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Dithiocarbamate to modify magnetic graphene oxide nanocomposite (Fe_3O_4-GO): A new strategy for covalent enzyme (lipase) immobilization to fabrication a new nanobiocatalyst for enzymatic hydrolysis of PNPD

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ABSTRACT

Immobilization of lipase was successfully achieved on the surface of magnetically separable Fe_3O_4/graphene oxide (GO) via a post-modification. This post modification was achieved in alternation to glutaraldehyde post-modification. The activity of immobilized lipase had not a significant loss in the activity while on the other hand, it is simply extractable (by keeping its major activity) from reaction crude by a magnet. Each step of immobilization was carefully monitored by characterization and all were successfully proved. SEM, TEM, XRD, EDX, and FTIR were used to characterize the support and immobilization process.

1. Introduction

In recent decades, trends to immobilize enzymes have been more popular due to its advantages of keeping activity and easy recovery [1,2]. Among the various supports, [3] magnetic nanoparticles, graphene oxide nanosheets (GO), [3,4] biopolymers, [2] and silica nanosystems [5] are became in more attention. On the other hand, to produce a covalent linkage between support and enzyme, some methods are being proposed and developed. For example, post-modification of support's amine groups (amine itself can be produced on the surface by some precursors like 3-(aminopropyl)triethoxysilane (APTES) or adding some amine containing polymer like chitosan) with glutaraldehyde (which is a bifunctional aldehyde) or epichlorohydrin can make it qualificative support for enzyme immobilization. However, despite of appreciable efforts and developments for producing a nontoxic and less destructive linkage between enzyme and solid support, new demands are still remained. In this investigation, we have tried to propose and examine a new method for fine immobilization of lipase on magnetic Fe_3O_4/GO support through post modification with APTES and subsequent further post-modification with carbon dioxide to generate dithiocarbamate moieties on the surface. In our proposal mechanism, dithiocarbamate functions are the main agent for producing afore-mentioned linkage between enzyme and support.

Lipase [6] has been investigated as a highly active and applicable enzyme in various fields of biotechnology especially in bio-catalysis reactions including hydrolysis, [7] aminolysis, [8] and transesterification [9] of triglyceride and long chained esters to biodiesel or amide products [7]. Therefore, recovery and reuse of lipase is as a major concern to convert it to an economical options in the industrial and large scale production of bioproducts [10,11]. Magnetic nanoparticles are playing as an important role in recovery process of lipase. Besides, GO [12] with higher surface area and biocompatibility and higher affinity to bind lipase can be as a compliment partner to magnetic nanoparticles in the form of mag-

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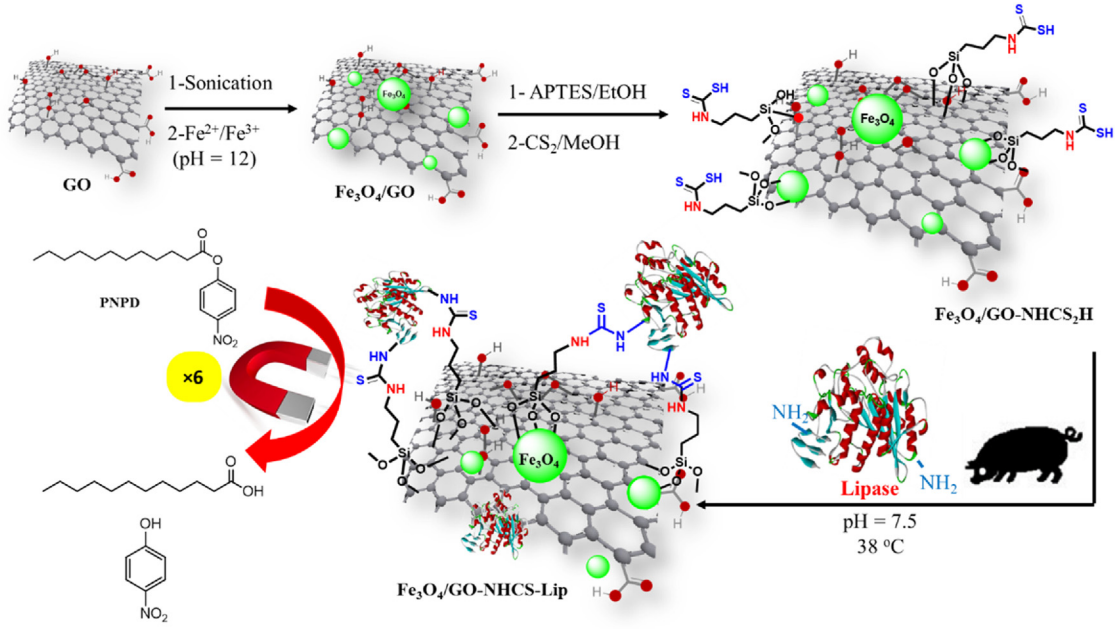
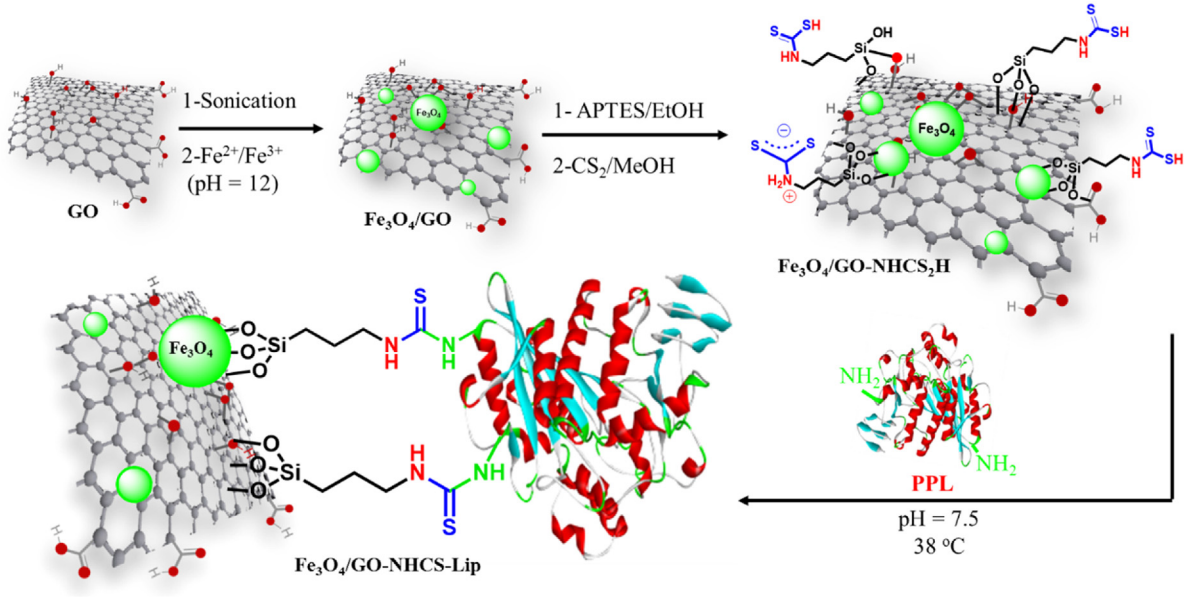


Fig. 1. Schematic representation of the production of biocatalysts by Lipase immobilization onto magnetic GO.



Scheme 1. General procedure of synthesis of Fe_3O_4/GO-NHCS-Lip.

netic Fe_3O_4/GO nanocomposite [13]. Hence, functionalization of Fe_3O_4/GO with APTES and its post-modification with carbon disulfide (CS_2) to produce dithiocarbamate functionalities can convert Fe_3O_4/GO nanocomposite to an active material to support lipase enzyme. Following to our previous experiences, this immobilization may be achieved by covalent attachment (Fig. 1).

2. Experimental

2.1. Materials and apparatus

p-Nitrophenyl dodecanoate (PNPD) was purchased from Sigma-Aldrich and used as a substrate for the measurement of lipase activity. Lipase (Lipase from porcine pancreas-PPL) was purchased from Sigma-Aldrich and used without any further purification. Homogeneous stirring was done by ultrasonic (Ultrasonic Homogenizer-model APU500 Advanced Equipment Engineering Company-Adeeco, Iran). The enzyme activity was monitored by measuring the concentration of hydrolyzed and released *p*-nitrophenol (PNP) from interaction of enzyme and substrate though UV–vis spectrophotometer.

Scanning electron microscopy (SEM) and Electron dispersive scanning (EDS) were recorded on TESCAN Vega, Transmission electron microscopy (TEM) was recorded on JOEL JEM 3010 instrument, FTIR was recorded on Shimadzu, X-ray diffraction spectra were recorded on Philips PW1800, and UV–vis spectra and analyses were measured and obtained by Shimadzu UV-1800 Spectrophotometer.