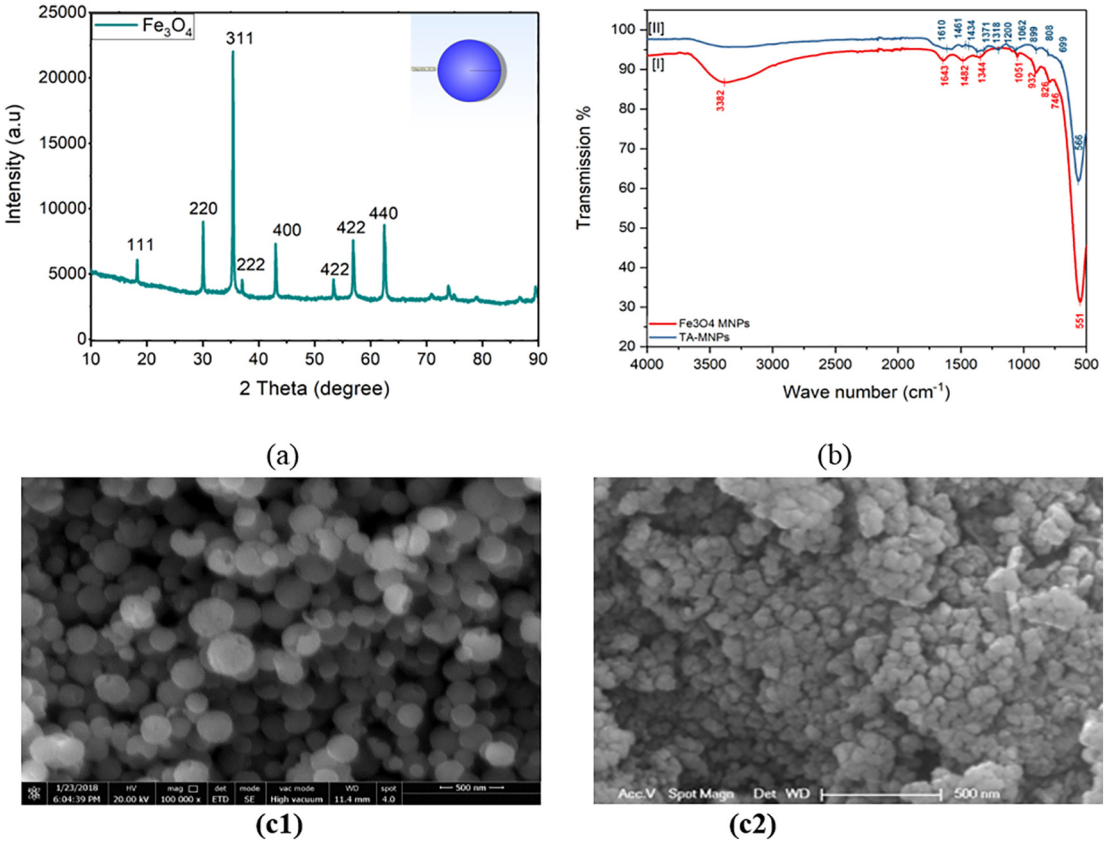


**Fig. 1.** (a) XRD pattern of synthesized $Fe_3O_4$ nanoparticles, (b) FTIR spectra for $Fe_3O_4$ MNPs, and TA-MNPs. (c) SEM images of $Fe_3O_4$ nanoparticles (c1), TA-MNPs (c2).

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