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Electrospun nanofibrous membranes containing epoxy groups and hydrophilic polyethylene oxide chain for highly active and stable covalent immobilization of lipase

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Abstract: Immobilization of enzymes on nanofibrous membranes could offer easy recycling and feasible continuous operations. In this study, a novel terpolymer poly (glycidyl methacrylate-co-methylacrylate)-g-polyethylene oxide (P(GMA-co-MA)-g-PEO) containing reactive epoxy groups and hydrophilic polyethylene oxide branch chain was synthesized. Electrospun P(GMA-co-MA)-g-PEO nanofibrous membrane was used for the immobilization of lipase molecules by covalent binding with the epoxy groups. The influences of the enzyme loading and activity by nanofibrous membranes with different content of monomers and immobilization temperature were investigated. The immobilized lipase achieved high enzyme loading of 150 mg/g and the maximum activity of 0.673 U/mg under the optimum immobilization conditions. The hydrophilic PEO branch chain was beneficial for the stabilization of the enzyme conformation which would promote the improvement of enzyme activity and stability. The results of FTIR spectra and SEM images of nanofibrous membranes before and

after immobilization demonstrated that lipase has been successfully covalently immobilized on the nanofibrous membranes. The optimal pH and temperature were 7.0 and 35 °C for catalysis reaction of the immobilized lipase. The stabilities of the immobilized lipase were also investigated. The results demonstrated that the immobilized lipase has good thermal stability, reusability and organic solvent stability. The good stabilities of immobilized lipase revealed that P(GMA-co-MA)-g-PEO nanofibrous membrane is an excellent carrier for enzyme immobilization.

Keywords: Nanofibrous membranes; Enzyme immobilization; Electrospinning; Covalent binding; Lipase

1. Introduction

Enzymes are green catalysts which are widely used in many fields due to their high degree of specificity and high level of catalytic efficiency. Lipase as one kind of the high efficient enzymes are widely used in textile, medical, papermaking and organic synthesis. However, there exist various practical problems which restrict their applications, for example, instability and nonreusability [1-3]. Immobilizing enzyme onto insoluble or solid supports is an effective way to overcome these problems [4-7]. Additionally, immobilization could also improve many other properties of enzymes such as the durability of organic solvents, pH tolerance and thermal stability[8,9].

In recent years, there is a trend to use nanostructured materials as supports for enzyme immobilization due to the large surface area to volume ratio of nanosize materials could effectively improve the enzyme loading and catalytic efficiency of the immobilized enzymes. Both nanoparticles and nanofibers were explored for this purpose [10-14]. But it is difficult for nanoparticles dispersion in reaction solution and the subsequent recovery for reuse. On the contrary, nanofibers can be easily

recovered from reaction media and be applied for continuous operations [15]. Immobilization of enzymes on nanofibers can be carried out by different methods which are mainly classified as physical and chemical methods. The enzyme stability is one of the keys to implement a successful bioprocess. Physical methods have weak interactions between matrix and enzymes which are easily affected by external factors. Whereas chemical methods are more stable than physical methods for the formation of covalent bond between the supports and enzymes [16]. Covalent immobilization of enzymes to supports occurs due to their side chain amino acids and degree of reactivity based on different functional groups [17]. Sometimes functional groups on the support material are activated by certain reagents, and then enzyme is coupled to the support material via covalent linkage.

Both natural and synthetic polymers have been electrospun into nanofibrous membranes (NFMs) for covalent immobilization of lipase, chymotrypsin, cellulose and lysozyme [18–27]. Electrospun NFMs from natural polymers are suitable candidates for enzyme immobilization due to the functional groups are in the polymer backbone for covalent bindings of enzyme molecules. However, proteins or polysaccharides need to be electrospun in aqueous solution which is more difficult than using organic solvents. Besides, NFMs from natural polymers are generally less chemical and mechanical stable than those from synthetic polymers. There are a lot of researches about synthetic polymers NFMs for enzymes covalent immobilization[28]. Li et al [29] reported that PAN NFMs were used directly for lipase immobilization by the nitrile groups through amidination reaction with lipase.

Ye et al [30-31] synthesized poly(acrylonitrile-co-maleic acid) (PANCMA) containing reactive carboxyl groups and fabricated it into NFMs for lipase immobilization. Wong et al [32] reported that chymotrypsin was covalently immobilized onto amine functionalized nylon 6,6 nanofibers via glutaraldehyde. Epoxy supports/carriers for enzyme immobilization have been studied extensively [33]. NFMs containing reactive epoxy groups also have been used for enzyme immobilization. Wang et al [34] reported the epoxy group containing nanofibers of poly(ethylene-co-glycidyl methacrylate) (PE-co-GMA) were used for the immobilization of streptavidin–horseradish peroxidase. However, enzyme activity would be greatly influenced by the epoxy carriers due to their hydrophobic and rigid surface. It maybe resolved by using a hydrophilic reagents to improve its hydrophilicity [35-36].

Base on this, a novel terpolymer poly (glycidyl methacrylate-co-methylacrylate)-g-polyethylene oxide (P(GMA-co-MA)-g-PEO) containing reactive epoxy groups and hydrophilic polyethylene oxide branch chain was synthesized which was fabricated into NFMs by electrospinning in this study. The hydrophilic polyethylene oxide branch chain on these NFMs would weaken the hydrophobicity and rigidty of the surface and alleviate the collision of the enzyme with the carriers which are beneficial for the improvement of the enzyme activity. The Lipase was immobilized on these NFMs by covalent bonding with the epoxy group on them. The influence on the enzyme loading and activity of the immobilized lipase by the different content of monomers and temperature were investigated. The chemical structure and morphology of the immobilized lipase were characterized by FTIR and SEM. The kinetic parameters of the free and immobilized lipase were measured by

using the Lineweaver-Burke method. Influence of the catalysis reaction of immobilized lipase (pH and temperature), thermal stability, reusability and organic solvent stability of the immobilized lipase were systematically investigated.

2. Experimental

2.1 Materials

Polyethylene glycol methacrylate (PEGMEMA, M_n=950), glycidyl methacrylate (GMA), methyl acrylate (MA), monopotassium phosphate (KH_2PO_4), monosodium phosphate(Na_2HPO_4), coomassie brilliant blue, phosphoric acid, 95% ethanol, olive oil and parahydroxyphenol were purchased from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China). Dimethylformaide (DMF) was purchased from Wuxi City Yasheng Chemical Co.,Ltd (Jiangsu, China). Azo diisobutyronitrile (AIBN) was purchased from Shanghai No.4 Reagent & H.V. Chemical Co.,Ltd (Shanghai, China). Candida antarctica lipase B (CALB) (1U/mg) was of biological grade and purchased from Hangzhou Novocata Biotechnology Co.,Ltd (Zhejiang, China).

2.2 Synthesis of P(GMA-co-MA)-g-PEO terpolymer and preparation of nanofibrous membranes by electrospinning

PEGMEMA 2.536 g and DMF 48 mL were fed into three-necked round bottom flask. After PEGMEMA was absolutely dissolved, GMA 2.536 g, MA 2.536 g and AIBN 0.303 g were added under N_2 atmosphere. Then the round bottom flask was put into 65 °C water bath oscillator. After a period reaction time, parahydroxyphenol was added into the mixture to terminate the reaction. The solvent was evaporated to remove, then the reaction product were dried under vacuum till the mass constant. The crude product was washed alternately by distilled water and anhydrous ethanol for three times, then the product was dried under vacuum till the mass constant to obtain the purified P(GMA-co-MA)-g-PEO terpolymer.

The purified P(GMA-co-MA)-g-PEO terpolymer was dissolved in DMF to prepare spinning solution which was thoroughly stirred for 24 h by magnetic stirring until it turning uniform and transparent. The prepared spinning solution was placed into a 5 mL syringe with a inner diameter of 0.7mm needle. The applied voltage, the flow rate of the polymer solution and the distance between needle tip and collector were controlled. The electrospinning was under the temperature of 20 °C and the humidity of 25%. After a period of time, the NFM was peeled from the aluminum foil, then dried in the vacuum drying oven.

2.3. Immobilization of lipase

A certain amount of prepared NFMs was washed alternately by methanol and distilled water for three times before adding into 5 mL centrifuge tube, and CALB solution with certain pH and concentration was added. Then the centrifuge tube was put into 30 °C water bath oscillator for immobilization. The centrifuge tube was token out after a period of reaction, and then the immobilized lipase was obtained by filtering the enzyme solution. The immobilized lipase was washed by phosphate buffer solution (0.05 mol/L, pH=7.0) until the protein couldn't be detected in the washing solution. The filtered enzyme solution and washing solution were collected for the determine of enzyme loading. The immobilized enzyme was kept at 4 °C. The detailed procedure for making electrospun P(GMA-co-MA)-g-PEO NFMs immobilized with lipase is schematically depicted in Fig. 1.

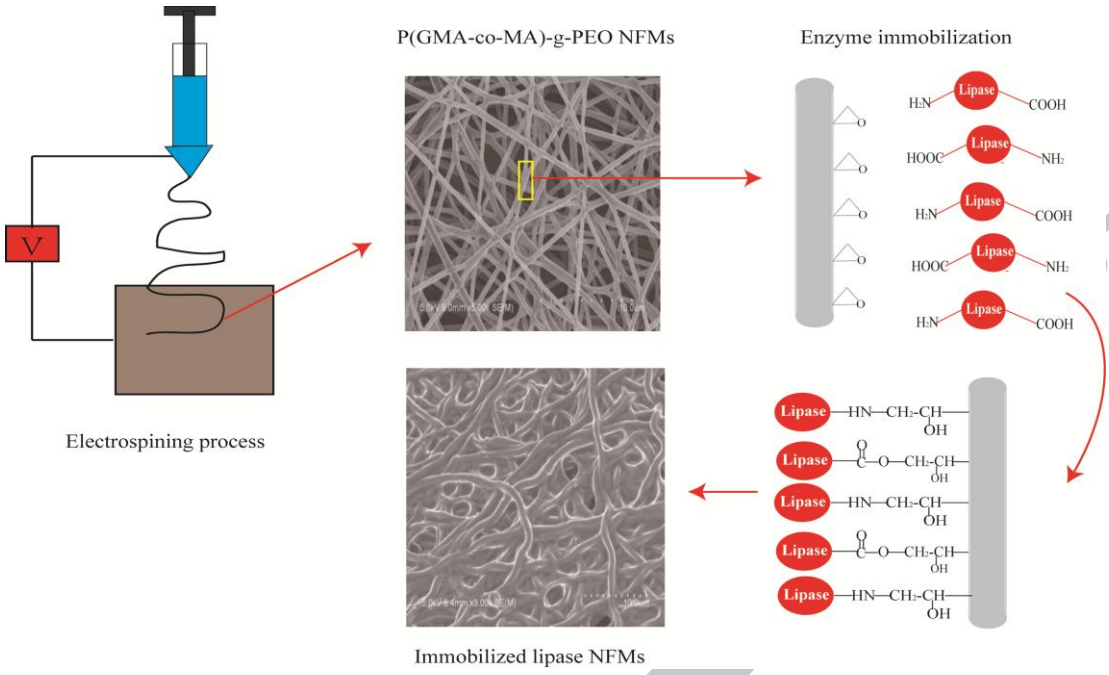

Fig. 1. Schematic illustration showing the detailed procedure for the preparation of electrospun P(GMA-co-MA)-g-PEO NFMs immobilized with lipase

2.4. Determination of enzyme loading and activity

The enzyme loading of immobilized lipase on the NFMs was estimated using the Bradford method [37]. The enzyme protein solution 0.3 mL and Bradford working solution 3.0 mL were added into the centrifuge tube and mixed thoroughly, then put it into the 25 °C water bath oscillator for chromogenic reaction 10 min. At the same time, phosphate buffer solution (0.05 mol/L, pH=7.0) 0.3 mL and Bradford working solution 3.0 mL were mixed as the blank sample. The absorbance of the enzyme solution before and after immobilization was determined by the ultraviolet spectrophotometer. The enzyme loading was calculated by the Eq. (1), the mean value of it was obtained by three times.

$$A_e(\%) = \frac{(C_0 - C) \times V - C_W \times V_W}{W} \times 100 \qquad (1)$$

Where A_e is the enzyme loading (mg/g), C_0 is the concentration of enzyme before immobilization (mg/mL); C is the concentration of enzyme after immobilization

(mg/mL); V is the volume of enzyme; C_W is the enzyme concentration of phosphate buffer washing solution after immobilization (mg/mL); V_W is the volume of phosphate buffer washing solution after immobilization (mL); W is the weight of immobilized NFM.

The catalysis activity of lipase was measured by using the method of hydrolyzing olive oil [38]. Olive oil 1 mL was added into 3 mL of phosphate buffer solution (0.05 mol/L, pH=7) which was put it into the 37 °C water bath for 5min, and then a certain amount of immobilized or free lipase was added into the solution for reaction 10min. The reaction was stopped by addition of toluene 8 mL. The amount of generated fatty acid was measured the absorbance at 710 nm with cupric acetate as a color indicator. An activity unit(U) of lipase was defined as the amount of lipase that catalyzes olive oil to generate 1umol fatty acid per minute under the above assay conditions. Therefore, the activity of lipase was calculated according to the following Eq.(2) and (3).

The activity of immobilized lipase was calculated as Eq. (2).

$$a = \frac{C_1 V_1}{tM} \qquad (2)$$

Where a is the activity of immobilized lipase (U/mg), C_1 is the concentration of fatty acid (mmol/L), V_1 is the volume of fatty acid about 8.8 mL, M is the weight of immobilized lipase, t is the catalysis time of 10 min.

The activity of free lipase was calculated as Eq. (3).

$$a = \frac{1000 C_1 V_1}{t C_2 V_2} \qquad (3)$$

Where a is the activity of free lipase (U/mg), C_1 is the concentration of fatty acid

(mmol/L), V_1 is the volume of fatty acid about 8.8 mL, C_2 is the concentration of free lipase solution, V_2 is the volume of free lipase solution, t is the catalysis time of 10 min.

2.5. Characterization of P(GMA-co-MA)-g-PEO and the immobilized NFMs

The chemical structure of P(GMA-co-MA)-g-PEO terpolymer was characterized by H-proton nuclear magnetic resonance (^{1}H-NMR) and Fourier transform infrared spectroscopy (FTIR). ^{1}H-NMR was conducted on Bruker Avance spectrometer (400 MHz) at room temperature. FTIR spectra was recorded on a IR Prestige-21 FTIR spectrometer (Shimadzu, JAPAN). The chemical structure of NFMs before and after immobilization was characterized by FTIR. The morphology of NFMs before and after immobilization was viewed by a Hitachi S-4800 scanning electron microscopy (SEM) after being sputtered with gold. Static water contact angles were determined by contact angle apparatus (DSA-25, Kruss, Germany).

2.6. Determination of the kinetic parameters

The kinetic parameters of free and immobilized lipase were measured by using the Lineweaver-Burke method [39]. The different concentration of substrates (olive oil solution) were prepared (0.0526, 0.0789, 0.1052, 0.1315, 0.1578, 0.1841 and 0.2104 g/mL), the initial rate of the lipase catalysis the hydrolysis of olive oil was determined. The reciprocal of initial rate as Y-axis and the reciprocal of the concentration of substrates as the X-axis to draw curve which was fitted to obtain a linear equation. The kinetic parameters K_m and V_{max} could be obtain from Eq. (4).

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (4)$$

Where V_0 is the initial rate of lipase catalysis reaction ($mmol \cdot L^{-1} \cdot min^{-1}$), [S] is the mass concentration of substrate ($g \cdot mL^{-1}$).

2.7. Effect of pH and temperature on lipase catalysis reaction

The influences of pH and temperature on the free and immobilized lipase was investigated by determining the free and immobilized lipase activities of catalysis the hydrolysis of olive oil at different pH (4~10) and temperature (15~55 °C). The enzyme loading of the P(GMA-co-MA)-g-PEO NFMs immobilized lipase was 150 mg/g. The relative activity was calculated by Eq. (5).

$$R_r = \frac{v}{v_{max}} \times 100\% \qquad (5)$$

Where R_r is the relative activity, v is the lipase activity under different pH or temperature (U), v_{max} is the highest enzyme activity (U).

2.8. Stability of immobilized lipase

The thermal stability, reusability and organic solvent stability of the immobilized lipase were investigated. The thermal stability was determined by the residual activity of the free and immobilized lipase which were kept at different temperature for 3 h. The residual activity was calculated by Eq. (6). The immobilized lipase NFM was separated from the substrate solution after the catalysis reaction of olive oil which was washed by phosphate buffer solution. Then the reuse activity was determined by putting it into the new olive oil solution for catalysis reaction. The residual activity was also calculated by Eq. (6). The organic solvent stability of the immobilized lipase was determined by the residual activity of the immobilized lipase which were immersed in methanol at 35 °C per hour. The residual activity was calculated by Eq. (6).

$$R_a = \frac{A_t}{A_0} \times 100\% \qquad (6)$$

Where R_a is the residual activity (%), A_t is the activity of lipase after different

treatment (U), A_0 is the initial activity (U).

3. Results and discussion

3.1 Characterization of P(GMA-co-MA)-g-PEO

3.1.1 ^{1}H-NMR spectra of P(GMA-co-MA)-g-PEO

The chemical structure of P(GMA-co-MA)-g-PEO was as shown in Fig. 2.

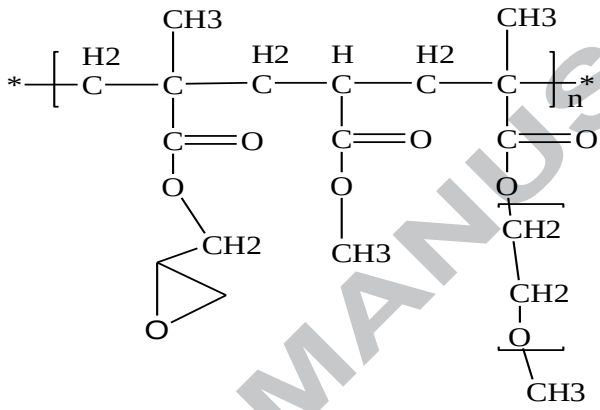

Fig. 2. Chemical structure of P(GMA-co-MA)-g-PEO

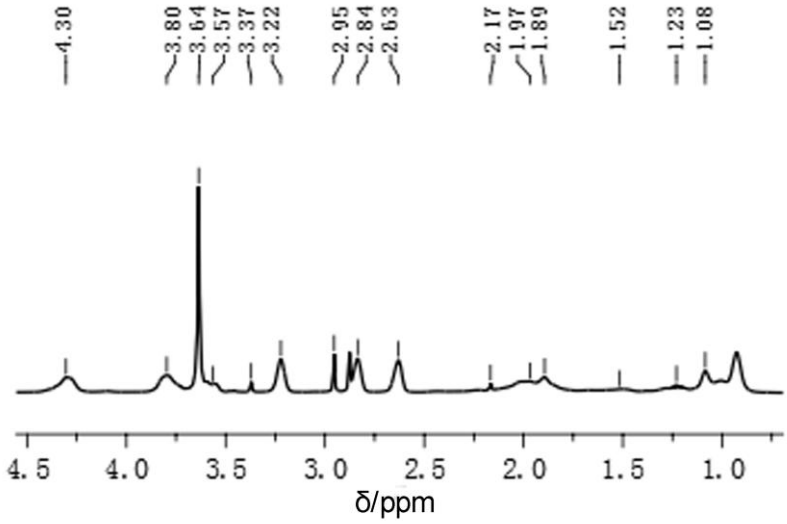

Fig. 3. ^{1}H-NMR spectra of P(GMA-co-MA)-g-PEO

The ^{1}H-NMR spectra of P(GMA-co-MA)-g-PEO is shown in Fig. 3. The characteristic peaks at 1.08 and 1.23 belong to the -CH_3 protons of PEGMEM and GMA, respectively. The peaks at 1.52~1.97 ppm are assigned to -CH_2 protons and at

2.17 ppm is assigned to -CH protons in main chain of this terpolymer. The characteristic peaks at 2.63 and 2.95 represent the -CH and -CH_2 protons of the epoxy group, respectively. The peak at 3.22 is assigned to -OCH_3 protons of PEGMEMA, while those at 3.37~3.64 arise from the -CH_2CH_2O protons. The peak at 3.80 arises from the -OCH_3 protons of MA, and the peak at 4.30 is attributed to the -CH_2O protons.

3.1.2 FTIR spectra

The FTIR spectra of PEGMEMA, GMA and P(GMA-co-MA)-g-PEO are shown in Fig. 4. As can be seen from Fig. 4, the absorption peak at 1633 cm^{-1} which belongs to the stretching vibration of C=C bond of GMA and PEGMEMA was disappeared in the P(GMA-co-MA)-g-PEO. This demonstrate that all the monomers have been completely reacted. In the spectrum of P(GMA-co-MA)-g-PEO, the peak at 908cm^{-1} is assigned to the skeletal vibration of the epoxy group, the peak at 1151 cm^{-1} is attributed to the stretching vibration of (C-O-C) of PEGMEMA, at 1722 cm^{-1} is assigned the stretching vibration of ester C=O, the peaks at 2996cm^{-1} and 2946cm^{-1} are attributed the stretching vibration of -CH_2 and -CH_3.

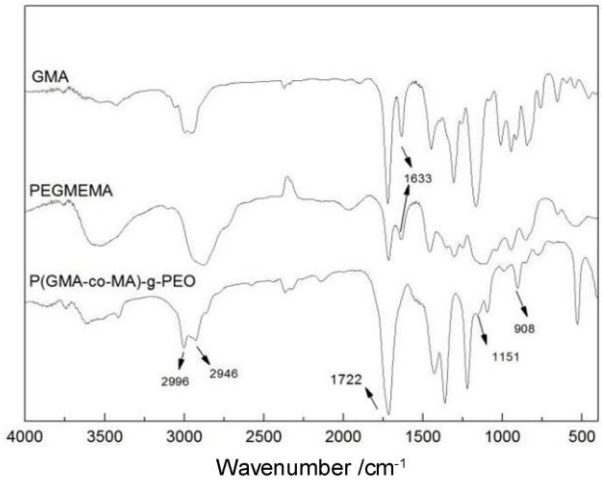

Fig. 4. FTIR spectra of P(GMA-co-MA)-g-PEO

The results of ^{1}H-NMR and FTIR indicated that the synthesized product was P(GMA-co-MA)-g-PEO.

3.2 Influence of enzyme loading and activity

3.2.1 Different content of monomers of the NFM

The influence of enzyme loading and activity of the immobilized lipase by the different content of monomers was determined. The immobilized process was as follow: the pH of the immobilized solution was 7, the concentration of CALB lipase was 12 mg/mL, the immobilized temperature was 30 °C and the time was 5 h. The results were shown in Fig. 5.

As can be seen from Fig. 5, the enzyme loading was low when the content of GMA was low which means the low content of epoxy group in the NFM. The enzyme loading increased with the content of GMA which demonstrated that the immobilization of lipase mainly by covalent binding with the reactive epoxy group on the NFM. As Fig. 5 shows, the specific activity of free enzyme was 1 U/mg, while the specific activity of the immobilized enzyme decreased. The specific activity of immobilized NFM_d and NFM_e were 0.67 U/mg and 0.11 U/mg. Therefore, the enzyme loading of NFM_e was a little higher than NFM_d, while the activity of immobilized NFM_e was much lower than that of NFM_d. This shows that the higher activity of the immobilized lipase would obtain due to the hydrophilic PEO branch chain on the NFMs which would stabilize the conformation of lipase. The results demonstrate that the optimal mass ratio of PEGMEMA, MA and GMA was 12.5%, 12.5% and 75%, the enzyme loading and activity of lipase immobilized on NFM_d were 149 mg/g and 0.67 U/mg.

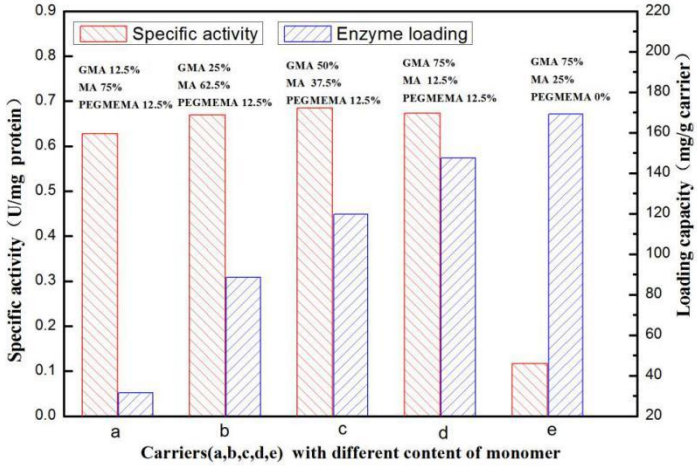

Fig. 5. Effect of the monomers content on enzyme loading and specific activity

Static water contact angles was used to characterize the hydrophilicity of the NFMs. The static water contact angles of the NFM_d (containing 12.5% PEO) and NFM_e were as shown in Fig. 6. The static water contact angle of NFM_e and NFM_d were 126.76° and 81.25°, respectively. This means hydrophilicity of the NFM would improved by introducing the hydrophilic PEO branch chain. Therefore, the prepared P(GMA-co-MA)-g-PEO possessed a certain hydrophilicity which was beneficial for the stabilization of the enzyme conformation. Thus, it would promote the improvement of enzyme activity and stability.

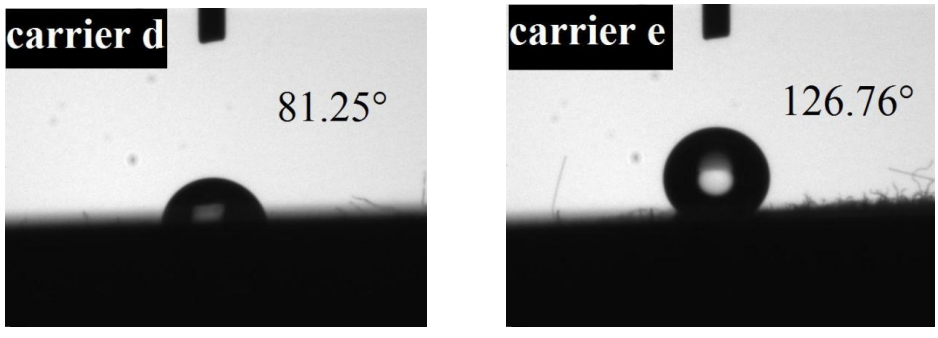

Fig. 6. Static contact angles of NFM_d and NFM_e

3.2.2 Immobilization temperature

NFM_d was chosen as the carrier to investigate the influence of enzyme loading and activity of the immobilized lipase by different temperature from 15 to 50 °C .

As shows in Fig. 7, the enzyme loading increased greatly when the temperature increased from 15 °C to 35 °C, the reason was that the higher temperature was beneficial for the covalent binding between epoxy groups of NFM and amino groups of lipase. When the temperature increased continually, the increasing of enzyme loading wasn't obviously, while the activity of lipase was decreased greatly. The reason about this maybe due to the easier inactivation of lipase at higher temperature. Therefore, the optimal temperature for lipase immobilization was 35 °C. The immobilized lipase achieved high enzyme loading of 150 mg/g and the maximum activity of 0.673 U/mg under the optimum immobilization conditions.

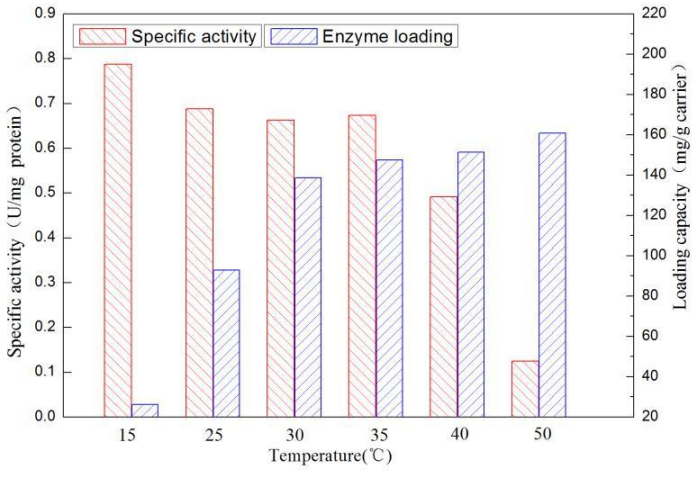

Fig. 7. Effect of immobilized temperature on enzyme loading and activity

3.3 Chemical structure of the immobilized NFM

The chemical structure of NFMs P(GMA-co-MA)-g-PEO before and after immobilization was characterized by FTIR as shown in Fig. 8. As can be seen from Fig. 8, both of the NFMs before and after immobilization exist the absorption peaks at $908cm^{-1}$ and $846cm^{-1}$ which are assigned to the stretching vibration of epoxy group,

the peaks at 1722cm^{-1} and 1161cm^{-1} are attributed to ester carbonyl and ether bond, respectively. The absorption peaks at 2996cm^{-1} and 2946cm^{-1} are assigned to -CH_2 and -CH_3. These demonstrated that all the characteristic absorption peaks of the NFMs exist after immobilization. The wide and strong absorption peak at 3660~3272cm^{-1} is assigned to the stretching vibration of -OH which means lipase was on the NFMs after immobilization. The intensities of the characteristic absorption peaks at 908cm^{-1} and 846cm^{-1} which are assigned to the epoxy group were decreased. It also demonstrated that the epoxy group in the NFM was covalent binding with lipase.

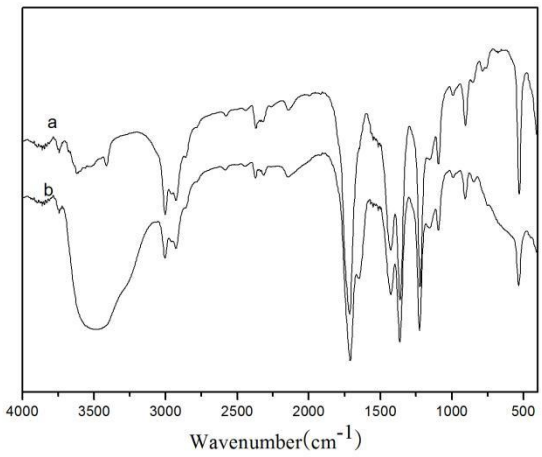

Fig. 8. FTIR of NFM before（a）and after（b）lipase immobilization

3.4 Morphology and hydrophilicity of NFMs

The morphology of NFMs before and after lipase immobilization was viewed by SEM which was shown in Fig. 9. It shows that the immobilized lipase of the NFM swelled slightly comparing with before immobilization, but it also maintained the nanoscale structure.

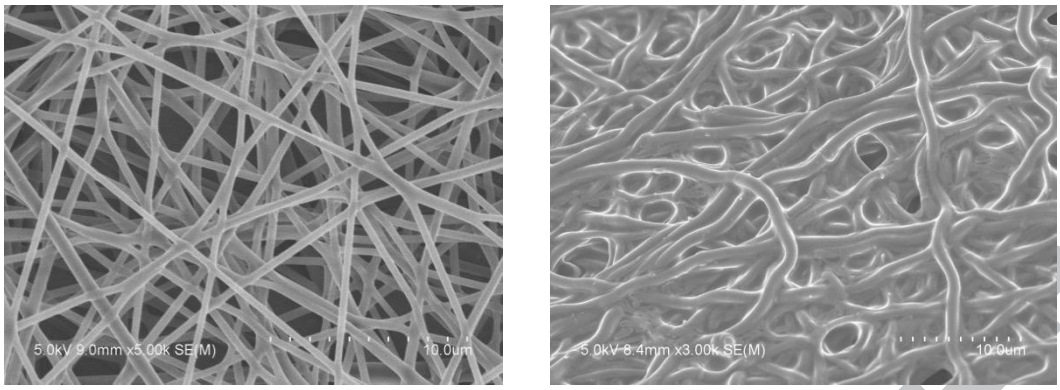

Fig. 9. SEM of NFM before（left）and after（right）lipase immobilization

3.5 Kinetic parameters

Lineweaver-Burke method was used to study the kinetic parameters of free and immobilized lipase by investigating the relationship between the initial rate of lipase catalysis reaction (V_0) and the different concentration of substrates ([S]) from 0.0526, to 0.2104 g/mL. The reciprocal of initial rate ($1/V_0$) as Y-axis (ordinate) and the reciprocal of the concentration of substrates (1/[S]) as the X-axis (abscissa) were as shown in Fig. 10. The kinetic parameters K_m and V_{max} from the double reciprocal plot are listed in Table1. K_m reflects the affinity between enzyme and substrate, the lower of K_m value means the higher of the affinity, otherwise the lower. V_{max} reflects the rate of the enzyme catalysis reaction [40]. As can be seen from Table 1, the V_{max} of the immobilized lipase was lower than that of free lipase which demonstrated that the catalysis reaction rate was decreased after the immobilization. The reason about this maybe due to the conformational changes and the steric hindrance effect after the immobilization. From Table 1, the K_m of immobilized lipase was higher than that of free lipase which demonstrated the lower affinity between immobilized lipase and the substrate. The reason about this maybe due to the steric hindrance after the immobilization reduced the contact area.

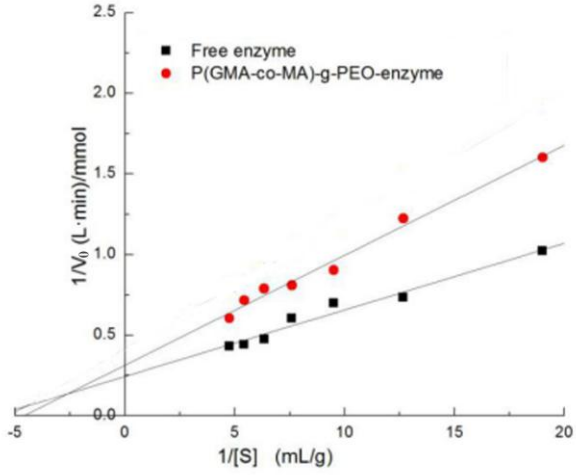

Fig. 10. Lineweaver-Burke plots of free and immobilized lipase

Table 1 Kinetic parameters from hydrolysis of olive oil

Enzyme	K_m/（g·mL^{-1}）	V_{max}/(mmol·L^{-1}·min^{-1})
Free enzyme	0.169	4.096
P(GMA-co-MA)-g-PEO-enzyme	0.218	3.193

3.6 Effect of pH and temperature on lipase catalysis reaction

3.6.1 Effect of pH

pH has a great influence on the catalytic performance of enzymes due to the pH change would make the change of enzyme spatial structure. The activities of the lipase in the pH range from 4 to10 were determined to obtain the optimal pH for lipase catalysis reaction. As can be seen from Fig. 11, the activities of the immobilized lipase were higher than the free lipase in the pH range from 4 to 10. The results indicate that the immobilized lipase has the better pH tolerance than the free lipase. The optimal pH of immobilized lipase was about 7.0, and the free lipase was about 8.0.

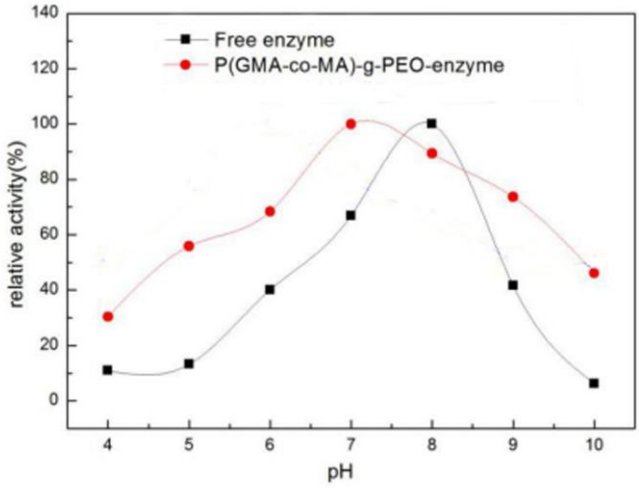

Fig. 11. The relationship between the relative activity of enzyme and pH value

3.6.2 Effect of temperature

Fig. 12 shows the different catalysis activities of the free and immobilized lipase at different temperature from 15 to 55 °C. The temperature of the maximal relative activity for free lipase was 30 °C, while the optimal temperature for immobilized lipase was 35 °C. In addition, the relative activity of the immobilized lipase was higher than the free lipase in this temperature range. The result indicates that the immobilized lipase has better temperature stability than the free one in some extent owing to the multipoint connection between lipase and NFMs, which stabilizes the conformation of lipase and prevents the extensional deformation of the folding peptide chain at high temperature [41].

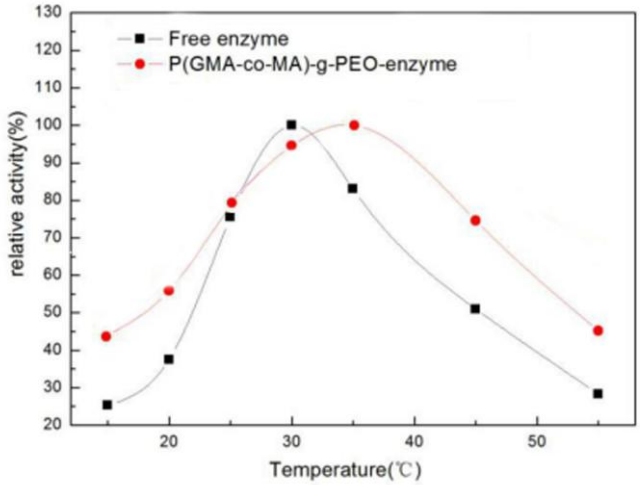

Fig. 12. The relationship between the relative activity of enzyme and temperature

3.7 Stability of immobilized lipase

3.7.1 Thermal stability

The thermal stability of free and immobilized lipase are given in Fig. 13. It can be seen that the thermal stability of immobilized lipase decreased with the increasing of temperature. The retained relative activity of the immobilized lipase was greatly different from free lipase. The retained relative activity was 43% for free lipase and 81% for the immobilized lipase at 40 °C. When the temperature was 70 °C, the activity of free lipase was lost seriously which was only about 15%, while the relative activity of P(GMA-co-MA)-g-PEO-enzyme was much higher about 40%. Therefore, the immobilization of lipase could prevent the conformation transition at high temperature which could reduce the activity loss of the enzyme[42].

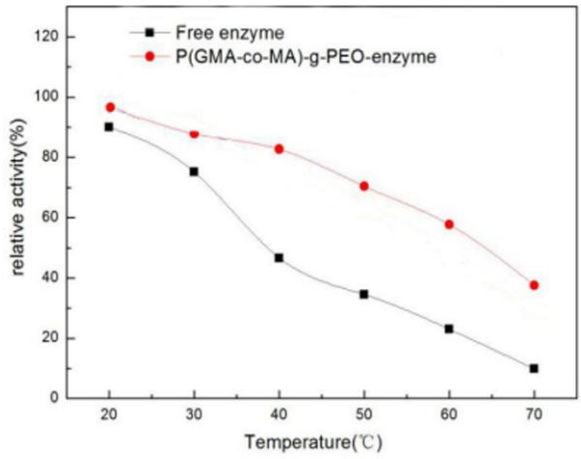

Fig.13. Thermal stability of free and immobilized lipase

3.7.2 Reuse stability of immobilized lipase

Comparing with the free enzyme, one of the most important advantages of the immobilized enzyme is reuse stability. Fig. 14 shows the effect of repeated use on the residual relative activity of immobilized lipase. The residual activity of P(GMA-co-MA)-g-PEO-enzyme is 45% after 5 reuses which demonstrated good reuse stability.

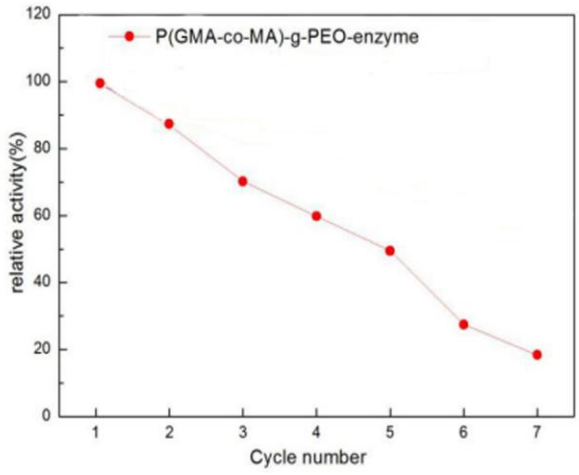

Fig. 14. Ruseability of immobilized lipase

3.7.3 Stability of organic solvent

The organic solvent stability of immobilized lipase was as shown in Fig. 15. To evaluated organic solvent stability, the immobilized lipase was treated in methanol at 35 °C. The residual relative activity of immobilized lipase was nearly 70% after being treated in methanol for 12 h which reveals good organic solvent stability.

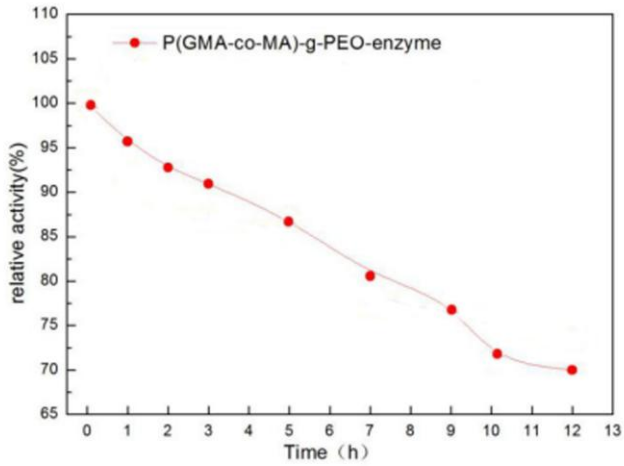

Fig. 15. Stability of organic solvent of immobilized lipase

4. Conclusions

The novel P(GMA-co-MA)-g-PEO terpolymer containing epoxy groups and hydrophilic PEO branch chain was synthesized which was electrospun into nanofibrous membranes for lipase immobilization. The influence on the enzyme loading and activity of the immobilized lipase by different content of monomers and temperature have been investigated. The immobilized lipase achieved the higher enzyme loading and activity when the content of the three monomers was 75% for GMA, 12.5% for MA and 12.5% for PEGMEMA, the immobilized temperature was 35 °C. The enzyme loading of the immobilized lipase was 150 mg/g under the optimal conditions. The hydrophilic PEO branch chain was beneficial for the stabilization of enzyme conformation which would promote the improvement of

enzyme activity and stability. The results of FTIR and SEM demonstrated that lipase was successfully covalent binding on the NFMs. The K_m values was increased while V_{max} decreased after immobilization which arisen from the steric hindrance effect of the immobilized lipase. The optimal pH and temperature were 7.0 and 35 °C for catalysis reaction of immobilized lipase. The thermal stability of the lipase was improved after the immobilization. The residual relative activity of immobilized lipase was higher than 40%, while the free lipase was only 15% which were treated under 70 °C for 3 h. The immobilized lipase possessed good reuse and organic solvent stability. The residual relative activity of immobilized lipase was 45% after 5 reuses and nearly 70% after being treated in methanol for 12 h. The good stabilities of immobilized lipase revealed that P(GMA-co-MA)-g-PEO nanofibrous membranes is an excellent carrier for enzymes immobilization.

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P(GMA-co-MA)-g-PEO) terpolymer containing epoxy groups and hydrophilic PEO branch chain was synthesized

P(GMA-co-MA)-g-PEO nanofiber membrane was prepared by electrospinning process

Lipase was covalently immobilized on the P(GMA-co-MA)-g-PEO nanofibrous membranes

Immobilized lipase achieved high enzyme loading and activity

Immobilized lipase possessed good thermal, reuse and organic solvent stability