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Incorporating Mobile Nanospheres in the Lumen of Hybrid Microcapsules for Enhanced Enzymatic Activity

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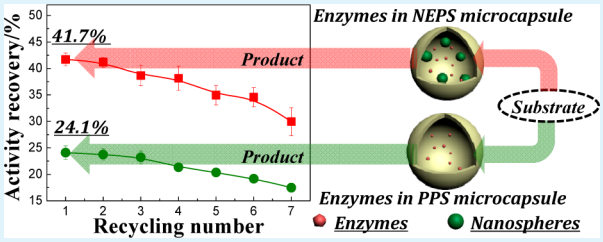
Supporting Information

ABSTRACT: Physical encapsulation of enzymes in microcapsules, as a mild, controllable method, has been widely utilized for enzyme immobilization. However, this method often suffers from the big mass transfer resistance from the capsule lumen. In this study, a novel biocatalysis system with enhanced catalytic activity is constructed through coencapsulating enzymes and nanospheres in the lumen of protamine/silica hybrid microcapsules, which are synthesized through the synergy of biomimetic silicification and layer-by-layer (LbL) assembly. When utilized as the host for catalase (CAT) encapsulation, the hybrid microcapsules maintain high mechanical stability, high enzyme loading, and low enzyme leaching. Particularly, because of the existence of mobile nanospheres, the mass transfer resistance in the microcapsules is significantly reduced because of the vigorous agitation, thus acquiring an enhanced catalytic activity. Our strategy may also find applications in drug delivery and biosensor fields.



KEYWORDS: *biocatalysis system, hybrid microcapsules, mobile nanospheres, enhanced mass transfer rate, enzymes*

INTRODUCTION

Enzymes are nature's sustainable catalysts, which can catalyze variety of chemical reactions with impressive levels of regio-, chemo-, and stereoselectivity.[1−3] However, the industrial application of enzymes in many fields is often hampered by their low operational stability and difficulties in recovery and reuse.[4,5] Immobilization of enzymes on varies scaffolds (e.g., nano/microparticles,[6,7] gels,[8] films,[9] etc.) provides a straightforward approach to overcome these drawbacks.[10−12] Particularly, during the past decades, microcapsules have proven to be effective supports for enzyme immobilization because they can maintain the encapsulated enzyme in the free state and protect enzymes from leakage/environmental attacks, thus significantly enhancing the enzyme stability.[13−16] Nevertheless, the enclosed system usually has a low mass transfer rate for the substrate(s)/product(s), which then results in a relative lower enzymatic activity. Theoretically, for a catalytic reaction enabled by enzyme-containing microcapsules, the total mass transfer resistance during the reaction process is composed of the following three parts: $N_{\text{bulk solution}}$ (mass transfer resistance resulting from the bulk solution), $N_{\text{capsule wall}}$ (mass transfer resistance resulting from the capsule wall), and $N_{\text{capsule lumen}}$ (mass transfer resistance resulting from the capsule lumen).[17,18] In general, $N_{\text{bulk solution}}$ can be diminished through increasing the magnetic/mechanical stirring. To decrease the mass transfer resistance through the capsule wall (that is, to decrease $N_{\text{capsule wall}}$), some efforts have been devoted to generating nanopores within the capsule wall by using surfactants or nanoparticles as sacrificial templates.[17,19] However, to the best of our knowledge, rare investigations focus on decreasing $N_{\text{capsule lumen}}$, which are primarily dependent on the flow state of the encapsulated solution (the solution in the lumen of microcapsules). Therefore, developing a novel and efficient approach to improve the flow state of the solution in the capsule lumen may open an alternative and promising way for increasing the mass transfer property and finally enhancing the catalytic activity.

In the present study, we constructed a novel enzymatic catalysis system enabled by nanoparticles-encapsulated protamine/silica (NEPS) microcapsule scaffolds. Specifically, hydrophilic silica nanospheres and enzymes are entrapped during the synthesis of $CaCO_3$ microparticles, which are then utilized as sacrificial templates for alternatively depositing the positively charged protamine layer and the negatively charged silica layer. During the deposition process, the protamine layer induces the hydrolysis and condensation of sodium silicate to form the silica layer, which allows a new cycle of deposition step of the protamine layer. Up to a desirable assembled layer number, the protamine/silica-coated $CaCO_3$ microparticles are acquired and then treated with ethylene diamine tetraacetic acid

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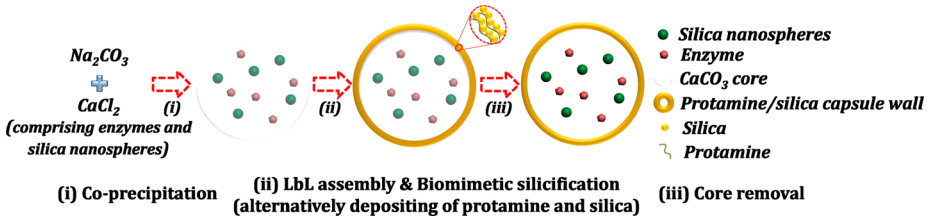
Scheme 1. Fabrication Process of Enzyme-Containing NEPS Microcapsules



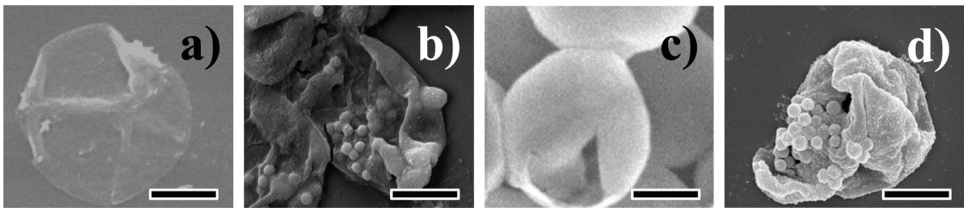


Figure 1. SEM images of (b, d) NEPS and (a, c) PPS microcapsules with (a, b) one and (c, d) two protamine/silica layers (scale bar: 2 μm).

(EDTA) to remove $CaCO_3$. Finally, enzyme-encapsulated NEPS microcapsules are obtained. Taking catalase (CAT) as the model enzyme for the synthesis of enzyme-encapsulated NEPS microcapsules, the resultant biocatalysts exhibit desirable stability as a result of the hybrid composition of the capsule wall; and show enhanced catalytic activity in comparison to enzyme-encapsulated pristine protamine/silica (PPS) microcapsules due to the improved flow state of the encapsulated solution.

RESULTS AND DISCUSSION

In general, the hierarchical NEPS microcapsules as well as PPS microcapsules are both synthesized by using synergy of biomimetic silicification and LbL assembly as described in the Experimental Section (see the Supporting Information). The synthesis procedure of enzyme-encapsuled NEPS microcapsules is illustrated in Scheme 1. Briefly, the $CaCO_3$ microparticles (the sacrificial templates) were first synthesized through coentrapping the hydrophilic silica nanospheres and enzymes during the coprecipitation of $CaCl_2$ and Na_2CO_3. Subsequently, the fabrication of NEPS microcapsules was initiated with the deposition of the protamine layer onto the surface of the $CaCO_3$ microparticles. The protamine-coated $CaCO_3$ microparticles were then kept in contact with the sodium silicate solution. The positively charged protamine layer attracted the silicate anions in the solution via electrostatic interactions, which led to an increased silicate concentration around the protamine layer, and the silicification process was triggered. Finally, a protamine/silica bilayer was built-up. The silica layer with negative charges triggered a new cycle of protamine deposition. The alternative assembly of protamine layer and silica layer was verified by monitoring the surface zeta-potentials of $CaCO_3$ microparticels coated with $(protamine/silica)_x$ bilayers (see Figure S1 in the Supporting Information). And, it was worth mentioning that the reason for fabricating PPS microcapsules is to clarify the influence of enclosed nanospheres on the morphology and permeability of NEPS microcapsules. Figure 1 presented the morphologies of the as-acquired NEPS and PPS microcapsules with different layer number. Seen from these figures, the number of the protamine/silica bilayers was found to significantly influence the morphology of the resulting capules.[13] More specifically, the microcapsules with one protamine/silica bilayer were in a collapsed state, and numerous folds and creases were observed on the surface of the microcapsules (Figure 1a), suggesting the hollow structure and low mechanical strength of the capsule wall. However, the microcapsules with two protamine/silica bilayers displayed a relatively intact spherical shape. The variation in the morphology of the microcapsules with different bilayers of protamine/silica revealed the tunability in mechanical properties. Additionally, the average diameter of the frozen-dried microcapsules was approximately 5 μm, consistent with the size of $CaCO_3$ microparticles. Notably, in comparison to PPS microcapsules, the existence of nanospheres in the interior of NEPS microcapsules verified their successful formation (Figure 1b, d). Besides, it could be found, from Figure 1b and Figure S2 in the Supporting Information, ca. 10–20 nanospheres were encapsulated in a single microcapsule. Meanwhile, few nanospheres were located in the capsule-free regions, indicating majority of the nanospheres were encapsulated in the microcapsules.

Besides the morphological characterizations, the chemical compositions of the NEPS and PPS microcapsules were also characterized through FTIR as shown in Figure 2. Specifically,

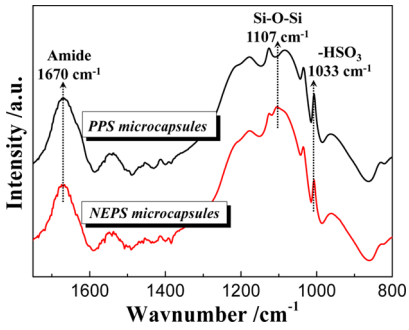


Figure 2. FTIR spectra of NEPS and PPS microcapsules.

the FTIR spectra for both kinds of microcapsules had a distinct peak at 1670 cm^{-1}, which was the characteristic of primary amines originated from the deposited protamine layers. Peaks arising from vibrations of Si–O containing species were identified at 1107 cm^{-1}, which were corresponded to Si–O–Si (symmetric stretching) and Si–O–Si (asymmetric stretching) bonds. This demonstrated that silica existed in both kinds

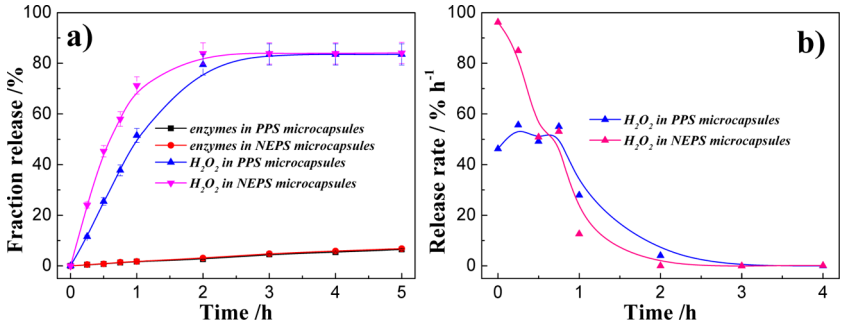


Figure 3. (a) Release profile of enzyme (CAT) and H_2O_2 from NEPS and PPS microcapsules; and (b) release rate of H_2O_2 from NEPS and PPS microcapsules.

Table 1. Several Immobilization Parameters[a] and Kinetic Parameters of Free and Immobilized Enzymes

	K_m (mM)	V_{max} (mM min^{-1})	immobilization yield[a] (%)	immobilization efficiency[a] (%)	activity recovery[a] (%)
free enzyme	50.7	19.61			
enzyme-encapsulated NEPS microcapsules	56.9	3.86	97.5	42.8	41.7
enzyme-encapsulated PPS microcapsules	53.0	2.92	98.4	24.5	24.1

[a]Specific definition of several immobilization parameters, including immobilization yield, immobilization efficiency, and activity recovery could be found in the Supporting Information.

of microcapsules. In particular, once the peak of $-HSO_3$ groups at 1033 cm^{-1} was normalized, it could be found the NEPS microcapsules possessed a stronger adsorption peak at 1107 cm^{-1}, which provided an alternative evidence of the successful encapsulation of silica nanospheres.

When utilized as the host for enzyme encapsulation, the NEPS microcapsules must serve two critical functions: (1) the capsule wall should prevent enzymes from leaching; (2) substrate(s) and product(s) could freely diffuse inside and outside the microcapsules. Hence, the release profiles of enzymes (CAT, ~9.2 nm) and the substrates (H_2O_2, <1 nm) from the capsule lumen to the bulk solution were investigated (Figure 3). For CAT, both NEPS and PPS microcapsules leaked only about 5% protein within 5 h. Therefore, it can be conjectured that the capsule wall can effectively prevent enzymes from leaching. Since the dynamics radius of H_2O_2 was smaller than 1 nm, its permeability through NEPS and PPS microcapsules may directly affect the catalytic behavior of the biocatalysis systems constructed upon these two kinds of microcapsules. As illustrated in Figure 3a, H_2O_2 transferred much faster through NEPS microcapsules compared to PPS microcapsules. The maximal difference exceeded nearly 20% once the release time prolonged to ca. 1 h. After calculation, the release rate of H_2O_2 was plotted in Figure 3b. Obviously, the initial release rate of H_2O_2 for NEPS microcapsules (94% h^{-1}) exhibited almost two times higher than that for PPS microcapsules (47% h^{-1}). Because the only difference between NEPS microcapsules and PPS microcapsules was the existence of enclosed mobile nanospheres, the quicker release rate would be mainly attributed to the enhanced flow of the encapsulated solution. Generally, this enhanced release rate could lead to increased catalytic activity. Correspondingly, a comparative study on the apparent kinetic parameters (K_m, V_{max}) of the reaction has been carried out among the enzyme-encapsulated NEPS, enzyme-encapsulated PPS and free enzymes. The higher value of K_m could be observed for the immobilized enzymes (Table 1) in comparison to that for the free enzymes, indicating the lower affinity between enzymes and substrates. Meanwhile, it was found that, after encapsulated in the microcapsules, the maximal reaction rate (V_{max}) of both immobilized enzymes decreased, exhibiting a V_{max} of 3.86 mM min^{-1} (enzyme-encapsulated NEPS microcapsules) and 2.92 mM min^{-1} (enzyme-encapsulated PPS microcapsules), respectively. Because the enzyme encapsulation process was performed under rather mild conditions, such as neutral pH, aqueous solution and room temperature,[6,20] and the capsule wall materials were composed of biocompatible protamine and silica,[21] the decrease in catalytic rate could be ascribed to the diffusion limitation of substrate(s)/product(s) other than the denaturation of enzymes. Besides, it was also observed that the V_{max} of enzyme-encapsulated NEPS microcapsules was about 1.3 times higher than that of enzyme-encapsulated PPS microcapsules. To further clarify the influence of enclosed mobile nanospheres on the catalytic performance, we investigated the activity recovery for both immobilized enzymes. Obviously, the activity recovery of enzyme-encapsulated NEPS microcapsules (41.7%) was much higher than that of enzyme-encapsulated PPS microcapsules (24.1%). This enhanced catalytic activity (V_{max} and activity recovery) should be attributed to the following reason: the enclosed silica nanospheres could improve the flow state of the encapsulated solution, which would lead to an enhanced mass transfer rate for small molecules (that is decreasing $N_{capsule\ lumen}$ as proven in Figure 3), finally leading to the increased catalytic rate (V_{max}) and higher activity recovery.

For practical applications, operational stability was quite essential and worthy of investigation. Especially, for the catalytic reaction conducted by enzyme-encapsulated NEPS microcapsules, mechanical strength of the capsule wall must be high enough to resist both the external shearing forces generated by magnetic stirring and internal collision from nanospheres movement. Therefore, we demonstrated the recycling stability of CAT-encapsulated NEPS as well as PPS microcapsules (Figure 4a). Generally, both of the CAT-encapsulated microcapsules maintained high recycling stabilities. Specifically, the CAT-encapsulated NEPS microcapsules lost only 29% (the activity recovery was decreased from 41.7 to 30.0%) of the initial activity after seven cycles, which was in comparable to that of CAT-encapsulated PPS microcapsules (28% of its initial activity was lost: the activity recovery was decreased from 24.1 to 17.5%). This desirable performance

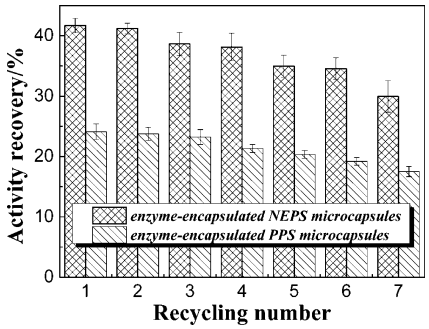


Figure 4. Recycling stability of CAT-encapsulated NEPS and PPS microcapsules.

could be mainly ascribed to the two following aspects: (1) the mechanical robustness of hybrid capsule wall ensured the resistance of external and internal impact, (2) the appropriate pore size in the capsule wall minimized the leakage of enzymes from the internal capsule lumen.

Collectively, similar to previous pristine protamine/titania (PPT) hybrid microcapsules synthesized through biomimetic silicification and LbL assembly,[13] the merits of this system included the following points: (i) the fabrication process was conducted in aqueous solution at neutral pH and room temperature; (ii) the leakage of CAT could be effectively inhibited by appropriately manipulating the number of protamine/silica bilayers; (iii) the strong mechanical strength of the hybrid capsule wall rendered the enzyme-encapsulated microcapsules with high operational stability. Besides, in comparison to PPS or PPT microcapsules, the significant advantages for the NEPS microcapsules was that the enclosed silica nanospheres could improve the flow state of the encapsulated solution, leading to an enhanced mass transfer rate, and consequently an increased catalytic activity.

CONCLUSIONS

In summary, we have constructed a novel and efficient biocatalysis system through coencapsulating nanospheres and enzymes in the lumen of protamine/silica microcapsules. The enclosed mobile nanospheres could lead to improved flow state of the encapsulated solution through vigorous agitation, subsequently enhancing catalytic activity of the biocatalysis system, whereas the mechanical robustness of the hybrid capsule wall could render this NEPS microcapsule with desirable stability, including recycling stability and storage stability. Besides biocatalysis, the merits of this hierarchical architecture will make it highly versatile for a broad range of other applications such as biosensoring, drug/gene delivery, etc.

ASSOCIATED CONTENT

Supporting Information

Experimental details and additional experimental data including zeta potential of $CaCO_3$ microparticles coated with different protamine/silica layers; the overview (SEM images) of NEPS microcapsules with one protamine/silica bilayer; and SEM images of NEPS microcapsules with two protamine/silica bilayers, which were frozen dried and manually grounded are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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