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Synthesis, characterization and kinetic analysis of chitosan coated magnetic nanobiocatalyst and its application on glucose oleate ester synthesis

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ABSTRACT

In this work, we report the covalent immobilization of lipase enzyme on the surface of chitosan coated magnetic nanoparticles (MNPs) using *N*-(3-dimethylaminoproyl)-*N*-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as coupling agents. Surface functional groups of chitosan coated MNPs, lipase enzyme, and immobilized lipase enzyme were characterized by Fourier transform infrared spectroscopic (FT-IR) analysis. The structural characteristics of the chitosan coated magnetic nanoparticles were analyzed by X-ray Diffraction (XRD) studies. The statistical methodology with central composite design (CCD) was applied to evaluate the effects of immobilization, including the magnetic nanoparticles, lipase enzyme, pH and immobilization time on the enzyme activity. Based on the statistical analysis, the optimum immobilization conditions of magnetic nanoparticles (40 mg), lipase enzyme (40 mg), pH (9), and immobilization time (6 h), the maximum enzyme activity obtained was 16.94 U/mL. The optimum pH and temperature for maximum enzyme activity of immobilized lipase are found to be pH 9 and 40 °C respectively. The immobilized lipase exhibited excellent catalytic activity over eight successive cycles and retain 64% original stability. The Michaelis–Menten enzyme kinetic studies of immobilized lipase on chitosan coated MNPs showed maximum activity (V_{max}) and Michaelis–Menten constant (K_m) of 33.7 (μmol/mL min) and 0.89 mM respectively. The thermal stability of lipase was significantly improved after immobilization. The thermal deactivation rate of immobilized lipase was studied to follow the Arrhenius law with deactivation energy of 73 kJ/mol. Glucose ester yield of 80.8% was achieved in this study revealed that immobilized lipase on MNPs have promising application in industrial scale for sugar ester production.

1. Introduction

Lipase (triacylglycerol ester hydrolase, EC 3.1.1.3) enzymes catalyze the hydrolysis of triglycerides to fatty acids and glycerol [1–3]. Lipases have been involved in several industrially significant catalytic biotransformation reactions such as esterification, transesterification, alcoholysis, and acidolysis [4]. Lipases have been utilized in various industries like food, cosmetic, detergent, oil, and fats, leather, and pharmaceuticals [5]. However, the industrial applications of lipase has few limitations due to high cost, low stability and inability of separation [6,7]. These drawbacks can be overcome by immobilization on supports material, which could improve enzyme stability, reusability, and easy recovery [8,9]. In the past few years, nanostructured materials have been dramatically used as supports for lipase immobilization [10]. Among these nanostructures, magnetic nanoparticles (MNPs) possess low toxicity and easy separation from reaction medium by applying magnetic field [11–13]. The strong magnetic field attraction between particles leads to increased agglomeration and enzyme loading capacity limitations [14–16]. The surface can be modified by various polymers such as gelatin, polyacrylamide, polyaniline, chitosan, poly (hydroxyethyl methacrylate) and copolymers of styrene to improve the catalytic efficiency and stability [17–21]. Chitosan, a biopolymer consists of linear chain of D-glucosamine and *N*-acetyl-D-glucosamine is widely used as a surface functionalization agent having reactive amino and hydroxyl groups which facilitate the enzyme linkage to MNPs [22]. Chitosan is considered to be non-toxic, safe biopolymer and able to form complexes by reacting with the polyanions. Response surface methodology (RSM) is a widely used statistical tool for optimization in bioprocess

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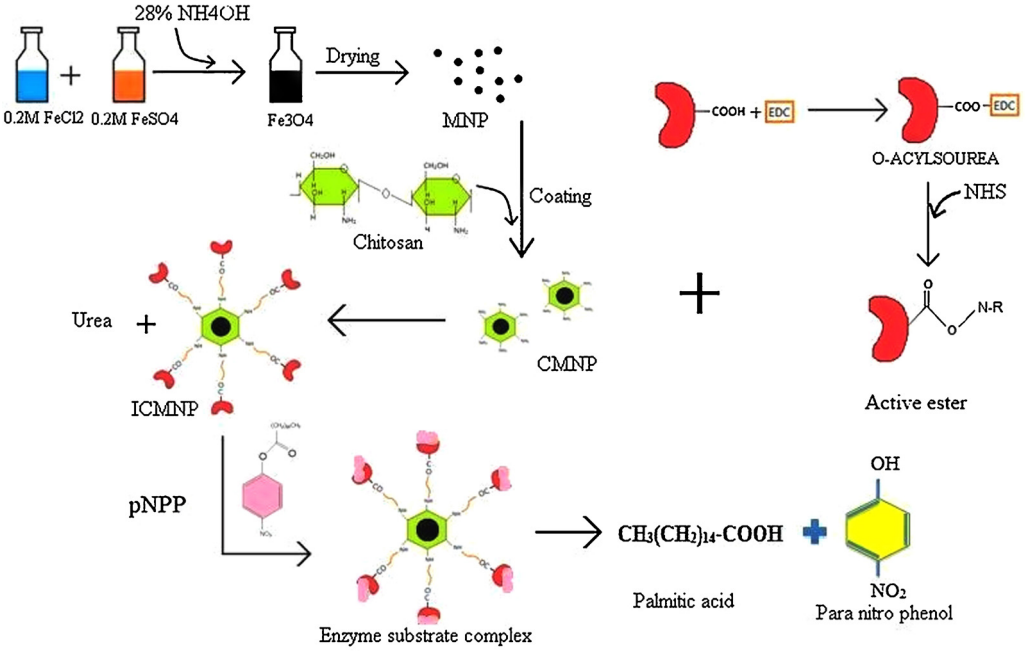

Fig. 1. Schematic representation of MNP synthesis, surface functionalization of MNPs with chitosan and lipase immobilization onto the chitosan coated MNPs.

field and literature on application of RSM in enzyme immobilization on MNPs is limited [8,23]. In our study, the prepared magnetic lipase biocatalyst was used to synthesize the glucose oleate ester, a sugar fatty acid ester (SFAE). SFAEs are nonionic surfactants posses significant applications in food, cosmetics, and pharmaceutical industries because of their biodegradability and nontoxic property [24]. SFAEs can be synthesized by enzymatic and non-enzymatic methods. Enzymatic synthesis of SFAEs is highly preferred than non-enzymatic method since chemical route has several disadvantages such as low selectivity, high energy requirement and toxic byproducts generation [25].

In the present work, the magnetic nanoparticles (MNPs) were prepared by simple co-precipitation method. The MNP coated with chitosan polymer employs the amino groups for covalent binding with lipase enzyme using EDC (*N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide) and NHS (*N*-hydroxysuccinimide) as linking agents. The structure and properties of the immobilized MNPs were characterized by Fourier transform infrared spectroscopic (FT-IR) and X-ray Diffraction (XRD) techniques. The optimal conditions for immobilization of lipase on MNPs were investigated by response surface methodology (RSM) based on the central composite design methodology. The effect of pH and temperature, reusability and storage ability of the immobilized lipase were also investigated. Further, prepared immobilized lipase was used for glucose oleate ester synthesis.

2. Materials and methods

2.1. Chemicals

Rhizopus niveus Lipase and *p*-nitrophenylpalmitate (pNPP) were purchased from Sigma–Aldrich. EDC (*N*-(3-dimethylaminoproyl)-*N*-ethylcarbodiimide), NHS (*N*-hydroxysuccinimide), chitosan, ethanol, and isooctane were purchased from Sisco Research laboratories Pvt., Ltd., Mumbai, India. Glucose, ferric chloride, ferrous sulphate, acetic acid, oleic acid, ammonium hydroxide and sodium chloride were purchased from Hi-media, India. All the chemicals used in this study are of analytical grade.

2.2. Preparation and surface modification of magnetic nanoparticles

Magnetic nanoparticles were prepared by the co-precipitation method [26,27]. 0.1 M of ferrous sulphate and 0.2 M of ferric chloride were dissolved in 50 mL deionized water and 28% of ammonia solution was added with vigorous stirring at 80 °C for 30 min and the pH adjusted to 10. The solution on cooling yields black precipitate. The precipitate was separated magnetically and washed several times with deionized water and ethanol to remove the excess ammonia. The resulting MNPs were dried at room temperature. 400 mg of nanoparticles were dispersed in 0.4 g chitosan containing 20 mL of 1% acetic acid solution. 1 N sodium hydroxide was slowly added to the reaction mixture to precipitate the chitosan coated MNP. The precipitate was isolated and rinsed several times with deionized water until it reaches pH 7 and then dried at 40 °C for 2 h.

2.3. Characterization of immobilized enzyme and chitosan coated MNP

The magnetic nanoparticles were characterized by XRD (the scan range of 2θ was selected from 20° to 80°) (PAN analytical, X'Pert power, Germany). Functional groups and surface bonding of the chitosan coated MNP was analyzed by FTIR spectroscopic technique using RX1 FTIR spectrometer (PerkinElmer, USA).

2.4. Covalent immobilization of lipase enzyme

Fig. 1 illustrates the synthesis of chitosan coated MNPs and lipase immobilization. In brief, 30 mg of lipase was added to 0.25% EDC containing 4 mL of 50 mM phosphate buffer solution (with 200 mM NaCl) and the solution was incubated at 25 °C for 1 h with shaking. Then, 30 mg of NHS was added to the solution and the incubation was continued for another 1 h. 50 mg of the chitosan coated MNP was added to this solution and incubated for further 4 h. EDC was used to activate the carboxyl groups of lipase with NHS and subsequently reacted the amino group of the chitosan coated MNPs. The activated carboxyl group of enzyme was combined with