Girish S. Nhivekar and Virendra K. Rathod*

Optimization of lipase-catalyzed synthesis of polyethylene glycol stearate in a solvent-free system

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Abstract: Polyethylene glycol stearate is widely used in pharmaceuticals and cosmetic industries. The current work describes the synthesis and optimization of polyethylene glycol stearate by esterification of polyethylene glycol 600 and stearic acid using Fermase CALB$_{ex}$ 10000, a commercial immobilized lipase B in a solvent-free system. The impact of various parameters that include temperature, reaction time, biocatalyst loading, agitation, acid to alcohol molar ratio, and amount of molecular sieves was optimized to achieve maximum conversion. The highest conversion of 86.98% was obtained in 6 h under the following optimized conditions: temperature 70°C, biocatalyst loading 0.5%, acid to alcohol molar ratio 1:4, speed of agitation 300 rpm, and molecular sieves 5% (w/w). The final condensate product was analyzed through Fourier transform infrared spectroscopy to confirm the functional group and also by ^{1}H nuclear magnetic resonance spectroscopy. The immobilized catalyst can be reused up to four cycles, exhibiting more than 60% of its initial activity.

Keywords: lipase; polycondensation; polyethylene glycol; solvent-free system.

1 Introduction

Low and high molecular esters are widely used in the field of pharmaceuticals, flavors, perfumery, and cosmetics. Polycondensation is a type of reaction where polymerization is carried out by condensation of alcohol with the acid to form the corresponding ester. This method is mostly applied in the synthesis of biodegradable polyesters and polymers [1]. The traditional method to synthesize esters includes the use of chemical catalyst such as acetates of manganese, zinc, calcium, cobalt, magnesium, and antimony oxide [2]. However, the traditional method needs extreme reaction conditions of temperature and pressure, which lead to the formation of unwanted side products that require further additional cost for its separation and purification from the reaction mixture. Hence, a newer method for ester synthesis is required to resolve the above issues. Use of enzymes or biocatalyst instead of chemical catalysts is a new era. The enzymatic route of esterification possesses many advantages over chemical catalyst as it has high enzymatic activity, requires milder reaction conditions, high control of enantio-, chemo- and regioselectivity, and is overall a green route of synthesis [3–5].

Lipases [3.1.1.3] are a group of enzymes that catalyze many esterification, transesterification, and hydrolysis reactions [6]. Because of such vast industrial applications, lipases can be found in immobilized form on various resins that imparts better stability in a broad range of temperature and pH, thus increasing its reproducibility [2]. Polyesterification can be performed in many ways, namely, esterification, transesterification, acidolysis, and alcoholysis, to obtain their respective polyesters [7].

Many aliphatic polyesters are synthesized by fermentation, i.e. *in vivo* using microbes that can produce enzymes such as polymerase in late 1980s. The synthesis of polyesters *in vitro* is the new field where they are synthesized by using an enzyme in a synthetic way because of their several advantages as mentioned above [8, 9]. There are many esters and polymeric materials synthesized using lipase-catalyzed polycondensation such as poly(ethylene terephthalate) and poly(butylene succinate) that are derived from an aromatic polyester and poly(ε-caprolactone) obtained from aliphatic polyesters. Such polymers are obtained from either ring opening or condensation polymerization method [10]. Lipases initiate the ring-opening polymerization of small or large rings, i.e. lactones, cyclic lactides, and carbonates, to produce their corresponding polyesters and polycarbonates and their polymeric materials. The condensation of hydroxides with diols is also carried out using lipases. Thus, lipases provide greener route to synthesize esters by polycondensation reactions.

***Corresponding author: Virendra K. Rathod,** Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India, e-mail: vk.rathod@ictmumbai.edu.in
Girish S. Nhivekar: Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India

Polyethylene glycol (PEG) is a nontoxic, hydrophilic polymer that is widely applied in the field of medical biology [11]. Also, PEG contains alcoholic group that can be useful in the synthesis of polyesters. PEG provides a small fraction of end group that takes part in the synthesis of polyester via polycondensation [12]. Polyethylene glycol stearate (PEG stearate), a surfactant formed from condensation of polyethylene glycol and stearic acid, is used in the pharmaceutical industry, cosmetic industry, and as softener in the textile industry. The traditional way of synthesizing PEG stearate includes polymerization of polyethylene glycol with stearic acid in the presence of an acid catalyst such as *p*-toluenesulfonic acid or phosphoric acid, sulfuric acid in the presence of toluene and xylene at the higher temperature of 150°C. The use of such acid catalyst and higher temperature, the process makes nongreener [13, 14]. To overcome such problems of higher temperature and use of acid catalyst, the lipase can be applied to synthesize PEG stearate. However, limited work has been performed on the polymerization of PEG-based alcohol and stearic acid using lipase. Thus, there is indeed a need to understand the effect of the various parameters of enzyme-catalyzed synthesis using PEG and stearic acid and also to study the reusability of the catalyst, which helps to produce higher amount of product at lower cost. Considering the overall factors involved, the present work was performed to carry out the synthesis of PEG stearate in a solvent-free system (SFS) using Fermase as a catalyst. In order to obtain maximum conversion, the parameters that drive the reaction such as temperature, mole ratio of the substrates, enzyme loading, speed of agitation, and effect of molecular sieves were optimized, and their influence was studied. Moreover, the immobilized lipase was also reused to determine its activity fall after repeated uses.

2 Materials and methods

2.1 Materials

Polyethylene glycol 600 (PEG 600) and stearic acid (99%) were purchased from Himedia Pvt. Ltd., Mumbai, India. Potassium hydroxide was procured from Sigma-Aldrich (St. Louis, MO, USA). Methanol, molecular sieves 4 Å, and ethanol were purchased from S.D. Fine chemicals Pvt. Ltd., Mumbai, India. Fermase (commercial enzyme immobilized on polyacrylate beads of size 0.3–0.5 mm and having stability in the range of pH 3–10 and temperature 40–70°C) from source *Candida antarctica* lipase B was kindly given by Fermenta Biotech. Pvt. Ltd., India. All other reagents used were of analytical grade.

2.2 Experimental setup

The experimental setup consists of a mechanically agitated flat bottom glass reactor of 50 ml capacity that is equipped with four baffles and three-bladed turbine impeller. The mixture was agitated using an electric motor that is provided with a speed controller system monitored using a digital tachometer. The reactor was placed in a thermostatic water bath, which was maintained at a desired temperature with the help of a temperature control system manufactured by Dakshin Pvt. Ltd., Mumbai, India. A typical reaction consisted of a 4:1 molar ratio of PEG 600 to stearic acid, respectively, without adding any solvent, and the reaction volume was maintained at 15 cm^3. Initially, the reaction mixture was agitated at 200 rpm and at 70°C temperature for 5 min to dissolve the stearic acid in PEG. After complete homogenization of the reaction, 0.5% (w/w) catalyst was added by keeping the temperature and the speed of agitation constant to initiate the reaction. At the same time, 5% (w/w) molecular sieves 4 Å were also added in the reaction mixture to remove water formed at the time of esterification. After regular time interval, very small amount of sample was withdrawn from the reaction mixture without taking any catalyst particles. After completion of reaction, the mixture was filtered through a filter paper, and enzyme particles were separated using a sieve having size of 0.5 mm, further washed with acetone two to three times, and kept in a desiccator for determination of enzyme activity.

2.3 Determination of enzyme activity

The activity of the enzyme was measured by using the method described by Divakar et al. [15]. The mixture of 1.35 ml butyric acid (0.16 M) and 2.7 ml butanol (0.33 M) in 85.95 ml *n*-heptane was prepared and stored as a stock solution. Further, 3 ml of stock solution was incubated with appropriate amount of enzyme at 60°C for an hour at 150 rpm. Similarly, a blank was kept for incubation without addition of the enzyme particles. After completion of an hour, the sample was taken and mixed with 1 ml of ethanol and titrated with 0.01 M of alcoholic NaOH using phenolphthalein as an indicator.

Esterification activity was measured by using the following equation (1):

$$\text{Enzyme activity} = \frac{(V_0 - V) \times M \times 100}{A \times T} \tag{1}$$

where V_0 is the initial value of NaOH, V is the final value of NaOH. M is the molarity of NaOH, A is the weight of the enzyme, and T is the time.

One unit of esterification enzyme activity is defined as 1 μmol butyric acid consumed per minute per milligram of lipase. The activity of fresh lipase was 6.5 (U/g).

2.4 Purification of the product

After completion of reaction, the reaction mixture was collected and dissolved in ethyl acetate, and enzyme particles were separated by filtration. The reaction mixture contains product with unreacted PEG and stearic acid. Unreacted PEG was removed by applying repeated washing between saturated sodium chloride solution and distilled water. After careful washing, the solvent was removed by rotary evaporation, and the product was collected and analyzed through Fourier transform infrared (FT-IR) spectroscopy (Bruker VERTEX 80V Vacuum FT-IR spectrophotometer, USA) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy (MR400, Agilant) using tetramethylsilane (TMS) as internal standard and $CdCl_3$ as a solvent.

2.5 Analysis of the reaction

After every interval, the progress of the reaction was measured by consumption of acid, and analysis of the sample was carried out to find out the unreacted acid in the reaction mixture by titration method. The sample aliquots were diluted with 20 ml of ethanol and titrated against the 0.1 N alcoholic KOH solution using phenolphthalein as an indicator to find out the unreacted acid in terms of its acid value. The percentage conversion was calculated based on the unreacted acid in the reaction mixture to find out the rate of esterification by using the following equation (3) [16]:

$$\text{Acid value } (A) = \frac{56.1 \times M \times V}{W} \tag{2}$$

where V is the volume of KOH (ml), M is the molarity concentration of titrant (mol/l), and W is the weight of the sample (g).

$$\%\text{Conversion} = \frac{A_0 - A_1}{A_0} \times 100 \tag{3}$$

where A_0 is the initial acid value and A_1 is the acid value at a particular interval.

3 Results and discussion

Various reaction parameters such as time, temperature, enzyme loading, molar ratio of reactants, speed of agitation, and amount of molecular sieves were optimized. Further, the reusability of the immobilized enzyme was determined as per economic feasibility of the process. Each of the reaction parameter has been discussed in detail in the following sections. All the parameters were optimized while altering one parameter at a time and keeping the others constant.

3.1 Influence of molar substrate ratio

The concentration of substrates plays a vital role in the esterification reaction, as excess of one of the reactants drives the reaction in a forward direction while another one can inhibit the reaction causing the negative effect. Generally, a large amount of alcohol in esterification reaction helps the reaction to move it in a forward direction. However, higher concentration of alcohol can inhibit the reaction by inactivating enzyme active sites. Conversely, the high amount of substrates can cause a decrease in reaction rate by inhibition action [15]. Hence, it is mandatory to use a proper reactant concentration during the reaction. Here as per the reaction stoichiometry, molar ratio of acid to alcohol is 1:2. However, in order to obtain the optimum molar ratio, experiments were carried at different mole ratios of stearic acid to PEG 600 as 1:1, 1:2, 1:3, 1:4, and 2:1 while keeping the other parameters constant. The reaction was carried out at 70°C temperature with 0.5% (w/w) enzyme loading and speed of agitation at 200 rpm in a solvent-free system. The results obtained are shown in Figure 1, which showed that as the ratio of stearic acid to PEG 600 was varied from 1:1 to 1:4, the conversion increased from 58.83% to 84.35%, indicating that the alcohol in excess drives the reaction in a forward direction. On the contrary, when the ratio of stearic acid to PEG 600 was changed from 1:1 (58.83%) to 2:1 (53.01%), the percent conversion decreased with an increase in the concentration of stearic acid [16]. This could be attributed to the fact that more concentration of stearic acid may cause an increase in the viscosity of the reaction mixture, which lowers the access of substrate molecules to the active sites of the biocatalyst [17]. Additionally, an excess concentration of the acid can perturb the enzyme activity and denature it, and thus, the conversion is lowered [18]. With consideration of the above effect with different mole ratios of substrates, a 1:4 ratio of stearic acid to PEG 600 was considered as optimum and used for further experiments.

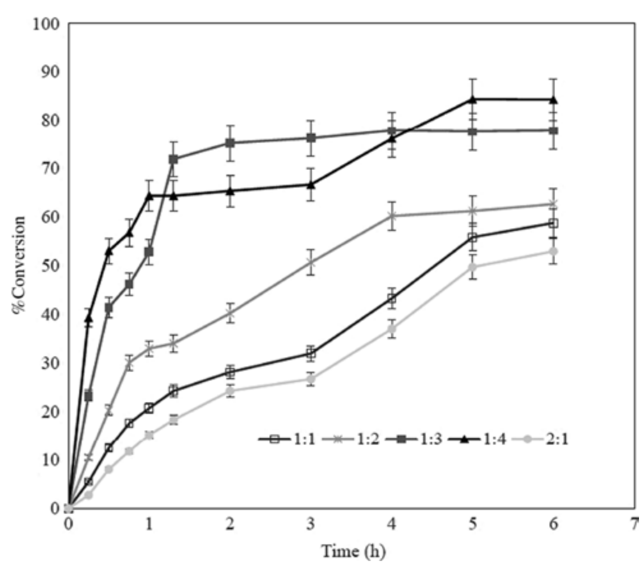

Figure 1: Influence of molar ratio of stearic acid to PEG 600 on reaction conversion at enzyme loading Fermase 0.5% (w/w), temperature 70°C, speed of agitation 200 rpm, and molecular sieves 5%.

3.2 Influence of temperature

Enzyme activity and its stability are important factors while considering the enzymatic reactions that are temperature dependent [13]. Above the optimum temperature, the lipases lose its tertiary structure, leading to the loss of its activity. From literature, it has been observed that the lipases are active in the range of 25°C–80°C in the most of its immobilized form depending upon their source of origin [19, 20]. Also, the enzyme used here has an operating range of temperature from 40°C to 70°C and pH 3–10. Therefore, the effect of temperature on the formation of PEG stearates was conducted at 60°C, 65°C, 70°C and 80°C (results not shown for 80) while keeping the other parameters constant, i.e. molar ratio acid to alcohol 1:4, speed of agitation 200 rpm, and 0.5% (w/w) catalyst loading. Figure 2 shows that as temperature increased from 60°C to 70°C, the conversion also increased from 57.10% to 84.35%. At lower temperature, the reaction mixture is more viscous, limiting the reactants to come in contact with the active sites of the enzymes. With an increase in temperature, the viscosity decreases, and thus, the solubility of reactants, mass diffusion, rate of reaction, and conversion increase. The maximum conversion of 84.35% was obtained at 70°C, but with further rise (80°C) in temperature, the reaction did not occur. This is attributed to the fact that at higher temperature, the enzyme leads to lose its activity denoting that it is not stable at higher temperature [17]. Thus, temperature of 70°C is determined as the optimum temperature for the synthesis of PEG-stearate.

3.3 Influence of the amount of biocatalyst

The amount of biocatalyst is the key factor in enzymatic reactions as it adds cost in the final production of the product. Thus, it becomes crucial to consider the amount of enzyme considering the economy of the reaction process. The reactions were carried out without any solvent for different amounts of enzyme ranging from 0.25% to 1% (w/w) by keeping other parameters constant, i.e. 70°C temperature, 1:4 acid to alcohol molar ratio, and speed of agitation at 200 rpm. Figure 3 illustrates that at lower concentration of catalyst, the conversion was lower, and it increased with concentration of enzyme. At enzyme loading of 0.5% (w/w), the conversion obtained was 84.36%; with further addition of enzyme, the conversion did not improve significantly; and at 1%, the conversion reduced to 70%. The initial lower amount of the catalyst is not sufficient for efficient interaction of substrates, and lower conversion is obtained at 0.25% enzyme loading. With gradual increase in concentration, the conversion also increased as more active sites were available and reaction rate was also enhanced further. With the presence of excess amount of enzyme at 1%, the active sites remained unexposed to the substrates because of agglomeration of immobilized enzyme in solvent-free system and lowered the conversion [21]. At 0.5% (w/w) enzyme loading, the conversion was at maximum because of efficient substrate and enzyme interaction. Besides, higher amount of enzyme hampers the mixing by increasing viscosity and causes negative effect on transport and diffusion of the substrates to the active sites of the excess enzyme

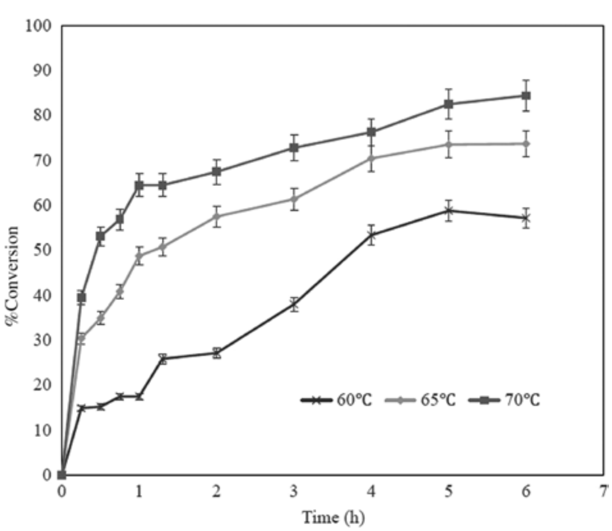

Figure 2: Influence of temperature on reaction conversion at enzyme loading Fermase 0.5% (w/w), stearic acid to PEG 600 ratio 1:4, speed of agitation 200 rpm, and molecular sieves 5%.

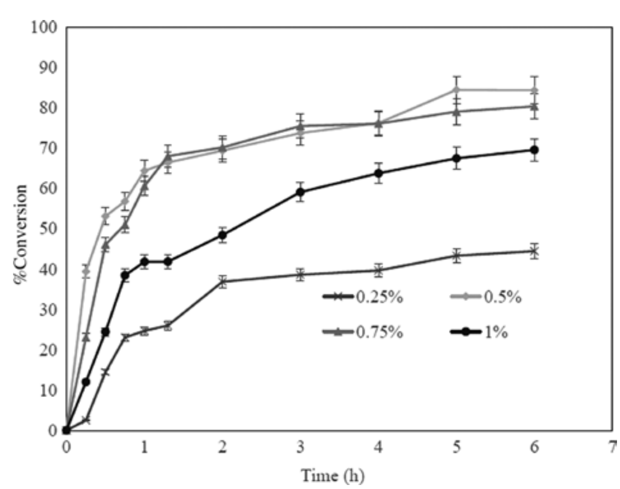

Figure 3: Influence of amount of catalyst Fermase on reaction conversion at stearic acid to PEG 600 ratio 1:4, temperature 70°C, speed of agitation 200 rpm, and molecular sieves 5%.

molecules, which reduces the overall mass transfer [22]. Based on the above results, 0.5% (w/w) of catalyst loading was set to be the optimum condition for the synthesis of PEG stearate.

3.4 Influence of the speed of agitation

While catalyzing the reaction with the help of immobilized enzymes, the participating reactants should travel through the reaction media to the active sites of the enzymes where the substrate is converted into the product. For effectual travel from the reaction mixture to the active sites, the substrate has to diffuse from the bulk environment of the reaction mixture to the external surface of the enzyme and also into the interior active sites where the actual reaction takes place. At the same time, the formed product needs to diffuse from the active sites to make it available for the next substrate. Such mass transfer limitations can be minimized by applying external agitation [23]. To check the agitation speed where the mass transfer resistance is negligible in the SFS, reactions were carried out by varying the speed of agitation from 200 to 400 rpm while keeping other parameters constant, i.e. mole ratio of acid to alcohol 1:4, catalyst loading to 0.5% (w/w), and temperature 70°C. From Figure 4, it shows that as the speed of agitation increases from 200 to 300 rpm, the conversion increases from 81.36% to 86.98%, respectively [24]. But as the speed of agitation is raised further from the 300 to 400 rpm, it decreases to 65.29%. An increase in %Conversion with the speed of agitation is due to the increase in the turbulence that reduces external mass transfer resistance. However, the decrease in %Conversion at higher agitation is due to the detachment of the enzyme from the support, causing negative effect on the activity that resulted in the lower conversion of the reactant [25]. Considering the above results, the optimum agitation speed was chosen as 300 rpm.

3.5 Influence of the addition of molecular sieve

Water is formed in esterification reactions, independent of the type of reaction whether it is enzyme catalyzed or not [23]. Reaction may proceed in reverse direction if water is not removed from the reaction, causing a decrease in both reaction rate and yield of the ester [26]. Application of molecular sieves can help to remove water formed in the esterification reaction [27]. Considering this, different amount of activated molecular sieves (4 Å) in the range of 0–6% (w/w) were added to study its effect on esterification while keeping other parameters constant, which are ratio of acid to alcohol 1:4, temperature 70°C, speed of agitation 300 rpm, and catalyst loading 0.5% in SFS.

From Figure 5, it is observed that as the amount of molecular sieves is increased from 0% to 5%, the conversion increases from 66.18% to 86.98%. Thus, it can be concluded that 5% of molecular sieves is sufficient to remove water formed in the reaction. When reaction was performed without molecular sieves, i.e. at 0%, only 66.18% conversion was obtained, indicating that water generated in the reaction causes negative effect and gives rise to lower conversion of substrate to product. As the

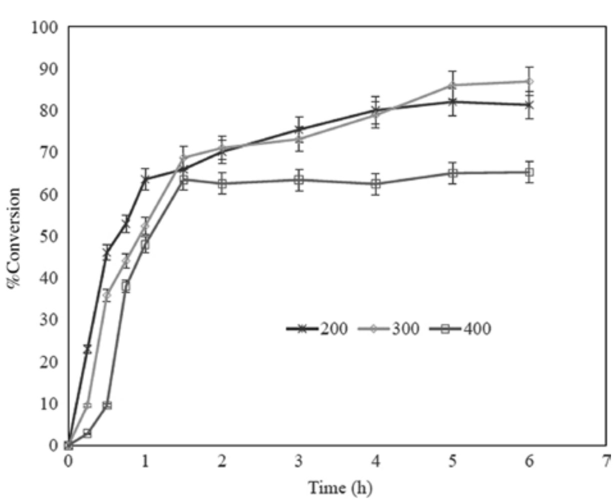

Figure 4: Influence of speed of agitation on reaction conversion at stearic acid to PEG 600 ratio 1:4: temperature 70°C, enzyme loading Fermase 0.5% (w/w), and molecular sieves 5%.

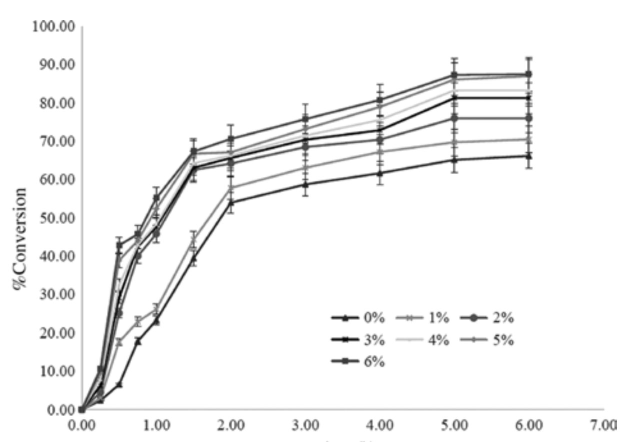

Figure 5: Effect of molecular sieves on reaction conversion at stearic acid to PEG 600 ratio 1:4, temperature 70°C, catalyst loading Fermase 0.5% (w/w), and speed of agitation 300 rpm.

amount of molecular sieves increases, the conversion also increases, showing water is gradually removed from the reaction that increases the conversion. Further increase in the amount of molecular sieves from 5% to 6% shows a marginal change in conversion. Hence, the optimum amount of molecular sieves chosen is 5% (w/w).

3.6 Reusability of enzyme

Enzyme reusability is a very important factor for economical process. For the reusability study, the reaction mixture was filtered through a filter paper to separate the enzyme and the molecular sieves from the reaction mixture and further passed through a sieve having a size of 0.5 mm for separating enzyme particles from the molecular sieves. Separated enzyme was then washed with the acetone two to three times and kept overnight for drying in a desiccator. Figure 6 depicts the effect of enzyme reusability on the conversion, which indicated that the conversion was reduced after each cycle and it was decreased from 86.98% to 65.39% after the fourth time of reuse. The repeated use of enzyme in acidic reaction conditions hampers the active sites of the enzymes that causes the enzyme to lose its activity, resulting in lower conversion [28].

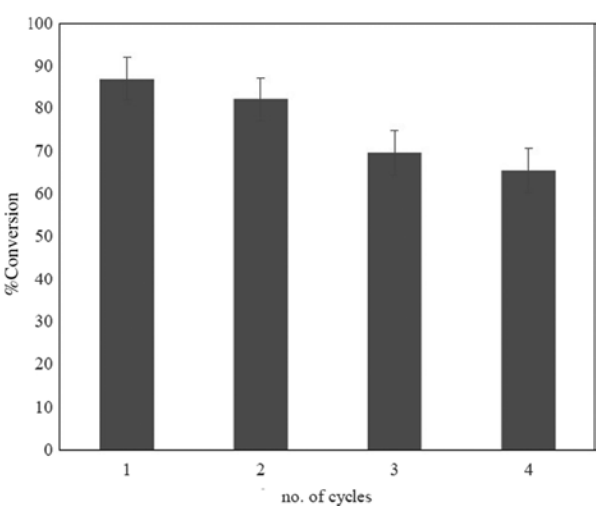

Figure 6: Reusability of biocatalyst.

3.7 Comparison between acid-catalyzed and enzymatic synthesis of polyethylene glycol stearate

Table 1 compares the various operating parameters for the synthesis of polyethylene glycol stearate between the current work (enzymatic synthesis) and the acid-catalyzed synthesis conducted by Abo-Shosha et al. [13] and Liwen and Honglu [14]. From the table, it is clear that in the case of the enzyme-catalyzed reaction, the maximum conversion was achieved with lower catalyst requirement, time, solvent, and temperature as compared with other acid-catalyzed processes. Thus, it can be concluded that hazardous acid catalysts such as H_2SO_4, phosphoric acid, and *p*-toluenesulfonic acid can be replaced by biocatalysts.

4 Characterization of polyethylene glycol stearate

4.1 FT-IR spectroscopy

The IR spectra of the parent acid, PEG, and the resulting product were obtained and shown in Figure 7A–C, respectively. The general results of the obtained spectra were similar with the variations concerning the intensity of bands. The spectra showed a band at 1250–1050 cm^{-1} in curve A, i.e. PEG 600, and at 1242 cm^{-1} in curve C, i.e. PEG stearate, representing the strong C–O–C bond. The peak at 1720 cm^{-1} in curve B is due to the strong C=O present in stearic acid, and similarly, the peak at 2870 cm^{-1} is due to the –OH stretching. The peak shown in curve C at 1734 cm^{-1} is due to the formation of a carbonyl bond, which is obtained because of the reaction between hydroxyl and

Table 1: Comparison between the enzyme-catalyzed and the conventional acid-catalyzed synthesis of polyethylene glycol stearate.

Parameters	Enzymatic synthesis [present work]	Abo-Shosha et al. [13]	Liwen and Honglu [14]
Catalyst (w/w)	Fermase (0.5%)	H_2SO_4 (4 g/kg)	Phosphoric acid and *p*-toluenesulfonic acid (2%)
Solvent	Solvent-free	Solvent-free	Xylene
Temperature (°C)	70	180	140–150
Conversion (%)	86.98	84	83
Reaction time (h)	6	8	4

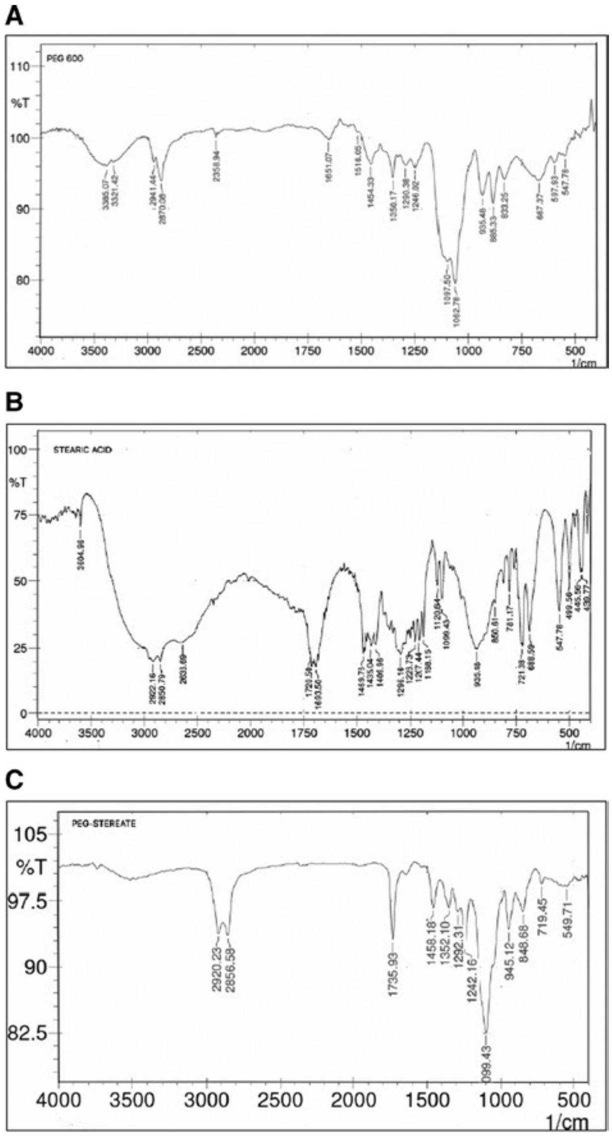

Figure 7: FT-IR spectra obtained before and after reaction between parent alcohol (A) PEG600 and acid (B) PEG600 and product (C) PEG stearate.

carboxyl groups of the reactant. This was not observed in the parent alcohol of PEG, i.e. curve A, but observed in the spectra of stearic acids (curve B) at a low value of 1697 cm^{-1}.

4.2 NMR analysis

The ^{1}H NMR of the product PEG stearate was done and reported in Figure 8. ^{1}H NMR (400 MHz, $CdCl_3$): 0.84 (t, $J = 6.6$ Hz, 6H), CH_3 protons of terminal stearic acid; 1.22 (m, 60H), $-[CH_2]_{15}-$ of linear stearic acid; 2.29 (t, $J = 7.5$ Hz, 4H), CH_2 adjacent to the linear $-[CH2]_{15}-$ group; 3 δ 3.76–3.52 (m, 21H), due to ethylene glycol chain. A summary of

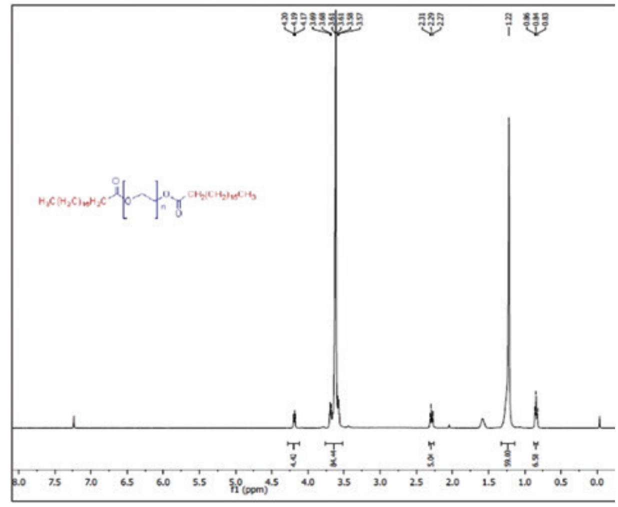

Figure 8: ^{1}H NMR of PEG stearate.

the results in ^{1}H NMR is described below i.e. Figure 8. The high intensity signal at 3.61 due to PEG CH_2OH protons and high-intensity signals at 1.22 due to $-[CH2]_{15}-$ of stearic acid confirmed that polyesterification of PEG and stearic acid occurred.

5 Conclusion

Polyethylene glycol stearate was successfully synthesized using Fermase $CALB_{ex}$ 10000, a commercial immobilized lipase B, by esterification reaction between PEG 600 and stearic acid in SFS. Various parameters were optimized to obtain maximum conversion. Maximum conversion of 86.98% was achieved at 4:1 molar ratio, temperature 70°C, biocatalyst loading 0.5%, time 6 h, speed of agitation 300 rpm, and molecular sieves 5% (w/w). Further, the product obtained was confirmed by using FT-IR and ^{1}H NMR spectroscopy. Reusability studies showed that the enzyme can be recycled up to four cycles, giving more than 60% of conversion. As solvent is not used during the reaction, it gives a greener approach for synthesis and eliminates drawbacks associated with their flammability and toxicity. Thus, the overall process becomes environmentally friendly and economical.

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References

[1] Kobayashi S, Uyama H. In *Biopolymers*, Doi, Y, Steinbuchel, A, Eds., Wiley/VCH: Weinheim, 2002, Vol. 3a, pp. 373–400.
[2] Gross RA, Ganesh M, Lu W. *Trends Biotechnol.* 2010, 28, 435–443.
[3] Varma IK, Albertsson AC, Rajkhowa R, Srivastava RK. *Prog. Polym. Sci.* 2005, 30, 949–981.
[4] Kamble MP, Yadav GD. *Ind. Eng. Chem. Res.* 2017, 56, 1750–1757.
[5] Kamble MP, Chaudhari SA, Singhal RS, Yadav GD. *Biochem. Eng. J.* 2017, 117, 121–128.
[6] Hasan F, Shah AA, Hameed A. *Enzyme Microb. Technol.* 2006, 39, 235–251.
[7] Houde A, Kademi A, Leblanc D. *Biotechnol. Appl. Biochem.* 2004, 118, 155–170.
[8] Yang Y, Yu Y, Wu D, Liu C, Zhao Z, Li Q. *Process Biochem.* 2012, 47, 1027–1036.
[9] Couturier L, Taupin D, Yvergnaux F. *J. Mol. Catal. B: Enzym.* 2009, 56, 29–33.
[10] Kobayashi S. *Macromol. Rapid Commun.* 2009, 30, 237–266.
[11] Chen J, Spear SK, Huddleston JG, Rogers RD. *Green Chem.* 2005, 7, 64–82.
[12] Sen S, Puskas J. *Molecules* 2015, 20, 9358–9379.
[13] Abo-Shosha MH, Nassar FA, El-Sayed Z, Hassabo AG. *Res. J. Text. Apparel* 2009, 13, 46–60.
[14] Liwen Z, Honglu X. *J. Dispers. Sci. Technol.* 2008, 29, 284–288.
[15] Kiran KR, Hari Krishna S, Suresh Babu CV, Karanth NG, Divakar S. *Biotechnol. Lett.* 2000, 22, 1511–1514.
[16] Zhong H, Fang Z, Zou B, Li X, Ouyang P, Guo K. *J. Mol. Catal. B: Enzym.* 2013, 90, 114–117.
[17] Tomke PD, Rathod VK. *Ultrason. Sonochem.* 2015, 27, 241–246.
[18] Bansode SR, Rathod VK. *Process Biochem.* 2014, 49, 1297–1303.
[19] Zhao Y, Liu J, Deng L, Wang F, Tan T. *J. Mol. Catal. B: Enzym.* 2011, 72, 157–162.
[20] Yadav GD, Trivedi AH. *Enzyme Microb. Technol.* 2003, 32, 783–789.
[21] Yadav GD, Devi KM. *Chem. Eng. Sci.* 2004, 59, 373–383.
[22] Gharat N, Rathod VK. *Ultrason. Sonochem.* 2013, 20, 900–905.
[23] Stergiou PY, Foukis A, Filippou M, Koukouritaki M, Parapouli M, Theodorou LG, Hatziloukas E, Afendra A, Pandey A, Papamichael EM. *Biotechnol. Adv.* 2013, 31, 1846–1859.
[24] Sun J, Yu B, Curran P, Liu SQ. *Food Chem.* 2012, 134, 89–94.
[25] Kuperkar VV, Lade VG, Prakash A, Rathod VK. *J. Mol. Catal. B: Enzym.* 2014, 99, 143–149.
[26] Duan Y, Zongliang D, Yao Y, Ruixia L, Dacheng W. *J. Agric. Food Chem.* 2006, 54, 6219–6225.
[27] Dange PN, Rathod K. *Resour. Technol.* 2017, 3, 64–70.
[28] Wang Y, Zhang DH, Chen N, Zhi GY. *Bioresour. Technol.* 2015, 198, 256–261.