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Pore size of macroporous polystyrene microspheres affects lipase immobilization

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ABSTRACT

Polystyrene (PST) microspheres are commonly chosen as immobilization carriers due to their unique advantages such as ideal mechanical strength, adjustable particle size, and favorable chemical stability. However, there were few reports on immobilization by using microspheres with large pore sizes (>100 nm). We have successfully prepared the PST microspheres with macropores and gigapores by a novel method. In this study, giga-/macro-/meso-porous PST microspheres (314 nm, 104 nm, and 14.7 nm in pore sizes) were employed to immobilize lipase (from *Burkholderia cepacia*) by strong hydrophobic interactions, and the effects of pore sizes on lipase distribution, relative activity, kinetic behavior, thermal stability, storage stability, and reusability were also investigated in detail. According to laser scanning confocal microscope (LSCM) observation, lipase penetrated into the center of those giga-/macro-porous microspheres. With regard to the mesoporous microspheres, lipase only adsorbed to the external shell. The relative activities of immobilized lipase were 146%, 126%, and 50.9% for giga-/macro-/meso-porous PST microspheres, respectively. Comparing with the kinetic constants of free lipase (0.441 mM), the K_m value for mesoporous PST-lipase (0.532 mM) was higher, whereas for giga-/macro-porous PST-lipase (0.402 mM and 0.411 mM), the K_m values were comparatively lower suggesting the accessibility of substrate to the enzyme active sites was unlimited. The thermal stability, storage stability, and reusability were all improved significantly with the increase of pore sizes. In stimulant system, even after 100 times of recycling, the activity of lipase immobilized on gigaporous and macroporous PST microspheres remained nearly 100% and 93%, respectively, while that of lipase-PST with 14.7 nm pores could only keep 64.1%. In real system, 73.5% and 68.8% activity of gigaporous PST-lipase and macroporous PST-lipase, respectively, were retained after being used 30 times, whereas only 49.4% activity was remained for mesoporous PST-lipase. Therefore, compared with other microspheres, the gigaporous PST microsphere exhibited obvious advantages as a potential enzyme support in industry.

1. Introduction

The use of enzymes as biocatalysts for synthesis of pharmaceutical products [1] and organic compounds [2] has gained more popularity in recent years, and the number of their industrial applications [3] is also growing rapidly. Generally, enzyme-catalyzed reactions operate in mild conditions, and produce fewer by-products due to its high specificity. In addition, they provide for an environmentally friendlier, more energy efficient and potentially more cost-effective techniques due to low-energy demanding operation and simpler downstream processing. However, using native enzyme as a biocatalyst presents some drawbacks, such as poor stability under operational conditions, difficulty of product recovery, and impossibility of multiple reuses in industrial process. Traditionally, these obstacles have been addressed with the immobilization of enzymes on heterogeneous matrixes by covalent binding [4–6], entrapment [7–9], or adsorption [10–15]. When an enzyme is immobilized on a hydrophobic carrier, the only interaction between the carrier and the enzyme is entropy driven. One enzyme molecule displaces a large number of water molecules both from the carrier and its own surface when it is immobilized. The interaction between two materials via this gain in entropy is also known as hydrophobic interaction [16].

In the immobilization, the support is a critical issue. A variety of solid supports including gels, resins, silica, and magnetic beads [17,18] have been investigated. The essential requirement for any carrier is the need to have a large surface area. In this respect, porous polymeric materials have attracted much attention due to their obvious advantage of high internal surface areas. In general, porous materials can be classified according to their pore sizes: microporous materials (less than 2 nm), mesoporous materials (2–50 nm), macroporous materials (50–200 nm), and gigaporous materials (more than 200 nm). To create more suitable porous supports, it

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is of great importance to understand the factors that influence the immobilizing behavior of enzyme within porous materials. It has been found that the pore sizes play an important role in the enzyme immobilization. Unfortunately, related studies have mainly focused on the mesoporous materials, such as MCM-41, SBA-15, KIT-6 [19–21]. In these cases, diffusion constraint is usually associated, which is not favorable to enzyme immobilization. On the contrary, macroporous materials can, in principle, avoid this problem, but the verification is still awaited.

The aim of this study is to investigate the influences of pore size on enzyme immobilization. A series of giga-/macro-/mesoporous PST microspheres with given diameter (30–40 μm) were employed as supports to study how the pore size affect the enzyme immobilization. Lipase (from *Burkholderia cepacia*) which molecular mass is 33.2 kDa, and an average molecular diameter is about 4–5 nm, was selected as a prototype due to its widely used in the processing of fats and oils hydrolysis, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, and production of cosmetics [22]. The lipase was often the only lipophilic component, and it could be extracted and purified by adsorption onto hydrophobic carriers in one single step. On the other hand, lipases broke down fats, and they were active at the interface of oil/fat and water. Therefore, the immobilization of lipases on hydrophobic carriers was thought to mimic this interfacial activation [13–15,23]. The lipase distribution within microspheres was observed using a LSCM method, and the influence of pore sizes on the PST-lipase hydrolysis activity was studied. The thermal stability, storage stability, reusability, and kinetic behavior of immobilized lipase were also estimated in detail to comparatively testify the feasibility of these microspheres as ideal immobilization carriers.

2. Experimental

2.1. Materials

Lipase PS Amano (lyophilized powder from *B. cepacia*) and *p*-nitrophenyl palmitate (*p*-NPP) were purchased from Sigma–Aldrich Company. Source 30 (mesoporous polystyrene microspheres with 14.7 nm pores) was obtained from GE Company. Styrene and divinyl benzene were of commercial grade (Beijing Chemical Reagents Co.), and they were distilled under a vacuum to remove the inhibitor. Benzoyl peroxide (25% water, Beijing Chemical Reagents Co.) was used as an initiator. Sorbitan monooleate (Span 80) (Bangde Technology and Trade Co., Beijing) was of reagent grade. Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%, Kuraray) was used as a stabilizer. Hexadecane (Wako Pure Chemical Industries Ltd.) was of reagent grade and was used as a diluent (porogen). All other reagents were of analytical grade and were obtained from Xingjin Chemicals (China).

2.2. Preparation of macroporous and gigaporous PST microspheres

Macroporous and gigaporous PST microspheres were synthesized by suspension polymerization process, which was developed in our previous study [24]. The mixture of monomer (styrene), surfactant (Span 80), crosslinking agent (divinyl benzene), diluent (hexadecane), and initiator (benzoyl peroxide) was used as the dispersed phase. Water, where the stabilizer [poly(vinyl alcohol)] and electrolyte (Na_2SO_4) were dissolved, was used as the continuous phase. An emulsion was prepared by dispersing the monomer phase into the aqueous phase in a four-neck glass flask equipped with an anchor-type agitator, a condenser, and a nitrogen inlet nozzle. After the emulsion was bubbled with nitrogen for 1 h, the nozzle was lifted up above the surface of the emulsion and the temperature was elevated to 75 °C for polymerization. The polymerization was carried out for 20 h under a nitrogen atmosphere. The polymer particles were washed in turn with water and ethanol, and followed by acetone extraction for 24 h. Finally, the particles were dried in vacuum at room temperature.

2.3. Enzyme immobilization

Firstly, microspheres (0.1 g) were wetted with 10 ml ethanol prior to use. Then, the carriers were washed with 50 ml phosphate buffer (0.1 M, pH 7.0) by filtration to replace ethanol. The supernatant was removed and replaced with 10 ml lipase solution (0.5 mg/ml). The vials containing the carrier and lipase solution were shaken gently at 25 °C and 30 rpm for 4 h. The solution was removed by filtration and the carriers were washed several times with phosphate buffer, until no protein was detected in the washing solution. Supernatant and washing solutions were collected to calculate the loading capacity by the bicinchoninic acid (BCA) protein assay. The amount of immobilized lipase was determined by measuring the initial and final concentrations of protein in the immobilization medium. A calibration curve was constructed from BSA solutions of known concentration (15–1000 μg/ml) and was used in the calculation of protein amount in the enzyme and washing solutions.

2.4. Activity assays of free and immobilized lipase

The activity of free and immobilized lipase was determined by the method of olive oil hydrolysis [4]. Briefly, an olive oil emulsion (100 ml) was prepared by mixing olive oil (50 ml) with a gum arabic solution (50 ml, 0.1 g/ml) under stirring at 18,000 rpm for 3 min with a high-performance disperser (IKA, T18 basic ULTRA-TURRAX, Germany). The assay mixture consisted of the emulsion (5 ml), phosphate buffer (4.0 ml, 0.1 M and pH 7.0) and free enzyme (1 ml, 0.5 mg/ml) or immobilized enzyme (10 mg support-lipase) in 1 ml. The oil hydrolysis was carried out at 37 °C and 60 rpm for 30 min in a water bath shaker. The reaction was stopped by adding 10 ml acetone–ethanol solution (1:1, v/v). The resulting fatty acids in the medium were determined by titration with 50 mM NaOH solution which was standardized with potassium hydrogen phthalate ($KC_8H_4O_4H$) using phenolphthalein as the indicator. One lipase unit was expressed as the release of 1 μmol fatty acid per min in the assay conditions, and related terms were used in this study as follows:

$$\text{Lipase activity(U/g} - \text{support)} = \frac{\text{activity of immobilized lipase}}{\text{amount of support used}} \quad \text{(A.1)}$$

$$\text{Specific activity(U/mg protein)} = \frac{\text{activity of immobilized lipase}}{\text{mass of protein loaded}} \quad \text{(A.2)}$$

$$\text{Activity yield(\%)} = \frac{\text{specific activity of immobilized lipase}}{\text{specific activity of free lipase}} \times 100\% \quad \text{(A.3)}$$

$$\text{Activity recovery(\%)} = \frac{\text{lipase activity}}{\text{initial lipase activity}} \times 100\% \quad \text{(A.4)}$$

$$\text{Residual specific activity(\%)} = \frac{\text{specific activity}}{\text{initial specific activity}} \times 100\% \quad \text{(A.5)}$$

2.5. SEM observation

The surface features of PST microspheres before and after immobilizing lipases were observed by a scanning electron microscope (SEM) (JEOL, JSM-6700F, Japan). Microspheres were resuspended in distilled water and the dispersion was dropped on a piece of aluminum foil and dried at ambient atmosphere. The sample was placed on a metal stub with double-sided conductive adhesive tape and was coated with a thin gold film under reduced pressure below 5 Pa with a fine coater (JEOL, JFC-1600, Japan).

2.6. LSCM analysis

A LSCM (Leica, TCS SP5, Germany) was employed to investigate the distribution of lipase within microspheres via observing Fluorescamine–lipase. Fluorescamine is a spiro compound used as a reagent for the detection of peptides and proteins, which is not fluorescent itself, but reacts with primary amines to form highly fluorescent products. It was soluble in acetone at 50 mg/ml, yielding a clear solution. Firstly, the microspheres immobilized with lipases were sliced with a freezing microtome (Leica, CM1850, Germany), and then Fluorescamine solution was added to react with sliced PST-lipase for 3 min. The samples were excited at 390 nm and the fluorescent images at 460–480 nm wavelengths were then taken.

2.7. Measurement of kinetic parameters

The Michaelis constant K_m and the maximum reaction velocity V_{max} for the free and immobilized lipase were determined by measuring the initial velocity of the reaction by varying the substrate (*p*-NPP) concentrations at a constant enzyme concentration. *p*-NPP was dissolved in acetone and diluted with phosphate buffer (50 mM, pH 7.0) containing 1.25% (w/v) Triton X-100. The reaction was started by adding 100 μl of free or immobilized enzyme (both containing 0.1 mg protein) to 1.9 ml of substrate solution and the absorbance was monitored at 410 nm. The initial rate was determined at 1 min. The final conversion was at 5 min for both free and immobilized lipase. From the measured activity of the free and immobilized enzymes, K_m and V_{max} was calculated using Lineweaver–Burk equation:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{[S]} \quad \text{(B.1)}$$

where V was the enzymatic reaction rate [(μmol/l)/min] [S] was the substrate concentration (mM), V_{max} was the maximum rate attained at an infinite concentration of substrate [(μmol/l)/min], and K_m was the Michaelis–Menten constant (mM).

2.8. Thermal stability

The thermal stability of free and immobilized lipase was determined by measuring the residual enzymatic activity at two different temperatures (50 °C and 70 °C) in a phosphate buffer (0.1 M, pH 7.0) for 6 h. After every interval, such as 15 min, 1 h or 2 h etc., given amount of immobilized enzyme was removed and assayed for enzymatic activity as described above.

2.9. Storage stability

The soluble lipase and immobilized lipases were immersed in phosphate buffer (0.1 M, pH 7.0) and stored at 25 °C. The storage stability was evaluated by determining the enzymatic activity of olive oil hydrolysis and the enzyme amount in supernatant solution at regular time intervals up to 15 days.

2.10. Recycling of immobilized lipase

Recycling of immobilized lipase was evaluated in a stimulant system as well as a real system according to the literatures [25,26]. In the stimulant system, the experiment was conducted by washing the immobilized lipase with fresh phosphate buffer (0.1 M, pH 7.0) for 3 min, and activity retention was then tested in hydrolysis of olive oil, which were repeated up to 100 cycles. In the real system, the activity of immobilized lipase was measured using *p*-NPP as substrate. The immobilized lipase was washed with ethanol (20%, v/v) and phosphate buffer (0.1 M, pH 7.0) after use in hydrolysis of *p*-NPP for 3 min and suspended again in fresh aliquot of substrate, this being repeated up to 30 cycles.

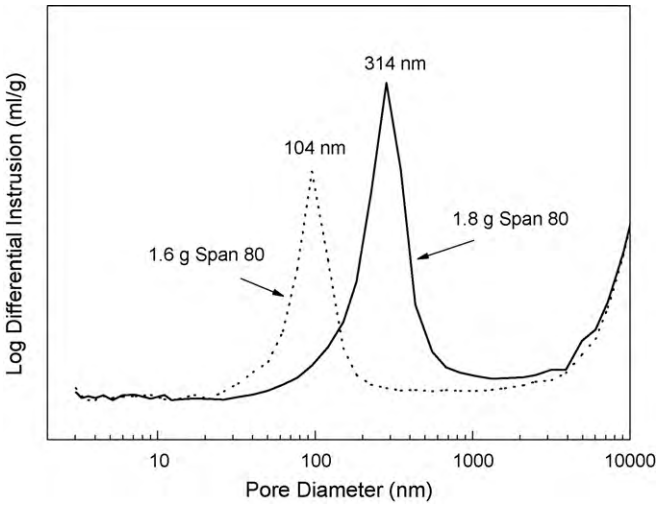

Fig. 1. Pore size distribution curves of PST microspheres prepared with different amounts of Span 80.

3. Results and discussion

3.1. Pore structure characterization of PST microspheres

In a previous study, Zhou et al. [24] have pointed out that the high concentration of surfactant (Span 80) in the oil phase was important for the formation of macropores. Therefore, gigaporous and macroporous microspheres were prepared via changing the Span 80 amount in reaction system (Fig. 1). These two giga-/macroporous microspheres, together with a commercially available mesoporous microspheres (Source-30), were employed for the following comparison. Generally, the pore sizes and total pore surface areas of microspheres were assumed to be inverse correlation. As expected, increasing the pore sizes from 14.7 nm to 104 nm and 314 nm resulted in a remarkable decrease in the surface areas (745 m^2/g, 31.3 m^2/g and 19.3 m^2/g). Other parameters, such as total pore volume and porosity, were not significantly different (Table 1).

3.2. Lipase distribution in PST microspheres

Fig. 2 displays LSCM images of Fluorescamine–lipase immobilized on polystyrene microspheres. It was observed that the distribution profiles of lipase within the microspheres highly depended on the pore sizes. The lipase penetrated into the center of gigaporous and macroporous microspheres, whereas the adsorption of lipase only occurred on the surface of mesoporous microspheres. In this case, the pore of mesoporous microspheres was not big enough to guarantee unhindered diffusion of the enzyme to the center, especially after some enzyme molecules were absorbed in pores. On the other hand, small pores are difficult to be wetted, and hence the diffusion of the enzyme was limited.

The surface features of microspheres before and after immobilization were further monitored by SEM observation (Fig. 3). The pore morphologies in gigaporous and macroporous PST microspheres were well maintained. Contrarily, the surface channels in mesoporous PST microspheres were plugged remarkably. These outcomes supported that, compared to the mesoporous microspheres, the gigaporous and macroporous PST microspheres were much better for lipase immobilization.

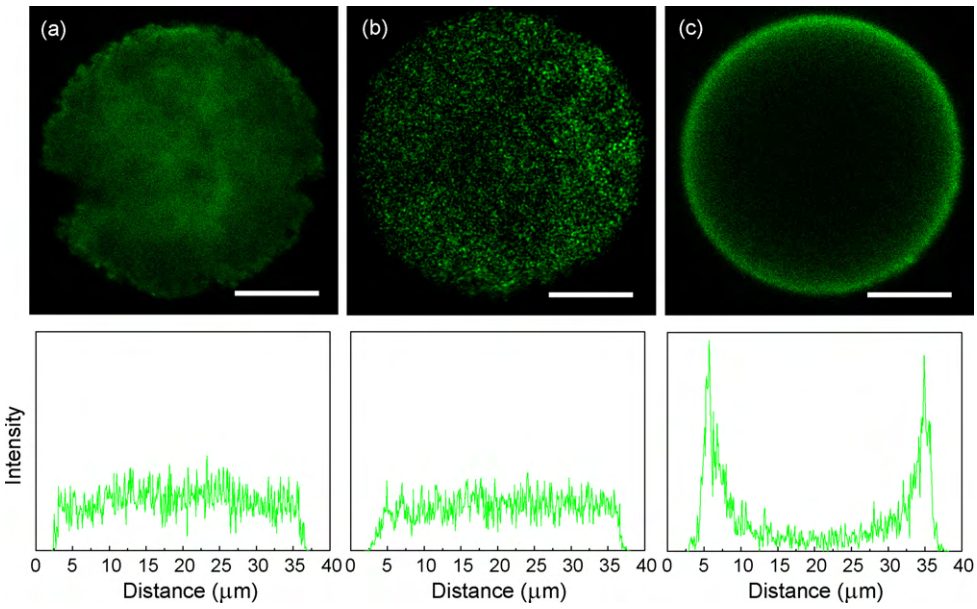

Fig. 2. LCSM images of Fluorescamine–lipase immobilized on (a) gigaporous, (b) macroporous, and (c) mesoporous PST microspheres, and scale bars represent 10 μm in each case. Corresponding fluorescence distribution profiles along diameter are displayed below.

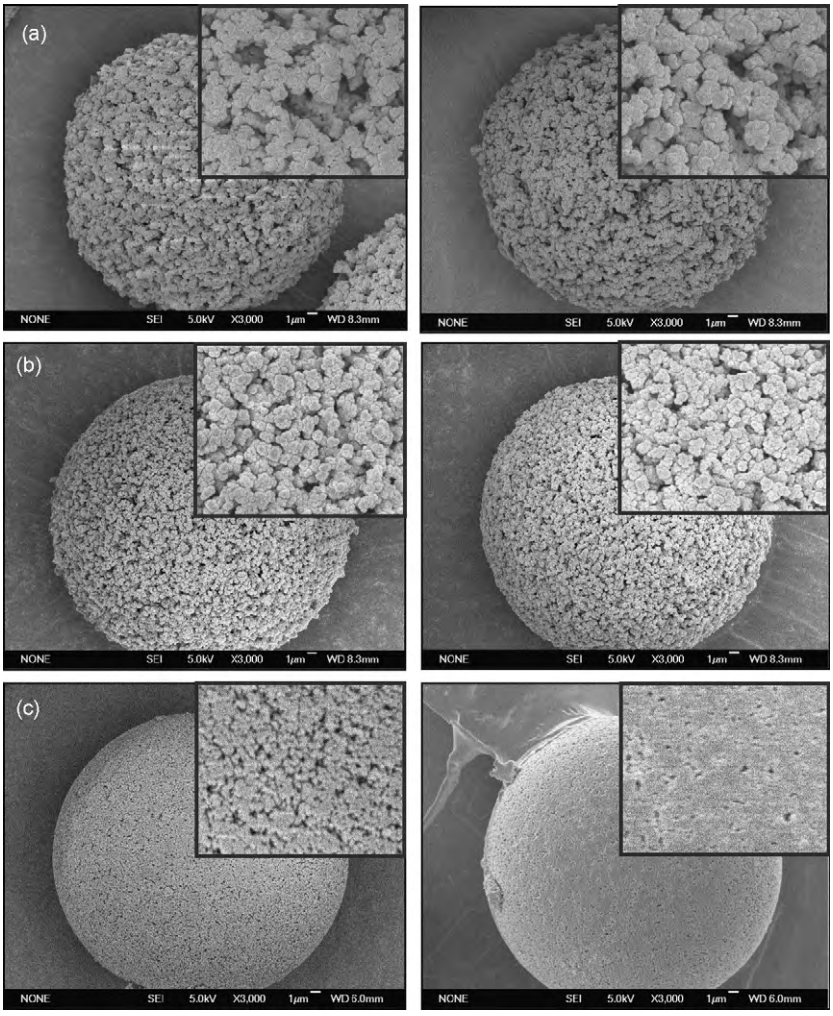

Fig. 3. SEM images of (a) gigaporous, (b) macroporous, and (c) mesoporous PST microspheres before (left) and after (right) lipase immobilization. The magnification is 10,000× in all enlarged pictures.

Table 1
Porosimetry measurement results of different PST microspheres.

Sample	Average pore size (nm)	Total pore surface area (m^2/g)	Total pore volume (cm^3/g)	Porosity (%)
PST-300	314	19.3	2.25	72.1
PST-100	104	31.3	1.74	67.7
Source-30	14.7	745	2.74	80.1

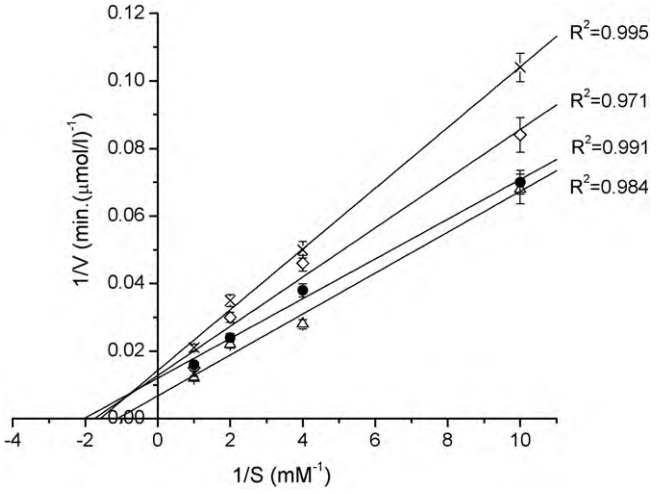

Fig. 4. Lineweaver–Burk plot for *p*-NPP hydrolysis of free and immobilized lipase. Free lipase (◊) and lipase immobilized on (△) gigaporous, (●) macroporous, and (×) mesoporous PST microspheres.

3.3. The loading and activity of immobilized lipase on different PST microspheres

To evaluate the enzyme loading capacity, the effect of specific surface areas was usually investigated. Compared to other two supports, theoretically, mesoporous Source-30 with higher surface areas (745 m^2/g) was expected to exhibit much higher loading. However, the differences were slight (see in Table 2). According to LSCM observation, a possible explanation was that the lipase could not penetrate into the center and only adsorbed to the external shell of Source-30. We noted that the activity of immobilized lipase was not positively related to the enzyme loading herein. Increasing the enzyme loading from 16.4 mg/g to 18.1 mg/g and 25.2 mg/g for different pore sizes microspheres (314 nm, 104 nm, and 14.7 nm) resulted in decreases in lipase activity and specific activity of immobilized lipase. For giga-/macro-porous PST-lipase, the specific activities were higher than that of free lipase due to its interfacial activation on hydrophobic PST microspheres to open the lid protecting the active sites and to make insoluble substrate (olive oil) easily access to lipase [19,21]. However, when mesoporous PST microsphere was used as carrier, the specific activity of immobilized lipase was only 50.9% relative to that of free enzyme. We proposed two possible hypotheses to explain these results. One hypothesis was that steric effects of pore sizes had significant influence on the lipase conformation, leading to the changes of enzyme activity. A second hypothesis was that diffusion limitations, especially in mesoporous PST microspheres, restricted the contact between lipase and olive oil (Fig. 3c). This point was further confirmed by the kinetic reactions of immobilized enzymes.

3.4. Kinetic parameters

Kinetics of hydrolytic activity of free and immobilized lipase were investigated using various initial concentrations (0.05–1 mM) of *p*-NPP as substrate. According to Lineweaver–Burk plot of $1/V$ versus $1/[S]$ (Fig. 4), Michaelis–Menten constant K_m and the initial maximum reaction velocity V_{max} of the free and immobilized lipase were calculated and the results are given in Table 3. It was worth pointing out that the value of $1/K_m$ could be used to reflect the affinity of enzymes and substrate. Notably, the K_m values for PST-300-lipase and PST-100-lipase were slight lower than that of free lipase, indicating the diffusion of substrate and product could be neglected, which could be attributed to their big enough pores to make substrate (*p*-NPP) easily access to lipase. However, the higher K_m value of Source-30-lipase was considered as an indication of a weaker affinity of the enzyme for substrate and a result of diffusion limitations in its small pores. Taken together, gigaporous PST microsphere was more suitable for lipase immobilization.

3.5. Thermal stability

The knowledge on thermal stability of an immobilized enzyme is useful in exploring its potential applications. We investigated the time profiles on the hydrolysis activity of free and immobilized enzymes at 50 °C and 70 °C (Fig. 5). It was observed that the hydrolysis activity of the immobilized enzymes was more stable than that of free enzyme under both two temperatures. After 6 h treatment at 50 °C, the immobilized lipase on the gigaporous microspheres presented more than 95% activity, while only 50% activity was maintained for the free lipase. As the temperature increased up to 70 °C, the activity recovery of both free and immobilized lipase decreased. For free lipase, almost no activity was observed after the heat treatment for 6 h, and the immobilized lipase on the gigaporous microspheres still maintained 85% activity, at least. Fig. 5 also illustrated that the activity recovery of immobilized enzymes was dependent on the pore size of supports. By increasing the pore size of the supports from 14.7 nm to 104 nm and 314 nm, the thermal stability increased significantly. These results might be ascribed to the distributions of lipase in the microspheres. When the support was with small pore size, lipase was primarily immobilized on the surface and cannot be well confined by the support at high temperature conditions, leading to the remarkable decrease in activity recovery. On the contrary, most lipase could be deeply confined in the internal regions of gigaporous and macroporous microspheres, which could provide well protection against high temperature.

3.6. Storage stability

The storage stability of immobilized lipase was determined via investigating the residual loading and the residual specific activity. As the profiles displayed in Fig. 6a, there was almost no leakage of lipase from gigaporous microspheres, whereas nearly 5% and 15% lipase leakages from the macroporous and mesoporous microspheres, respectively, were observed. It has been reported that lipase was immobilized on the PST microspheres by strong hydrophobic interactions between styrenic surfaces and lipase [10]. Therefore, lipase leakages were reasonable. As shown in Fig. 6b, compared with free lipase, there were significant increases in storage stability of lipase immobilized on gigaporous and macroporous microspheres. The free lipase was found to retain only 10.2% of its original specific activity after 15 days of storage at 25 °C. Under identical storage conditions, the specific activities of lipase immo-

Table 2
Yields of protein loading and activity of the immobilized lipase onto PST microspheres with different pore sizes.

Sample	Protein loading (mg/g-support)	Lipase activity (U × 10^2/g-support)	Specific activity (U/mg protein)	Activity yield (%)
PST-300	16.4 ± 0.1	32.3 ± 0.8	198 ± 7	146 ± 5
PST-100	18.1 ± 0.2	30.8 ± 0.8	170 ± 6	126 ± 5
Source-30	25.2 ± 0.3	17.3 ± 0.2	68.7 ± 5.8	50.9 ± 3.9

The specific activity of soluble lipase was 135 U/mg.

Table 3
K_m and V_{max} values of free and immobilized lipase from the Lineweaver–Burk plot of the Michaelis–Menten equation.

	Free lipase	PST-300-lipase	PST-100-lipase	Source-30-lipase
K_m (mM)	0.441 ± 0.022	0.402 ± 0.031	0.411 ± 0.021	0.532 ± 0.043
V_{max} [(μmol/l)/min]	70.4 ± 1.5	87.7 ± 1.3	72.5 ± 2.1	60.6 ± 1.4

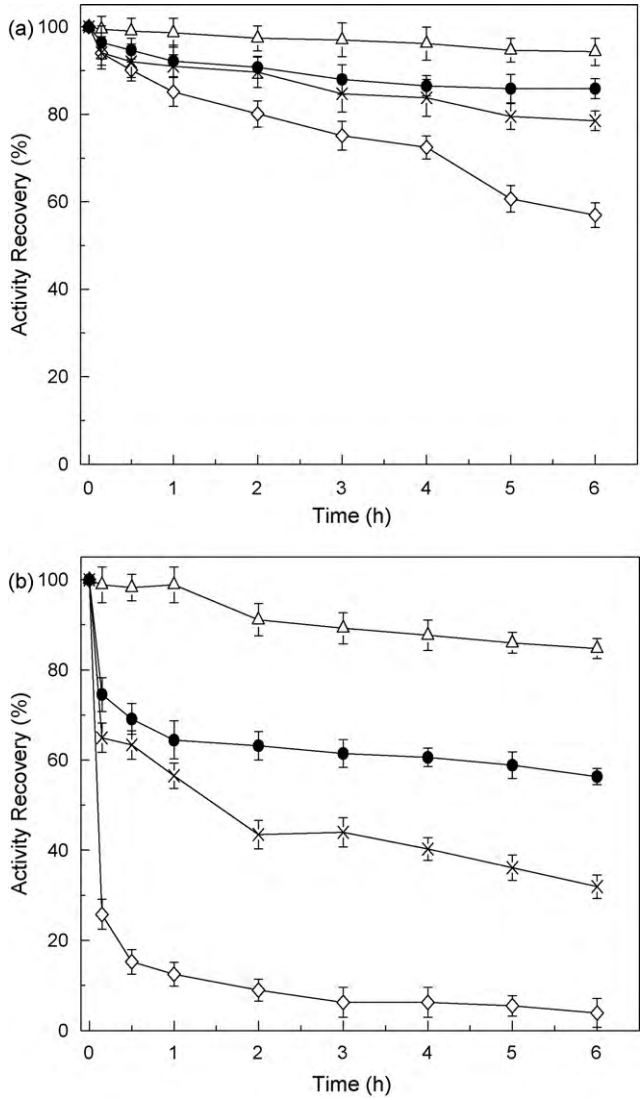

Fig. 5. Effect of temperatures on the hydrolysis activity of olive oil at two different temperatures, 50 °C (a) and 70 °C (b). Free lipase (◊) and lipase immobilized on (△) gigaporous, (●) macroporous, and (×) mesoporous PST microspheres.

bilized on gigaporous and macroporous microspheres were kept 67.5% and 63.3%, respectively. However, for mesoporous PST-lipase, its storage stability was performed in different phenomena, and its activity loss was more serious compared with free lipase. These results might be due to the fact that the pore sizes of microspheres affected the interaction between styrenic surfaces and lipase, leading to changes of lipase confirmation with increasing storage time.

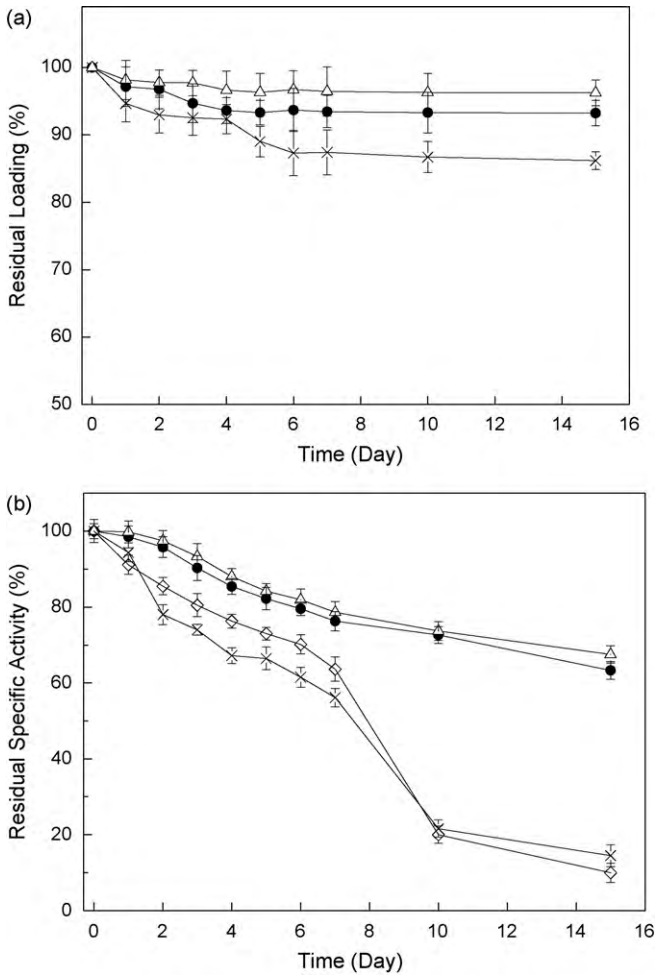

Fig. 6. Storage stability of immobilized lipase at 25 °C in loading (a) and specific activity (b). Free lipase (◊) and lipase immobilized on (△) gigaporous, (●) macroporous, and (×) mesoporous PST microspheres.

This result could further support that gigaporous PST microsphere was more suitable to immobilize lipase.

3.7. Effect of batch operation on activity of immobilized enzyme

In view of free enzyme hardly being reused in operational progress, the immobilized enzymes were expected to be repeatedly used for many times. Therefore, operational effect on the activity of immobilized enzyme should be examined. The effect of the reuse on the activity of immobilized lipase at pH 7.0 and 37 °C was plotted in Fig. 7 with activity recovery against the num-

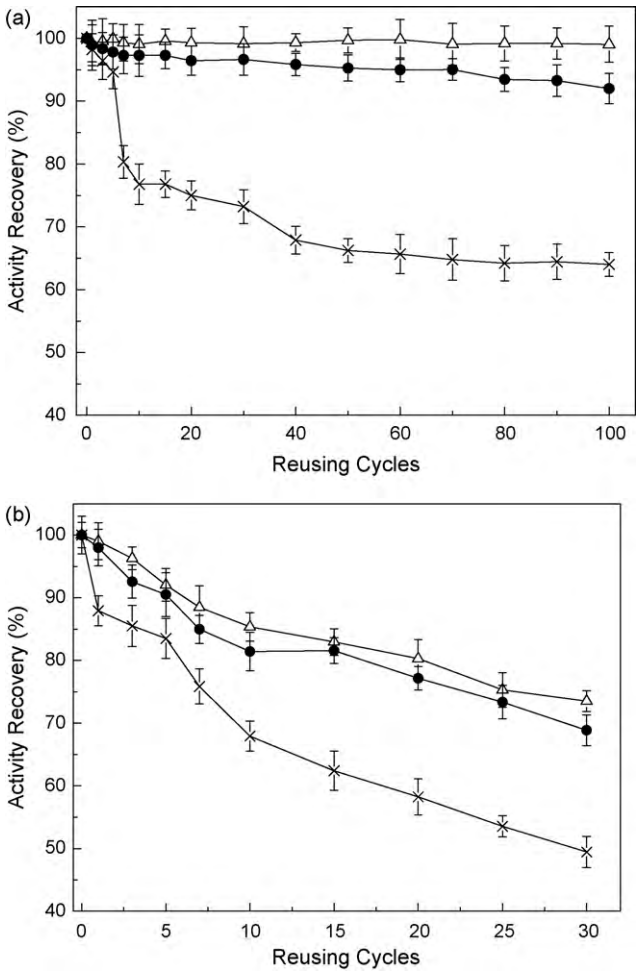

Fig. 7. Reusability of immobilized enzyme in stimulant system of olive oil hydrolysis (a) and in real system of *p*-NPP hydrolysis (b). Lipase immobilized on (△) gigaporous, (●) macroporous, and (×) mesoporous PST microspheres.

ber of multiple batch uses. In stimulant system, even after 100 times of recycling, the activities of lipase immobilized on gigaporous and macroporous PST microspheres remained nearly 100% and 93%, respectively, while that of lipase-PST with 14.7 nm pores could only keep 64.1% (Fig. 7a). In view of a true situation hardly being indicated in a stimulant system, we also investigated the reusability in a real system of *p*-NPP hydrolysis. As illustrated in Fig. 7b, the activity loss was more serious than that in the stimulant system, but the similar trend was observed in both systems. 73.5% and 68.8% activity of lipase immobilized on gigaporous and macroporous PST microspheres, respectively, were retained after being used 30 times, whereas for mesoporous PST-lipase, only 49.4% activity was remained. As the case of the storage activity, the activity loss of immobilized enzyme in the operation progress could mainly be attributed to two aspects. The one was the leaching out of lipase from the microspheres' surface. The second factor might be the conformational changes by repeated uses. Because most enzymes are very sensitive to environmental changes, treating them frequently would unavoidably make them denatured. The lipases on mesoporous microspheres were more affected in operational progress because most molecules were absorbed in shell. For gigaporous PST-lipase, most lipases were well protected because they penetrated to the interior of the microspheres. Compared with aforementioned two PST-lipases and other reported immobilized lipases [8,27], the reusability of gigaporous PST-lipase was much better.

3.8. Conclusion and outlook

In summary, giga-/macro-/meso-porous PST microspheres were employed to immobilize lipase by strong hydrophobic interactions, and the effects of pore size on lipase distribution, relative activity, kinetic behavior, thermal stability, storage stability, and reusability were investigated. It was found that lipase penetrated into the center of gigaporous and macroporous microspheres, whereas the adsorption only occurred on the surface of mesoporous microspheres. Another, the specific activity of immobilized lipase exhibited a close correlation with the pore size of the giga-/macro-/meso-porous PST microspheres. When the pore diameter of support was much larger than the molecular dimension of lipase, immobilized lipase showed higher specific activity. The specific activity of lipase immobilized on gigaporous polystyrene microspheres (314 nm pores) reached 1.5 times higher than that of free lipase. Further more, the results obtained from K_m showed that the enzyme affinity to substrate for mesoporous PST-lipase was lower compared with that of free lipase. Obviously, the higher enzyme affinities to substrate were detected in the immobilization systems with gigaporous and macroporous microspheres. In addition, the thermal and storage stability of immobilized lipase were improved with the increase in pore sizes of supports, and the similar tendency was also observed for the reusability of immobilized lipase. Conclusively, all these results in this study strongly supported that the gigaporous PST microsphere was an ideal candidate support for lipase immobilization.

Finding a suitable porous material to immobilize enzyme is of great signification. Although we investigated the influence of different pore sizes microspheres on the same molecular dimension enzyme immobilization, this study should be further extended to investigate other enzymes with different molecular dimensions. In this respect, next focal point is to choose the gigaporous PST microsphere to immobilize different molecular enzyme, and find a regulation between pore size of carrier and enzyme molecular dimension. In addition, we expect to modify the surface of gigaporous PST microsphere to immobilize other enzymes, and make this microsphere as a versatile immobilization support.

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