Active biocatalysts based on Candida rugosa lipase immobilized in vesicular silica

Cuicui Wu, Guowei Zhou*, Xiaojie Jiang, Jingyun Ma, Huayong Zhang, Hongbin Song

Key Laboratory of Fine Chemicals in Universities of Shandong, School of Chemistry, Shandong Polytechnic University, Jinan 250353, China

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Vesicular silica (VS) with hierarchical structure was prepared by utilizing cationic surfactant cetyltrimethylammonium bromide (CTAB) and anionic surfactant sodium dodecyl sulfate (SDS) as the structure directing agents, and 1,3,5-triisopropylbenzene (TIPB) as the micelle expander. The resulting unilamellar and multimamellar VS with interlamellar mean mesopore size of 15–20 nm and shell thickness of 5–15 nm were used as supports for immobilization of Candida rugosa lipase (CRL) through physical adsorption. Possible mechanisms for the formation of VS and the immobilization of CRL on VS are proposed. N2 adsorption-desorption experiments and Fourier transform infrared spectroscopy (FT-IR) measurements demonstrated that CRL was adsorbed into the curved channels of the VS. The catalytic activity, thermal stability, and reusability of VS immobilized CRL were assayed in phosphate buffer medium by hydrolysis of triacetin. The effects of pH and temperature on enzyme activity were also investigated. We report that VS immobilized CRL exhibited outstanding adaptability at higher pH and temperature, and excellent thermal stability and reusability compared with free CRL.

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1. Introduction

The past decade has witnessed a rapid evolution in the synthesis of silica mesoporous materials with high specific surface areas, large pore volumes and diverse morphologies such as rod-like [1], fibers [2], cage-like [3], hollow spheres [4], films [5], and vesicle-like [6–9] by choosing various templates, swelling agents and using different synthesis approaches. Jensen et al. [10] first reported the biomimetic synthesis of mesoporous amorphous silica in aqueous solution at room temperature. These materials have been extensively applied in diverse areas such as immobilization techniques [11,12], catalysis [13], drug delivery [14], sensors [15] and separation process [16].

In 1996, Pinnavaia et al. [6] first demonstrated the feasibility of vesicular silica with a high degree of framework cross-linking, and high specific surface area and pore volume by employing bolaamphiphiles \( H_2N(CH_2)_nNH_2 \) (\( n = 12–22 \)) as the structure directors through a biomimetic templating approach. Subsequently, silica materials with unique vesicle-like morphology and lamellar framework have been prepared by using various surfactants as templates [7–9]. Zhou et al. [11] used 1,3,5-triisopropylbenzene (TIPB) as a hydrophobic agent for adjusting the packing parameter of the surfactant to tune the size and morphology of the mesopores which resulted in a multimamellar vesicular silica with large uniform intershell spaces. They also immobilized enzyme in mesoporous vesicle-like and rod-like silica by physical adsorption. The properties of the lipase immobilized into the vesicle-like mesoporous silica are superior to that into the rod-like mesoporous silica. Besides the advantages of tunable pore size, large surface area and pore volume, vesicular silica possesses curved interlamellar mesochannel and multimamellar structure as well as the added inter-shell flexibility arising from the special vesicular structure. The features increase the affinity interaction between the silanol groups of vesicular silica and the lipase, resulting in a slower lipase leakage during the recycling process and are helpful to applications in proteins adsorption and enzyme catalysis. The higher stability and activity of the lipase immobilized in vesicular silica make it an excellent candidate for a new bioimmobilization host. However, they have not been widely studied in applied technological areas because of small pore size (interlamellar space) non-uniform morphologies.

Candida rugosa lipase (CRL) is a stable mesophilic lipase that has high activity and broad specificity in reaction medium. However, free lipase is often unstable and possesses low activity in organic solvents or in a harsh environment such as high temperature or extreme pH. Immobilization of CRL on various supports promotes the separation of products and lipases and improves catalytic activity, stability, and recyclability of immobilized lipase in continuous operations. A series of recent studies have reported investigating immobilization of CRL on different carriers [17–22]. Dyal et al. [17] reported immobilization of CRL on \( \gamma \)-Fe2O3 magnetic nanoparticles and studied the stability and enzymatic activity of the immobilized lipase. In 2010, effects of pore diameter and cross-linking method on the immobilization efficiency of CRL in SBA-15...
with different pore sizes were investigated by Gao et al. [20]. Ye et al. [22] demonstrated the feasibility of covalently immobilizing CRL onto the nanofibrous poly-(acrylonitrile-co-maleic acid) (PANMA) membrane formed by the electrophoresing procedure. Moreover, they explored the effects of fiber diameter on kinetic parameters, enzyme loading, and activity of the immobilized lipase.

In this paper, we report the synthesis of vesicular silica (VS-n (n = 0, 1)), where n is the molar ratio of 1,3,5-trisopropylbenzene (TIPB)/sodium dodecyl sulfate (SDS) in aqueous solution by utilizing cetyltrimethylammonium bromide (CTAB) and SDS as co-surfactants, and a small amount of the hydrophobic additive TIPB. VS-1 was prepared in presence of TIPB and VS-0 was synthesized in absence of TIPB. These materials were used as carriers for the immobilization of CRL by physical adsorption. The optimum catalytic conditions for free and immobilized CRL to function as biocatalysts in the hydrolysis of triacetin were determined. Simultaneously, the thermal stability and reusability of different immobilized CRL were also compared. The results indicate that the properties of lipase adsorbed on VS-1 surpass the properties of those absorbed on VS-0. The differences in the amounts of CRL adsorbed and the activities of CRL between VS-0 and VS-1 may be attributed to their pore sizes. The pore diameter of VS-1 by adding swelling agent TIPB is increased, which may improve the transport of the CRL molecule, making it favorable for the enzymatic reaction. Vesicular silica with large pores prepared in this manner has potential applications in separation, catalysis and drug delivery. However, both two catalysts had low stability and the further cross-linking among the adsorbed CRL in supports would be attempted to strengthen the stability of immobilized CRL.

2. Materials and methods

2.1. Chemicals

C. rugosa lipase (CRL), 1,3,5-trisopropylbenzene (TIPB), sodium dodecyl sulfate (SDS), and triacetin (99%) were purchased from Sigma-Aldrich. Cetyltrimethylammonium bromide (CTAB) was obtained from Shanghai Chemical Reagent Inc. of Chinese Medicine Group. Tetraethoxysilane (TEOS), and other chemicals were of analytical grade and were used as received without any further purification.

2.2. Synthesis of VS

In a typical synthesis, 0.28 g of SDS was dissolved in 266.5 g of deionized water with continuous stirring at 35 °C to form a clear aqueous solution, and 0.61 g of CTAB was dispersed into the solution. After stirring for 2–3 h, specific amounts of TIPB (molar ratio of TIPB/SDS being 0 and 1) were added drop-wise into the mixture for the preparation of two samples. The aqueous solution was stirred for another 18 h. Then, 4.2 g TEOS was added under vigorous stirring. The final molar ratio is 1 SDS/1.72 CTAB (x = 0, 1) TIPB/20.76 TEOS/1524.77 H2O. This reaction mixture was constantly stirred at 35 °C for 24 h, then sealed within a Teflon autoclave and heated at 100 °C for 24 h. The solid products were collected by filtration, washed with water, and dried at room temperature. Finally, the resultant powder was calcined at 550 °C for 6 h in air to remove the surfactant template. The surfactant-free samples were denoted as VS-n (n = 0, 1), where n is the molar ratio of TIPB/SDS.

2.3. Characterization

Field emission scanning electron microscopy (FESEM) was performed with a JEOL-JEM-6700F microscope operating at an accelerating voltage of 5 kV and electric current of 10 pA. Samples were mounted on the surface of a silicon wafer and sputtered with a thin film of gold to avoid charging under the electron beam prior to examination. Transmission electron microscopy (TEM) was carried out on a JEOL-JEM-1400 microscope (accelerating voltage of 120 kV). Samples for TEM measurements were suspended in ethanol by ultrasonication for 15 min and subsequently deposited on a carbon-coated copper grid. NaCl adsorption-desorption isotherms were measured at 77 K using a Gemini V2380 system. Before measurement, samples were outgassed at 120 °C for at least 10 h. The specific surface areas, Sm, were calculated using the Brunauer–Emmett–Teller (BET) method based on the adsorption data, while the pore diameter distribution was derived from adsorption branch using the Barrett–Joyner–Halenda (BJH) model. Fourier-transformed infrared (FT-IR) spectra were collected on samples in KBr tablets using a Bruker Tensor 27 Fourier transform infrared spectrophotometer.

2.4. Preparation of VS-n immobilized CRL

The immobilization of CRL was performed as following: first, 40 mg of VS-n was suspended in 20 ml of CRL phosphate buffer (pH 6.0) solution with a concentration of 2 mg/ml in a 50 ml capped vial. The mixture was stirred by a magnetic stirrer at 25 °C for 6 h and then was centrifuged at 6000 rpm and 4 °C for 10 min for obtaining CRL immobilized VS. All the protein concentrations were determined by the Bradford method [23], using bovine serum albumin (BSA) as standard. The immobilization yield (IVY) and the amount (P) of CRL loaded in support were calculated as follows:

\[ \text{IVY} = \left( \frac{C_1 - C_2}{C_1} \right) \times \frac{V_1}{V_2} = \frac{C_2 \times V_2}{C_1 \times V_1} \]  

(1)

where \( C_1 \) and \( C_2 \) are the protein concentrations of initial solution, final solution, and wash solution of CRL, respectively (mg/ml); \( V_1 \) and \( V_2 \) are the volumes of initial solution and wash solution of CRL (ml); \( W \) is the weight of support (g).

After immobilization of lipase, samples were designated as VS-0-CRL or VS-1-CRL depending on the molar ratio of TIPB and SDS used in the preparation.

2.5. Activity assays

A triacetin emulsion was prepared by vigorously stirring a mixture of triacetin (10 g), deionized water (25 ml), and phosphate buffer (pH 7.0, 12 ml) at 35 °C for 10 min. Then free or immobilized CRL was added to the emulsion once pH was stabilized. The mixture was continuously titrated with 0.02 M NaOH solution for 10 min to maintain a constant pH. The volume of NaOH consumed in 10 min was recorded to calculate the specific activity of free or immobilized CRL. CRL activity unit was denoted as U/g, with one lipase unit (U) defined as the amount of enzyme required to hydrolyze 1 µmol triacetin per min at 35 °C.

Simultaneously, blank experiments were performed through the same procedure using the same amount (40 mg) of VS-n or without adding any VS. The volumes of NaOH solution consumed by blank experiments were eliminated. Our results showed that neither support nor substrate could consume significant amounts of NaOH solution. All of the experiments were repeated in triplicate. The absolute activity of enzyme was calculated using the equation:

\[ E_a = \left( \frac{V_1 - V_0}{t} \right) \times \frac{C}{m} \]  

(3)

where \( E_a \) is the specific catalytic activity of enzyme (U/gprotein); \( V_1 \) and \( V_0 \) are the volumes of NaOH solution consumed in the lipase solution and blank experiments (ml), respectively; \( C \) is the concentration of NaOH solution (mol/l); \( t \) is the reaction time (min), i.e. 10 min and \( m \) is the weight of protein (g).

Relative activity (R) of immobilized CRL was calculated according to the equation:

\[ R = \frac{E_a}{E_{a,0}} \times 100\% \]  

(4)

where \( R \) is the relative activity of immobilized CRL (%); \( E_{a,0} \) is the specific activity of immobilized CRL (U/gprotein); \( E_a \) is the maximal specific activity of immobilized CRL (U/gprotein).

2.6. Effects of pH and temperature on CRL activity

The activity of free or immobilized CRL at different pH and temperature was measured as described previously. Optimum pH were compared in different buffers within the pH range of 5.0–9.0 (0.02 M K2HPO4–KH2PO4 buffer solution for pH 5.0, 0.02 M phosphate buffer solution for pH 6.0–8.0, and 0.02 M Na2CO3–NaHCO3 buffer solution for pH 9.0) at 35 °C. Thermal inactivation experiments were performed in phosphate buffer (pH 7.0) at temperatures ranging from 30 °C to 70 °C.

2.7. Reusability and deactivation stability of free and immobilized CRL

Phosphate buffer of free or immobilized CRL was heated in capped cuvettes in a water bath at 60 °C for desired time (15, 30, 60, 90, 120, and 150 min). Free or immobilized CRL was collected by centrifugation, and their relative activities were calculated as described above. The reusability of immobilized CRL was studied using the same substrate concentration, temperature, and reaction time as mentioned above. The initial activity of immobilized CRL was compared with the activity of the used CRL obtained after repeated use for 6 cycles.
3. Results and discussion

3.1. Structures and textural characterizations of VS-n

3.1.1. FESEM and TEM

Typical FESEM and TEM images of VS-0 and VS-1 are shown in Fig. 1. Most of the particles are intact spheres with diameters in the range of 20–400 nm and several particles are ruptured vesicular silicas (Fig. 1A and B), allowing one to see that they are hollow particles with relatively rough and thick shells. However, when VS-1 was prepared by adding a certain amount of TIPB, many particles appeared to be elongated, larger spherical shapes (Fig. 1B). TEM images illustrate the vesicular morphology of these particles. It is important to note that most of the particles are unilamellar silica vesicles but a few multilamellar (onion-like structure) silica vesicles exist (Fig. 1C). The dimensions of silica vesicles are polydisperse with diameters ranging from 20 nm to 400 nm, which is consistent with those observed in FESEM images. Furthermore, the vesicular structure has irregular hollow cavities and disordered wall structure. Interlayer spacing between two adjacent layers of multilamellar vesicular silica with multilayer shells is nearly 20 nm and layer thickness is about 5 nm (Fig. 1C). Therefore, the average pore diameter is large enough to entrap CRL (5 nm × 5 nm × 7 nm) molecules inside the pores. In contrast, VS-1 with multi-wall structures exhibits splintery vesicular silica because of the use of TIPB which can expand the pore sizes of the vesicles, as seen in Fig. 1D.

3.1.2. N2 adsorption–desorption isotherms

Fig. 2 shows the nitrogen adsorption–desorption isotherms of the various samples before and after CRL loading. All samples exhibit typical type II b isotherms with distinct H3-type hysteresis loops with capillary condensation at \( P/P_0 \) ranging from 0.45 to 0.99 [24]. The shapes of these sorption isotherms are similar to those observed for silica vesicles prepared by Lu et al. [25]. Moreover, the hysteresis loop that does not close until the saturation pressure is reached probably indicates the occurrence of desorption from the

![Fig. 1. FESEM and TEM images of the samples: (A) and (C) VS-0, (B) and (D) VS-1. The arrows in (A) and (B) point to the ruptured vesicular silicas. (C inset image is at a higher magnification).](image)

![Fig. 2. Nitrogen adsorption–desorption isotherms of the samples: (○) VS-0, (●) VS-0-CRL, (△) VS-1, and (▲) VS-1-CRL.](image)
slit-like pores between the walls of multilayer vesicular silica [26]. Fig. 2 also shows that the amount of nitrogen adsorbed in VS-1 or VS-1-CRL is markedly larger than that in VS-0 or VS-0-CRL before and after adsorption of CRL, respectively. It may be interpreted that the pore of VS-1 is enlarged by the use of TIPB, so it adsorbs much more amount of nitrogen, resulting in a relatively large pore volume. On the other hand, the amount of nitrogen adsorbed distinctly decreases in VS-1 (from 950 cm$^3$/g to 713 cm$^3$/g) and VS-0 (from 617 cm$^3$/g to 409 cm$^3$/g) upon CRL immobilization. Table 1 summarizes the textural properties of the diverse samples. One can see that the average pore diameter of VS-1 is larger than that of VS-0. Additionally, VS-1 exhibits a much larger $S_{BET}$ (362 m$^2$/g) than that of VS-0 (314 m$^2$/g), as well as a much larger total adsorption pore volume of 1.45 cm$^3$/g. Clearly, the $S_{BET}$ and $V_p$ of VS-0 and VS-1 sharply declined after CRL adsorption. Furthermore, immobilization of CRL led to an increase in the average pore diameter ($D_{bns}$) of VS-0 and VS-1 from 15.24 nm to 14.02 nm and from 20.51 nm to 17.98 nm, respectively. These results clearly indicate that most of the CRL molecules have been immobilized into the inner pores of vesicular silica [27].

### 3.1.3. FT-IR spectra

In order to further demonstrate that CRL has been adsorbed into the curved channels of silica vesicles, all the materials were analyzed by FT-IR spectra (Fig. 3). An intense absorption band at 1100 cm$^{-1}$ conforms to the asymmetric stretching vibration of Si–O–Si and the bands around 806 cm$^{-1}$ and 470 cm$^{-1}$ may reflect the symmetric stretching and deformation modes of Si–O–Si. The band between 3700 cm$^{-1}$ and 3200 cm$^{-1}$ and a dramatically weak peak at 966 cm$^{-1}$ could be attributed to Si–OH stretching vibration (silanol group) and stretching vibration of hydroxyl in water or hydroxyl at the surface of the silica. In CRL immobilized on vesicular silica, the characteristic peaks at around 1647 cm$^{-1}$ and 1540 cm$^{-1}$ are associated with C=O stretching and N–H deformation vibration (from –CONH– in CRL molecules). Furthermore, stretching vibrations from C–H at about 2930 cm$^{-1}$ were also observed in the spectra of free and immobilized CRL, but not in the spectra of raw materials. These results further confirm that CRL has been successfully adsorbed into vesicular silicas.

### 3.2. Mechanism for the formation of VS and immobilization of CRL into VS

The proposed structures and mechanism for the formation of VS-1 and immobilization of CRL into VS-1 is illustrated in Fig. 4. In the catanionic vesicle system, both hydrophobic and electrostatic interactions between the cationic and anionic surfactants drive the formation of the stable unilamellar and multilamellar vesicle aggregates. When TIPB, which acts as an expander, is added, it can interact with the hydrophobic chains of CTAB and SDS. The interaction enlarges the total volume of the hydrophobic chains, and then increases the value of the surfactant packing parameter $g$, which depends on the molecular geometry of the surfactant, as shown below:

$$g = \frac{V}{d_0 \times l_c}$$

where $V$ is the total volume of the hydrophobic chains in the catanionic surfactant, $d_0$ is the effective head surface area, and $l_c$ is the critical length of the hydrophobic tail.

When $g$ is in the range of 0.50–1.0, surfactant can form a vesicle phase. The majority of larger pore VS-1 with hierarchical structure is finally obtained by hydrolysis and polycondensation of TEOS and calcination. Then, van der Waals interaction, hydrophobic interaction, and hydrogen bonds form between the Si–OH groups on the silica surface and the residual groups on the amino acids side chains of lipase, which play a key role in the physical adsorption. Results of loading amounts and activities of immobilized CRL are listed in Table 2. It is noteworthy that VS-1 tends to load more lipase than VS-0 and the immobilization yield and enzyme activity of VS-1-CRL are larger than those of VS-0-CRL. The significant differences in the amounts of CRL adsorbed and the activities of CRL between VS-0 and VS-1 can be attributed to their pore sizes. The pore diameter of VS-1 is larger, which may improve the transport of the CRL molecule, making it favorable for the enzymatic reaction [28]. As expected, enzyme activities of immobilized lipases are higher than that of free lipases. This is in accordance with the results reported by Macario et al. [29]. The enhanced enzyme activity may be explained that the pores have enough exposed surface area to prevent protein denaturation and facilitate mass transport and substrate access to the immobilized enzyme. The structure of vesicular silica may also provide a beneficial confined space and thereby reinforce the trend toward favorable conformational changes, yielding much enhanced enzyme activity.

### 3.3. Optimum conditions for enzymatic activity

#### 3.3.1. Effect of pH on free and immobilized CRL activities

The effect of solution pH on $E_d$ and $R$ of CRL, VS-0-CRL and VS-1-CRL for triacetin hydrolysis was studied by varying pH from 5.0 to 9.0 at 35 °C (Fig. 5a). Our results clearly indicate that the optimal pH for CRL is 7.0, whereas the optimal pH for VS-0-CRL and VS-1-CRL shift to 8.0. Furthermore, VS-0-CRL and VS-1-CRL could retain about 63% of their highest enzyme activity in the range of pH

**Table 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$S_{BET}$ (m$^2$/g)</th>
<th>$V_p$ (cm$^3$/g)$^a$</th>
<th>$D_{bns}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS-0</td>
<td>314</td>
<td>0.92</td>
<td>15.24</td>
</tr>
<tr>
<td>VS-0-CRL</td>
<td>167</td>
<td>0.62</td>
<td>14.02</td>
</tr>
<tr>
<td>VS-1</td>
<td>362</td>
<td>1.45</td>
<td>20.51</td>
</tr>
<tr>
<td>VS-1-CRL</td>
<td>209</td>
<td>1.07</td>
<td>17.98</td>
</tr>
</tbody>
</table>

$^a$ Pore volume was determined by nitrogen adsorption volume at the relative pressure of 0.99.

**Table 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Immobilization yield (%)</th>
<th>$P$ (mg substrate/g protein)</th>
<th>Activity (U/g protein)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL</td>
<td>–</td>
<td>–</td>
<td>3066.889</td>
</tr>
<tr>
<td>VS-0-CRL</td>
<td>89.480</td>
<td>44.716</td>
<td>11456.139</td>
</tr>
<tr>
<td>VS-1-CRL</td>
<td>95.793</td>
<td>47.871</td>
<td>15186.647</td>
</tr>
</tbody>
</table>

$^a$ The activities were measured at the optimum catalysis condition (pH 7.0, 35 °C for CRL; pH 8.0, 50 °C for VS-0-CRL; and pH 8.0, 60 °C for VS-1-CRL).
7.0–9.0, while only 33% of its enzyme activity was retained by CRL at pH 9.0. These results indicate that immobilized CRL possesses excellent adaptability over a wider pH range compared to free CRL. A possible explanation for this observation is that polyionic matrices cause the partitioning of protons between the bulk phase and enzyme microenvironment causing a shift in the optimum pH value [30]. Additionally, hydrogen bonds formed between the support surface and CRL protect the active sites from being directly exposed to the medium and thus limit the sensitivity of immobilized lipase to a higher pH [31]. The screen function of the structure would also play an important role in enhancement of the adaptability [32].

3.3.2. Effect of temperature on free and immobilized CRL activities

Fig. 5b demonstrates the effect of temperature on enzyme activities of CRL, VS-0-CRL and VS-1-CRL within the range of 30–70 °C. It can be seen that the optimum temperature for lipase activity shifts from 35 °C for CRL to 50 °C and 60 °C for VS-0-CRL and VS-1-CRL, respectively. In other words, the immobilized lipases have better resistance to thermal denaturation than free lipases between 30 °C and 70 °C. A similar change in the optimum temperature upon immobilization of lipases has been reported by other researchers [33,34]. In addition, immobilized lipases have broader endurance for higher temperature compared to free lipases. VS-0-CRL and VS-1-CRL hold about 61% and 82% of their highest activities at 70 °C, respectively, while CRL retains only 22% of its highest activity under the same condition. This effect can be attributed to the increased protection afforded by the hydrogen bond and electrostatic interaction between the enzyme and the support [35].

3.3.3. Thermal stability of free and immobilized CRL

Thermal stability of enzymes is important for bioprocesses operated at high temperature in industrial application to improve productivity and to lower production costs. In order to survey the thermal stability of immobilized lipase, the relative activities of free and immobilized CRL for catalytic hydrolysis of triacetin were assessed by incubating the lipase at 60 °C for different time intervals (Fig. 6a). After 1.5 h of incubation, VS-0-CRL and VS-1-CRL maintained more than 59% of their respective initial activities, while CRL lost 59% of its activity after 1.5 h. This indicates that the immobilization procedure enhances the thermal stability of the lipase. Similarly, Nara et al. [36] reported that racemase adsorbed on folded-sheet mesoporous silica showed higher thermal stability than free racemase. The excellent thermal stability of immobilized lipase could be attributed to the architecture of the support which may provide protection to the enzyme molecules from direct exposure to high temperature.
were compared with those of free CRL. The significant differences in the amounts of CRL adsorbed and the activities of CRL between VS-0 and VS-1 can be attributed to their pore sizes. The sample VS-1-CRL is more stable than the sample VS-0-CRL. It may be because that the added TIPB increases the pore size of VS-1, which may improve the transport of the CRL molecule, making it favorable for the enzymatic reaction. VS-1-CRL was found to be superior to both free CRL and VS-0-CRL, making it the best option for industrial use in separation, catalysis, and drug delivery. However, both two catalysts had low stability and the further cross-linking among the adsorbed CRL in supports would be attempted to strengthen the stability of immobilized CRL.

Acknowledgments

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References

[25] Lu T, Yao XD, Lu GQ, He YH. Controlled evolution from multilamellar vesicles to hexagonal mesostructures through the addition of 1,3,5-trimethylbenzene. J Colloid Interface Sci 2009;336:368–73.