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A surfactant-coated lipase immobilized in magnetic nanoparticles for multicycle ethyl isovalerate enzymatic production

Iram Mahmood [a,c,*], Ishfaq Ahmad [b], Guo Chen [c], Liu Huizhou [c]

[a] School of Chemical and Materials Engineering, National University of Sciences and Technology, Islamabad, Pakistan
[b] Department of Mathematics and Statistics, International Islamic University, Islamabad, Pakistan
[c] National Key Laboratory of Green Process and Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

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ABSTRACT

Gum arabic coated magnetic Fe_3O_4 nanoparticles (GAMNP) were prepared by chemical co-precipitation method and their surface morphology, particle size and presence of polymer-coating was confirmed by various measurements, including transmission electron microscopy (TEM), X-ray diffraction (XRD), thermo gravimetric analysis (TGA), and Fourier transform infra red (FTIR) analysis. Magnetic particles were employed for their potential application as a support material for lipase immobilization. Glutaraldehyde was used as a coupling agent for efficient binding of lipase onto the magnetic carrier. For this purpose, the surface of a *Candida rugosa* lipase was initially coated with various surfactants, to stabilize enzyme in its open form, and then immobilized on to the support. This immobilized system was used as a biocatalyst for ethyl isovalerate, a flavor ester, production. The influence of various factors such as type of surfactant, optimum temperature and pH requirement, organic solvent used, amount of surfactant in coating lipase and effect of enzyme loadings on the esterification reaction were systematically studied. Different surfactants were used amongst which non-ionic surfactant performed better, showing about 80% esterification yield in 48 h as compared to cationic/anionic surfactants. Enhanced activity due to interfacial activation was observed for immobilized non-ionic surfactant–lipase complex. The immobilized surfactant coated lipase activity was retained after reusing seven times.

1. Introduction

Esters of short chain fatty acids and alcohols are important components of natural aromas and flavors [1]. Hence they are in high demand and frequently used in the food, beverages, pharmaceutical and cosmetic industries. For instance, ethyl isovalerate is a derivative of valeric acid, mainly found in fruits (one of the principal component of blueberry) and widely used in perfumery and fragrance. However, plant extracted natural flavor esters are often either too scarce or expensive for commercial use.

The use of enzymes to catalyze esterification has become a more promising method than acid- or base-catalyzed reactions for ester production [2,3]. In this context, the concept of a natural ester made by enzymatic synthesis with lipase and natural substrate is an attractive alternative to those routes [4,5]. However, stability and reusability of the enzymes have been of major concern in non-aqueous enzymatic synthesis [6,7]. Hence, utility of such enzymes in industrial processes is limited by their tendency to being denature and become inactivated when exposed to organic solvents.

Many researchers have recently overcome the problem and use of enzymes in organic media has attracted much interest for effective biocatalysis [8,9]. Some approaches to overcome this problem have been successfully developed [10,11] such as modifying the enzyme with high molecular weight synthetic polymers [12,13], or coating them with surfactants. The most significant advantages of the surfactant coated enzymes are their simple preparation procedure and good solubility in a wide range of organic solvents [13].

The physical modification of enzymes with surfactants is more suitable for surface hydrophobic enzymes as it can be applied in lipase assay to increase the lipid–water interfacial area by maintaining surface charge and hydrophobicity which, in turn, enhance the observed rates of lipase-catalyzed reactions [14], a process called bio-imprinting. In this regard, Mingarro et al. [15] reported a novel concept for the activation of lipolytic enzymes, interfacial activation-based molecular bioimprinting (IAMI), for use in nonaqueous media. Since the preparation procedure for surfactant coated enzymes is conceptually identical to the IAMI method, the conformation of lipase in the resultant complex could be considered to be an open active form. However, to fully exploit the technical and economical advantages of lipases, it is recommended to use

* Corresponding author at: School of Chemical and Materials Engineering, National University of Sciences and Technology, Sector H-12, Islamabad 44000, Pakistan. Tel.: +92 51 9085 5112; fax: +92 51 9085 5002.
E-mail address: dr.iram@scme.nust.edu.pk (I. Mahmood).

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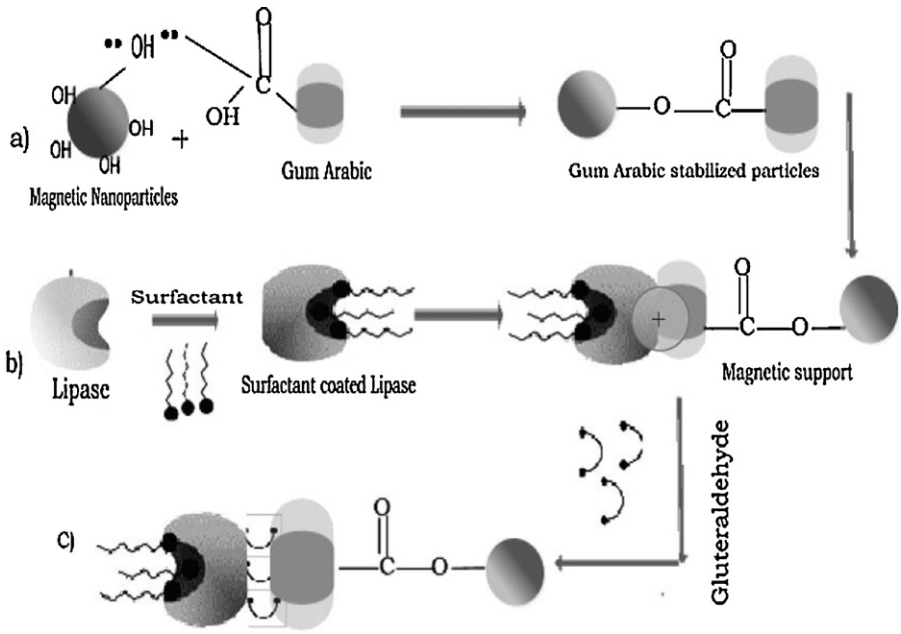

Scheme 1. Synthesis route to preparation of (a) gum arabic coated magnetic nanoparticles, (b) preparation of surfactant coated lipase and (c) immobilization on magnetic support.

them in an immobilized form to reduce cost and poor stability of the free lipase.

The selection criteria for immobilization technique and carrier are largely dependent on the particular lipase type, type of reaction system (aqueous, organic solvent or two-phase system), process conditions (pH, temperature and pressure) and goal of immobilization [16]. A good immobilization protocol should maintain a higher catalytic activity after enzyme immobilization. Hence, the design of an efficient lipase immobilized system is a rather difficult task.

Currently, the focus in enzyme immobilization technology is shifting toward the use of nanosized materials as supports due to their high specific surface area [17]. Since the high surface area: volume ratios of nanoparticles can effectively improve the enzyme loading, preventing aggregation enhancing the catalyst efficiency and is the simplest solution to solubility problem of these interesting biocatalysts. However, the recovery of nanoparticles immobilized enzyme is often limited.

Regarding industrial use, attention should also be paid to magnetic carriers because of their low cost and excellent separability in combination with relatively good durability and high activity of immobilized lipase. Magnetic immobilization has been shown to be a very promising method for enzyme catalyzed reactions [18,19]. Magnetic microparticles/nanoparticles have been used to immobilize lipase by our group and other groups in the world [20–24].

Several requirements must be met for magnetic carriers to be successfully used in immobilization: good dispersability in physiological medium and biocompatibility. These nanoparticles are easy to prepare. However due to their paramagnetic behavior, they are easy to aggregate. Therefore, it is necessary to engineer the surface of magnetic nanoparticles to minimize their aggregation during immobilization.

Many methods have been proposed to stabilize the magnetic particles, however not all of them yield known biocompatibility [25]. A natural polymer known as gum arabic (GA) has shown the ability to sustain colloidal stability of carbon nanotubes in aqueous solutions due to nonspecific physical adsorption [26]. It has also been used in the preparation of colloidal silver particles as a steric stabilizer [27]. Gum arabic coating offers two major benefits: it enhances colloidal stability and provides reactive functional groups suitable for coupling of bioactive compounds. Therefore, in present study, this natural polymer was used as a coating material to avoid magnetic nanoparticles agglomeration and enhancing their biocompatibility for use as a carrier for lipase immobilization.

Although there are hundreds of immobilization protocols, the design of new protocols that may permit to improve the enzyme properties during immobilization is still an exciting goal. Both properties of enzyme stability and reusability are necessary for successful industrial application. Hence, the present study aims to emphasize use of immobilized surfactant–lipase complex as a recyclable biocatalyst for ethyl isovalerate production [Scheme 1]. Enzymatic esterification with the surfactant-coated lipase for synthesis of short chain esters is limited [28]. In addition only few reports are available on the synthesis of ethyl isovalerate using *Candida rugosa* lipase source. Furthermore, surfactant-coated forms of the lipase from *Candida rugosa* source immobilized on magnetic support for ethyl isovalerate synthesis has not been described in the literature.

2. Materials and methods

2.1. Materials

Candida rugosa lipase (CRL) was purchased from Sigma–Aldrich Company. All other chemicals were purchased from commercial supplier (Beijing Chemical Reagent Co.) and were of the highest purity available, including ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ammonium hydroxide (25% [w/w]), isovaleric acid, ethyl isovalerate, gum arabic, glutaraldehyde, hexane, alcohol etc.

2.2. Synthesis of gum arabic coated magnetic nanoparticles

Magnetic Fe_3O_4 nanoparticles were prepared by the conventional co-precipitation method. Typically, 2.33 g of $FeCl_3{\cdot}6H_2O$ and 0.86 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 100 ml deionized water under nitrogen and 10 ml 25% $NH_3{\cdot}H_2O$ was added to the solution dropwise under vigorous stirring at 90 °C. After the color of