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Interfacial Polymerization of Dopamine in a Pickering Emulsion: Synthesis of Cross-Linkable Colloidosomes and Enzyme Immobilization at Oil/Water Interfaces

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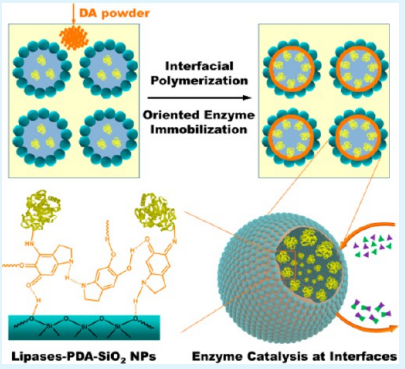
Supporting Information

ABSTRACT: Colloidosomes are promising carriers for immobilizing enzyme for catalytic purposes in aqueous/organic media. However, they often suffer from one or more problems regarding catalytic performance, stability, and recyclability. Here, we report a novel approach for the synthesis of cross-linkable colloidosomes by the selective polymerization of dopamine at oil/water interfaces in a Pickering emulsion. An efficient enzyme immobilization method was further developed by covalently bonding enzymes to the polydopamine (PDA) layer along with the formation of such colloidosomes with lipase as a model enzyme. In this enzyme system, the PDA layer served as a cross-linking layer and enzyme support for simultaneously enhancing the colloidosomes' stability and improving surface availability of the enzymes for catalytic reaction. It was found that the specific activity of lipases immobilized on the colloidosome shells was 8 and 1.4 times higher than that of free lipase and encapsulated lipase positioned in the aqueous cores of colloidosomes, respectively. Moreover, the immobilized lipases demonstrated excellent operational stability and recyclability, retaining 86.6% of enzyme activity after 15 cycles. It is therefore reasonable to expect that this novel approach for enzyme immobilization has great potential to serve as an important technique for the construction of biocatalytic systems.



KEYWORDS: *Pickering emulsion, colloidosomes, silica nanoparticles, poly(dopamine), enzyme immobilization*

INTRODUCTION

Enzymes are extremely attractive as biocatalysts for the green synthesis of chemicals and pharmaceuticals with high chemo-, stereo-, and regioselectivity.[1−3] In many cases, enzyme catalysis is performed in organic or organic-aqueous media, which leads to the low stability of free enzymes.[4,5] It is generally accepted that immobilization of enzymes is convenient for enhancing enzyme stability and facilitating enzyme recycling in practical biocatalytic synthesis.[6−9] Among various enzyme immobilization methods, encapsulation of enzymes is a simple, gentle, and general method that creates an aqueous microenvironment to enhance enzymatic activity in organic media.[10−12] Recently, numerous efforts have focused on preparing various capsules for enzyme immobilization, such as inorganic capsules,[10] organic capsules,[13,14] organic−inorganic hybrid microcapsules,[15−17] and colloidosomes (or capsules) enclosed by closely packed colloidal particles in Pickering emulsions.[5,18,19] Among these, the nanoparticle-stabilized capsule has proven to be a promising carrier for the immobilization of enzymes for catalysis purposes in aqueous/organic media.

A simple method to encapsulate enzymes within colloidosomes is to use nanoparticles with specific surface properties for stabilizing emulsions and the colloidosomes derived therefrom. One of the first investigations on this subject was carried out by Wu et al.,[18] who used hydrophobic SiO_2 nanoparticles to emulsify aqueous solutions of enzymes in organic reaction media. In this case, the specific activity of encapsulated CalB (lipase B from *Candida antarctica*) is more than 300 times higher than that of free enzymes for the esterification of 1-octanol and octanoic acid. Recently, we reported a monolayer capsule and a Pickering emulsion for the encapsulation of enzymes for catalysis purposes in organic media using amphiphilic silica Janus particles (JPs) as building blocks.[19]

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The irreversible physical attachment of nanoparticles at oil/water interfaces led to the high stability of the colloidosomes during the reaction process. Nevertheless, when the organic solvent is removed during the centrifugation operation, the colloidosomes are easily collapsed due to the loss of thermodynamic equilibrium and the strong hydraulic shear force, leading to enzyme leakage and unsatisfied recyclability. A promising strategy to overcome this drawback is to chemically link the nanoparticles during/after the formation of colloidosomes.[5,20−25] For instance, Morse et al.[23] used an oil-soluble polymer, tolylene 2,4-diisocyanate terminated poly(propylene glycol), as a cross-linker to immobilize the poly[2-(tertbutylamino) ethyl methacrylate] latex particles adsorbed at the *n*-dodecane/water interface, which significantly improved the resistance against acid-induced demulsification. Moreover, in such enzyme immobilization systems, enzymes are generally encapsulated in their free state in the aqueous cores of the colloidosomes.[18−20] In these cases, although the interfacial cross-linking enables us to enhance the colloidosomes' stability, it inevitably increases the mass transfer limitation of substrates and/or products through the capsule shells and thus reduces the catalytic efficiency. Therefore, there exists a dilemma in the development of colloidosomes for direct encapsulation of enzymes: while high recyclability requires a cross-linking layer between nanoparticles, high catalytic efficiency demands no blocking of the interparticle pores.

Oriented enzyme immobilization toward the aqueous phase at oil/water interfaces should be a promising strategy to solve this challenging problem. Previously, Samanta et al.[26] demonstrated that the enzyme−nanoparticle conjugates at oil−water interfaces provided an ideal geometry for the generation of biocatalysts, which retain the surface availability of the enzymes for catalytic reaction. Wang et al.[27] constructed a hollow polymersome-stabilized colloidosome for application in biphasic enzymatic catalysis. The encapsulated CalB inside the polymersome lumen had a high specific activity of up to 70.8 U mg $^{-1}$, which is 2.8 times higher than that of CalB positioned inside colloidosomes. Inspired by these architectures, we attempt to construct a novel stable colloidosome with a cross-linking layer, which enables us to simultaneously immobilize enzymes toward the aqueous core at oil/water interfaces.

Dopamine is a well-known biomolecule that polymerizes at alkaline pH to form adherent polydopamine (PDA) for coating diverse substrates, such as metals, metal oxides, and silica.[28−31] Catechol/quinone in PDA is the only functional element required for adhesion via different interaction forms (e.g., chelation bonding, hydrogen bonding) of catechol/quinone groups with surfaces.[30,32] On this basis, various colloidal particles were used as templates for the formation of PDA layers (or hollow capsules after template removal) at the aqueous-solid interfaces, for instance, SiO_2,[33,34] TiO_2,[35] and $CaCO_3$ particles.[36] In addition to solid templates, oil-in-water emulsion droplets were also utilized as soft templates for polymerization of dopamine at the aqueous-oil interfaces.[37,38] Xu et al.[38] demonstrated that strong interfacial basicity in oil-in-water emulsions significantly contributed to self-polymerization of dopamine preferentially occurring on the surfaces of the oil droplets. However, when surfactants were used to stabilize emulsions, the interfacial basicity was significantly reduced, leading to dopamine polymerization occurring in the aqueous phase. This finding suggests that it is a challenge to promote selective growth of the PDA layer at the oil/water Pickering emulsion interfaces due to the presence of surfactant-like nanoparticles. Indeed, to date, there have been no reports regarding the application of a Pickering emulsion as a template for directing the polymerization of dopamine at oil/water interfaces. To construct cross-linkable colloidosomes for oriented enzyme immobilization, as mentioned before, selective growth of PDA at Pickering emulsion interfaces is a prerequisite for enhancing the colloidosomes' recyclability and covalently immobilizing enzymes at oil/water interfaces via the quinone-amine reaction.

Herein, we develop a novel and facile approach for the synthesis of highly stable colloidosomes by the interfacial polymerization of dopamine at oil/water interfaces in a Pickering emulsion. (Octyl)-trimethoxysilane modified hydrophobic SiO_2 NPs were synthesized via a one-pot coprecipitation method as described previously.[39] We use such silica NPs to emulsify an aqueous solution in an organic medium, resulting in the formation of a Pickering emulsion composed of SiO_2 NPs-stabilized droplets (SiNDs). Then, dopamine powder is directly added into the emulsion for selective polymerization at oil/water interfaces, giving rise to cross-linkable and stable SiO_2 NPs/PDA colloidosomes (SiN/PDACs). Furthermore, we develop a simple and efficient enzyme immobilization method by covalently bonding enzymes to the PDA layer at oil/water interfaces along with the formation of such SiN/PDACs with lipase from *Candida sp.* as a model enzyme. The high catalytic performance and recyclability of the immobilized enzyme are demonstrated in the esterification of 1-hexanol and hexanoic acid in heptane/water media.

EXPERIMENTAL SECTION

Materials. Dopamine hydrochloride, fluorescein is othiocyanate (FITC), and lipase from *Candida sp.* expressed in *Aspergillus niger* (the specific activity for the first 1 h was measured to be 5.78 U mL^{-1} in the esterification of 1-hexanol with hexanoic acid in a water/heptane system) were purchased from Sigma-Aldrich. FITC-labeled lipases were prepared by overnight incubation at 4 °C in phosphate buffer (0.05 M, pH 8.0), followed by dialysis (molecular weight cutoff 8−14 kDa) against deionized water for 72 h. Tris(hydroxymethyl)-aminomethane (Tris), hydrochloric acid (HCl, 36−38 wt %) of reagent grade quality, and *n*-heptane of chromatographic grade quality were obtained from Guangfu Chemical Co. (Tianjin, China). Tetraethyl orthosilicate (TEOS), hexanoic acid, 1-hexanol, (octyl)-trimethoxysilane, and hexyl hexanoate were brought from Aladdin Industrial Corp. (Shanghai, China). All of the other chemicals, such as anhydrous ethanol, aqueous ammonia ($NH_3 \cdot H_2O$, 25 wt %) and aqueous hydrogen peroxide solution (H_2O_2, 30 wt %) were of analytical grade and were obtained from commercial sources.

Synthesis of Hydrophobic Silica Nanoparticles. The hydrophobic silica nanoparticles were synthesized by a coprecipitation method as described previously.[39] Briefly, the tetraethyl orthosilicate (TEOS) solution was freshly prepared by dissolving tetraethyl orthosilicate (4.65 g) in anhydrous ethanol (5.89 mL). Then, 8.1 mL of aqueous ammonia solution was mixed under vigorous shaking with 14 mL of deionized water and 200 mL anhydrous ethanol at 40 °C. Subsequently, the as-prepared TEOS solution (10.5 mL) was added dropwise to the ammonia/water/ethanol mixture (222 mL) within 3 min at 40 °C and 800 rpm. After a prehydrolysis period of 5 min, a total of 650 mg (octyl)-trimethoxysilane was added to the resulting mixture at regular intervals of 1 min (162.5 mg each time). After 30 min of incubation, the white precipitate was collected by centrifugation (10 000 rpm, 20 min) and repeatedly washed with deionized water until reaching neutral pH.

Preparation of the Silica Nanoparticles/Polydopamine Colloidosomes (SiN/PDACs). In a typical experiment, 20 mg of hydrophobic silica nanoparticles (NPs) was dispersed in 2 mL of

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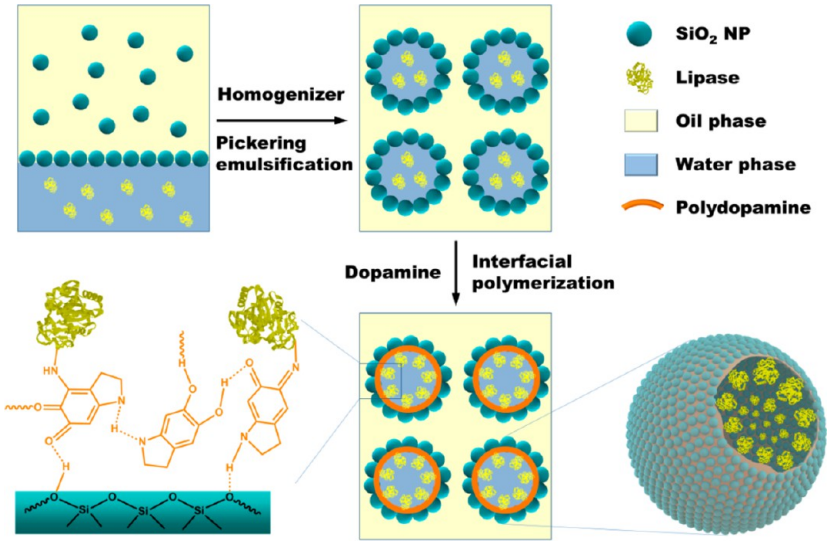
Scheme 1. Schematic Illustration of the Synthesis of Enzymes Bonding SiN/PDACs via the Simultaneously Selective Polymerization and Covalent Linking Enzymes at Oil/Water Interfaces in a Hydrophobic Silica NP-Stabilized Pickering Emulsion



heptane under ultrasonication for 10 min. Then, 333 μL of Tris buffer (10 mM, pH 8.5) was added to the silica NPs dispersion (the volume ratio of the oil to water, $R_{o/w}$, is 6). The resulting mixture was vigorously stirred in a modular homogenizer at 20 000 rpm for 2 min, giving rise to a white emulsion composed of silica nanoparticle-stabilized droplets (SiNDs). After the formation of a Pickering emulsion, 0.67 mg dopamine powder was added to the emulsion, followed by homogenization at 20 000 rpm for 1 min and incubation at 28 °C and 180 rpm for 24 h, giving rise to a dark gray emulsion composed of a large number of silica NPs/polydopamine colloidosomes (SiN/PDACs).

Preparation of the Silica Nanoparticles/Fluorescently Labeled PDA (F-PDA) Colloidosomes (SiN/F-PDACs). In a typical experiment, 2.2 mL of silica NP-stabilized Pickering emulsion (or SiNDs) at $R_{o/w}$ = 10 was prepared by following the procedure as described above. Then, 100 μL Tris buffer (10 mM, pH 8.5) was added into the resulting emulsions, followed by homogenization at 20 000 rpm for 1 min. It is worth noting that the volume of Tris buffer is the same as that of dopamine solution used for comparison. Then, 0.5 mg of dopamine powder was added, and the emulsion was homogenized again and allowed to prepolymerize for 1 h. According to the fluorescent labeling method previously described, 75 μL of aqueous H_2O_2 solution was added to the mixture, which was homogenized again and then allowed to react for another 15 h at 20 °C. The obtained SiN/F-PDACs were collected by centrifugation for subsequent fluorescence analysis. For comparison, the silica NPs/polydopamine stabilized emulsion (SiN/PDACs) was also used as the starting emulsion to confirm the further polymerization of dopamine at oil/water interfaces. In addition, the dopamine solution with the same dopamine dosage as given before, prepared by dissolving dopamine in Tris-buffer (10 mM, pH 8.5), was used to form SiN/F-PDACs instead of dopamine powder.

Immobilization of Enzyme at Oil/Water Interfaces along with the Formation of SiN/PDACs. First, 20 μL aqueous stock solution of lipases (obtained from Sigma without any treatment) was added to 313 μL Tris-buffer (10 mM, pH 8.5) solution. Subsequently, the resulting lipase solution (333 μL) was added to 2 mL of heptane solution containing 20 mg of well-dispersed hydrophobic silica NPs, followed by homogenization at 20 000 rpm for 2 min. After encapsulation of lipases within SiNDs, 0.67 mg dopamine powder was added to the Pickering emulsion, followed by homogenization at 20,000 rpm for 1 min and incubation at 28 °C and 180 rpm for 24 h, as described above. The reinforced Pickering emulsion composed of lipase-loaded SiN/PDACs (lipase-SiN/PDACs) was formed and used for subsequent experiments. For comparison, the dosage of Tris-buffer, silica nanoparticles and dopamine was optimized, while the enzyme loading was held constant at 20 μL.

Assessment of the Catalytic Performance of Free and Immobilized Lipases. The catalytic performance of free and immobilized lipases was investigated via the esterification of 1-hexanol with hexanoic acid in heptane/water media. Specifically, 2 mL of heptane solution containing 400 mmol L^{-1} 1-hexanol and 400 mmol L^{-1} hexanoic acid was added into a Pickering emulsion (2.333 mL) containing lipase-SiN/PDACs prepared as before. For comparison, the free lipases, a mixture of free lipases and dopamine, and lipase-SiNDs were also used to catalyze this esterification reaction. The default conditions unless otherwise noted were the same as given for lipase-SiN/PDACs, such as substrate concentration, lipase dosage, and $R_{o/w}$. All esterification reactions were carried out on a rotating shaker at 37 °C and 200 rpm for the appropriate amount of time. Aaliquots of 1 μL were taken out at different time points for gas chromatography (GC) analysis. All reactions were repeated three times.

The concentrations of 1-hexanol, hexanoic acid and hexyl hexanoate were determined using a GC system (SP 6890, Shandong Lunan Inc., China GC-7090A), equipped with a flame ionization detector (FID) on a PEG-20 M column (30 m × 0.32 mm × 0.25 μm). The temperatures of the gasify room, column, and detector were held at 260, 160, and 260 °C, respectively. All the measurements were repeated three times.

The specific activity of free or immobilized lipases was measured for the esterification of 1-hexanol and hexanoic acid in heptane/water media at 37 °C and 200 rpm for 1 h, respectively, by following the procedure as described before. Enzymatic activity is given in units (U) per milliliter (mL) of free enzymes, and 1 U is defined as the synthesis of 1 mmol hexyl hexanoate per minute.

The reusability stability of lipase-SiN/PDACs for the synthesis of hexyl hexanoate was investigated by measuring the enzyme activity (U mL^{-1}) in each successive reaction cycle (1 h per cycle). After each reaction batch, the lipase-SiN/PDACs was collected, washed with heptane three times through centrifugation (4500 rpm, 3.5 min)/redispersion cycles to remove any residual substrates and product, and then used for the next reaction cycle. The storage stability of free lipases and lipase-SiN/PDACs were assessed by storing them at 4 °C for 10 days.

Characterization. Optical microscopy imaging was performed on a polarized light microscope (Shun Yu XP, China) equipped with a video camera. The average diameters of the emulsion droplets were obtained through the statistical analysis of more than 100 droplets.

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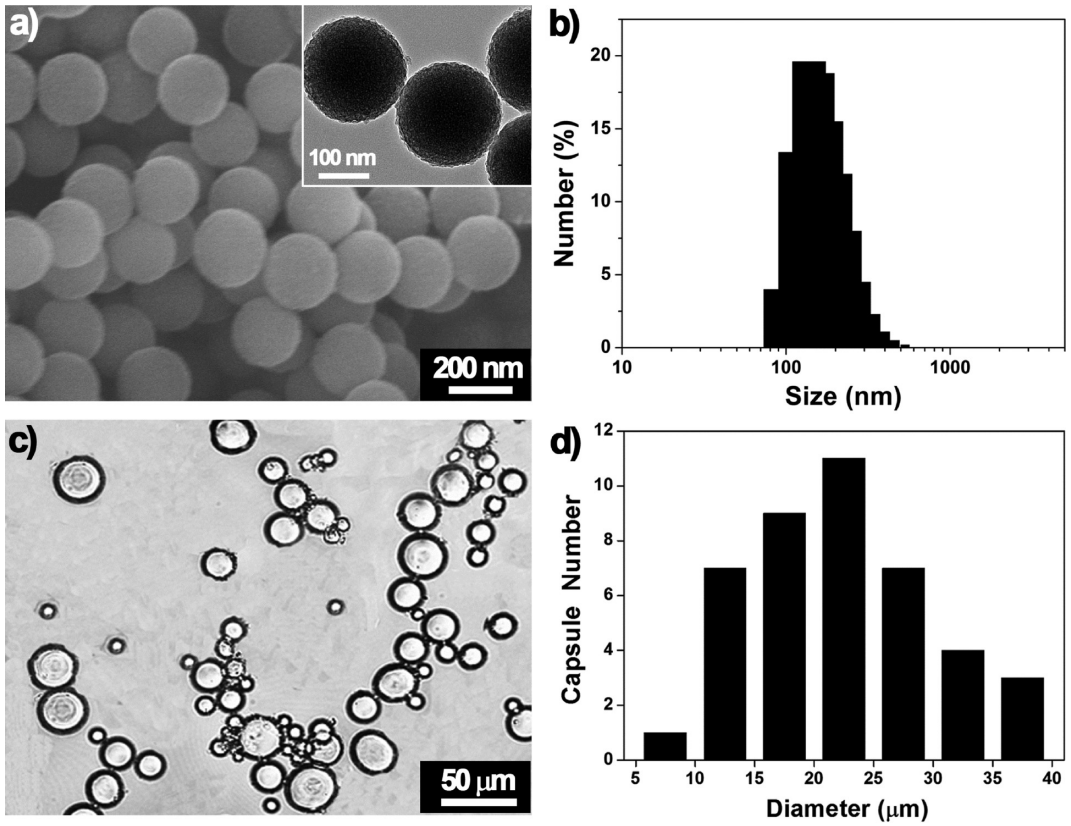

Figure 1. (a) SEM and (inset) TEM images of the hydrophobic silica NPs. (b) Size distribution of the silica NPs derived from the DLS analysis. (c) Confocal laser scanning microscopy (CLSM) image of the SiN/PDACs at a $R_{o/w}$ value of 6 under bright field. (d) Histogram of the size distribution of the SiN/PDACs, derived from the CLSM image.

Scanning electron microscopy (SEM) images were recorded using a field-emission scanning electron microscope (FESEM, S-4800, Hitachi High-technologies Co., Japan) at an acceleration voltage of 3 kV. All samples were sputter-coated with platinum using an E1045 Pt-coater (Hitachi High-Technologies Co., Japan) before SEM observation. Transmission electron microscopy (TEM) analysis was performed on a field-emission transmission electron microscope (JEM-2100F, JEOL, Japan) at an accelerating voltage of 120 kV. The optical and fluorescence microscope images were taken using a laser scanning confocal microscope (LSCM, FV-1000, Olympus, Japan) with an excitation wavelength of 488 nm. Elemental analysis was conducted with an energy dispersive X-ray spectrometer (EDS) equipped in the S-4800 FESEM at an accelerating voltage of 10 kV and X-ray photoelectron spectroscopy (XPS) with PHI1600 ESCA System (PerkinElmer, Waltham, MA). Fluorescence measurements were carried out using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA). The emission spectra were recorded in the wavelength range of 420−600 nm upon excitation at 405 nm. The static contact angles of silica NPs were measured with a contact angle measuring device OCA15EC (Data Physics Instruments, Germany) equipped with SCA 202 software. The reported values are averages of four measurements recorded at different positions on each substrate. The zeta potential and dynamic light scattering measurements were performed on a NANO ZS ZEN3600 apparatus. Thermogravimetric analysis (TGA) was carried out on a PerkinElmer Pyris analyzer.

RESULTS AND DISCUSSION

Synthesis and Structural Characterization of SiN/PDACs. Our novel method for oriented immobilization of enzymes at oil/water interfaces along with the formation of SiN/PDACs is shown in Scheme 1. Hydrophobic SiO_2 NPs are used to emulsify an aqueous enzyme solution in an organic medium, forming a Pickering emulsion in which enzymes are directly encapsulated within the aqueous core of SiNDs. Dopamine powder is then added into the emulsion for simultaneously selective polymerization and covalent linking of enzymes at oil/water interfaces, leading to the synthesis of enzyme-bonding SiN/PDACs.

To demonstrate the synthesis of SiN/PDACs, we prepared silica NPs by a coprecipitation method using (octyl)-trimethoxysilane as a hydrophobic precursor. As shown in Figure 1a,b, the silica NPs have an average diameter of approximately 140 nm and a distribution in diameter ranging from 100 to 340 nm. The polydispersity index (PDI) of the silica NPs is 0.296 from dynamic light scattering (DLS) measurement, indicative of a good dispersibility. The static water contact angle of such SiO_2 NPs is ca. 118° (Figure S2, SI), indicating the successful hydrophobization of the NP surface. We further applied the silica NPs to form a Pickering emulsion. Briefly, silica NPs were dispersed in heptane at a loading of 1% (w/v, heptane) under ultrasonication and then were mixed with water at an oil/water volume ratio ($R_{o/w}$) of 6. After homogenization at 20 000 rpm for 2 min, a white emulsion composed of SiNDs was formed. Subsequently, dopamine powder was added to the emulsion at a final concentration of 2 mg mL^{-1} buffer, followed by homogenization at 20 000 rpm for 1 min and incubation at 28 °C and 180 rpm for 24 h, giving rise to a dark gray emulsion composed of SiN/PDACs. As shown in Figure 1c,d, the emulsion droplets (SiN/PDACs) were spherical and had a diameter range of 5−40 μm, similar to that of silica JP-stabilized emulsion droplets as previously reported by us.[19] In addition, no change in the size

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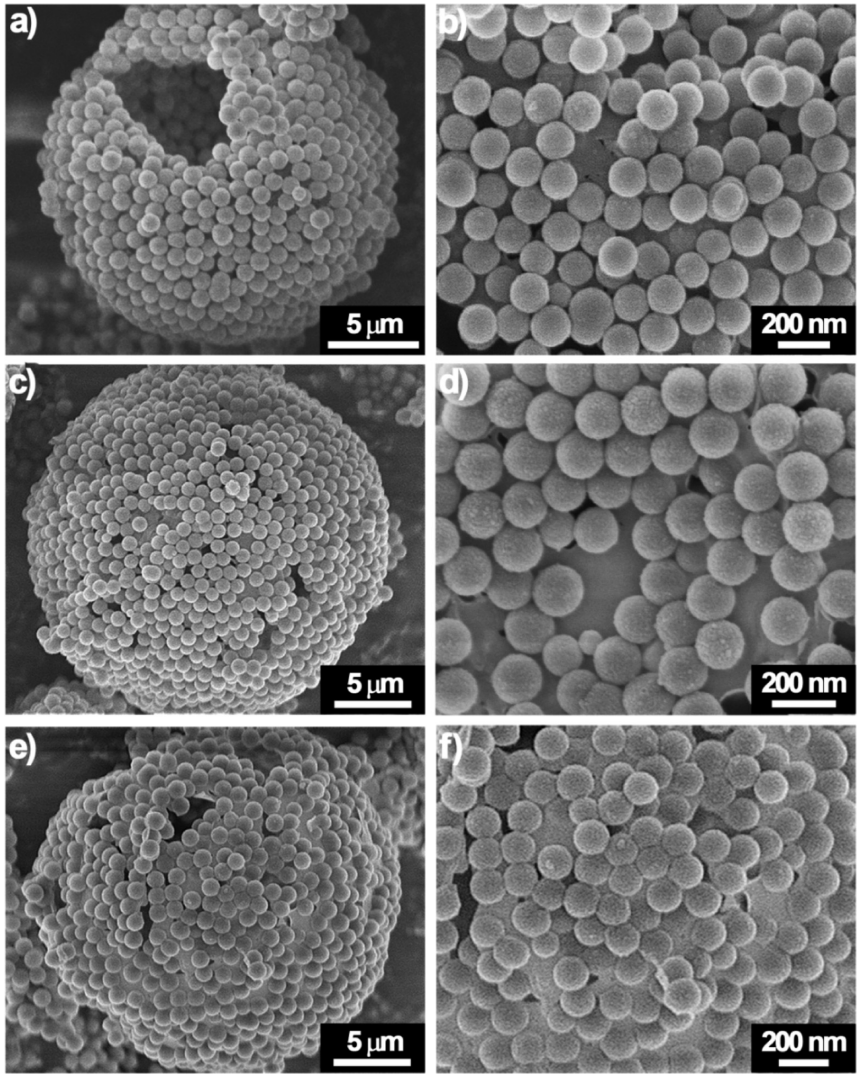

Figure 2. SEM images of the SiN/PDACs and their outer surface formed at different $R_{o/w}$ and dopamine concentrations. (a and b) $R_{o/w}$ = 10, 2 mg dopamine mL^{-1} Tris-buffer; (c and d) $R_{o/w}$ = 6, 2 mg dopamine mL^{-1} Tris-buffer; (e and f) $R_{o/w}$ = 6, 5 mg dopamine mL^{-1} Tris-buffer.

of emulsion droplets was observed before and after the polymerization process (Figure S3, SI), which should be attributed to no water added along with the addition of dopamine powder. We further investigated the change in the size of emulsion droplets at different $R_{o/w}$ values and silica loadings. As shown in Figure S4 (SI), the average diameter of SiNDs increased from 17 to 27 μm with decreasing $R_{o/w}$ values from 10 to 2. When the $R_{o/w}$ value was held constant at 10, an increase of silica loading from 0.2 to 1.5% led to a significant decrease in droplet size but no change in emulsion volume. Additionally, it is worth noting that no DLS signal was observed for the residual oil phase above the emulsion, indicative of no free SiO_2 NPs included in this phase. These results suggest that almost all of the silica particles are involved in the formation of emulsion and most of them should be adsorbed to droplet interfaces.

To observe the morphology of SiN/PDACs, we prepared the emulsions from different $R_{o/w}$ and dopamine concentrations, freeze-dried and characterized by scanning electron microscopy (SEM). As shown in Figure 2 and Figure S5 (SI), all the colloidosomes are spherical and hollow, and the shell is composed of packed silica NPs and a polymer layer. The EDS and XPS results show the presence of nitrogen atoms in the polymer layer (Figure S6, SI) and the detached SiO_2 nanoparticles (Figure S7, SI), which should be derived from the PDA molecules. When 2 mg mL^{-1} dopamine was used, the PDA layer was positioned in the inner surface of the colloidosome shell (Figure 2a–d) . After the dopamine concentration was increased to 5 mg mL^{-1}, a significant coverage of PDA on the outer surface of the shell was observed (Figure 2f), indicating that more PDA was formed around the SiO_2 NPs. Overall, the SEM images provide direct evidence in support of the polymerization of dopamine at oil/water interfaces. The SiNDs were also prepared as a control with no addition of dopamine. As shown in Figure S8 (SI), no PDA layer was found on the SiNDs shell. Moreover, we find that the presence of a PDA layer renders the arrangement of SiO_2 NPs more orderly compared to that of hydrophobic silica NPs alone, as previously reported.[18] In particular, at $R_{o/w}$ = 6, most of the SiO_2 NPs were easily arranged in a monolayer manner at oil/water interfaces, similar to the SiO_2 JPs that self-assembled into a monolayer capsule developed recently by us.[19]

We next employed a fluorescent labeling method to confirm the position of the PDA layer in the colloidosomes. Previous studies had demonstrated that the polymerization of dopamine in the presence of hydrogen peroxide (H_2O_2) in an alkaline

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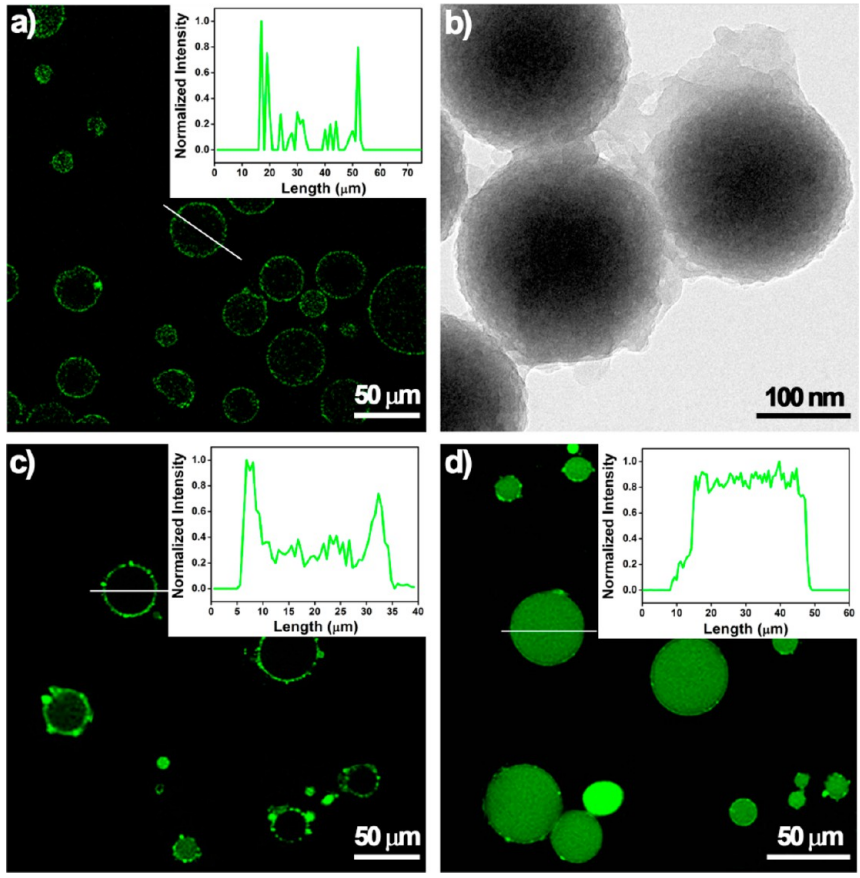

Figure 3. (a) Confocal laser scanning microscopy (CLSM) image of SiN/F-PDACs. (b) TEM image of detached SiO_2 NPs coated with PDA, which was collected from SiN/PDACs after repeated high-speed centrifugation, ultrasonic and cleaning treatment with water and ethanol for multiple cycles to remove the aqueous phase. (c) CLSM image of the SiN/PDACs containing FITC-labeled lipase. (d) CLSM image of the SiNDs containing FITC-labeled lipase. (insets a, c, and d) Graphs of normalized intensity of F-PDA positioned in a colloidosome.

environment led to the formation of fluorescently labeled PDA (F-PDA).[40,41] In this process, a fraction of dopamine, rather than PDA, was oxidized by H_2O_2 into small fluorescent molecules, which were incorporated into the simultaneously formed PDA chains.[41] On this basis, we added dopamine powder to the Pickering emulsion for 1 h prepolymerization. Then, an aqueous H_2O_2 solution was added, homogenized, and allowed to react for another 15 h at 20 °C, forming the fluorescently labeled colloidosomes (denoted as SiN/F-PDACs). The CLSM image clearly reveals that the colloidosome shell has much higher fluorescence intensity compared to the core of the colloidosome (Figure 3a). Additionally, the SiO_2 NPs were detached from SiN/PDACs via high-speed centrifugation and collected for transmission electron microscopy (TEM) analysis. As shown in Figure 3b, the SiO_2 NPs were coated by a PDA layer, exhibiting high adhesion toward the silica surface evidenced by no detaching during the ultrasonication. This morphology of SiO_2/PDA is significantly different from that of initial ones, as shown in the inset of Figure 1a. For comparison, the dopamine powder was dissolved in a Tris-buffer solution (10 mM, pH 8.5) to a final concentration of 5 mg mL^{-1} and then mixed with heptane containing 1% (w/v) SiO_2 NPs at $R_{o/w}$ = 6 for emulsification. As shown in Figure S9 (SI), the as-prepared colloidosomes also exhibit hollow structure, whereas no PDA layer was observed in the shell. The fluorescence analysis of such colloidosomes (Figure S10, SI) suggests that the polymerization of dopamine occurred in the aqueous core (the inner of the colloidosomes) rather than at oil/water interfaces. Overall, these results from SEM, CLSM, and fluorescence analysis indicate that dopamine selectively polymerized at oil/water interfaces to form a PDA layer adherent to the arranged SiO_2 NPs when the dopamine powder is directly added to the Pickering emulsion.

On the basis of the above-mentioned results, a possible mechanism for the selective polymerization of dopamine at water-in-oil Pickering emulsion interfaces was proposed. As shown in Scheme 2, the dopamine powder was added to the Pickering emulsion and dispersed in the oil phase upon the homogenization due to its low solubility. In this process, a fraction of dopamine likely transports through the colloidosome shell and is dissolved at oil/water interfaces and the bulk aqueous phase, simultaneously leading to the self-polymerization of dopamine. Due to the high adhesion of PDA to silica NPs via hydrogen bonding, the formed PDA chains around the colloidosome shell easily adhere to the SiO_2 NPs.[30] As the incubation proceeds, the dopamine in the oil phase gradually diffuses into the oil/water interfaces and simultaneously polymerizes with the existing PDA chains, giving rise to a thin PDA layer positioned at the interior of the colloidosome shell. This PDA layer creates a diffusion barrier and limits dopamine transfer into the bulk aqueous phase, which leads to the growth of PDA layer via the interfacial polymerization until the dopamine is completely consumed. In contrast, dopamine was dissolved in an aqueous solution and then encapsulated within the SiNDs in a Pickering emulsion. In this case, there is no preference for the oil/water interface over the bulk aqueous

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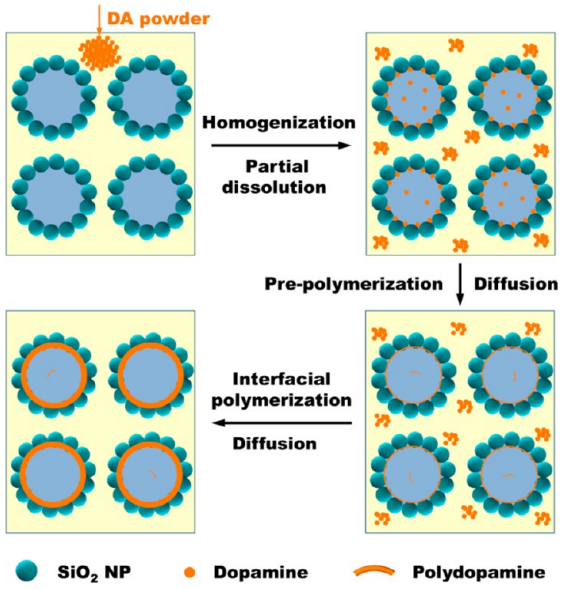
Scheme 2. Schematic Illustration of the Selective Polymerization of Dopamine at Oil/Water Interfaces in a SiO_2 NP-Stabilized Pickering Emulsion



phase to dictate the polymerization of dopamine; PDA was thus formed in the aqueous core of SiNDs. Due to the interactions between PDA and silica, we expect that a fraction of the formed PDA likely adheres to the silica nanoparticles at oil/water interfaces. However, no uniform and thin PDA layer was formed on the colloidosome shell.

Immobilization of Enzymes onto PDA Layer Localized at Oil/Water Interfaces along with the Formation of SiN/PDACs. To demonstrate this novel process of interfacial dopamine polymerization in a Pickering emulsion for enzyme immobilization, we chose lipase from *Candida sp.* as the model enzyme and the esterification of 1-hexanol and hexanoic acid into hexyl hexanoate as the target reaction. The enzyme immobilization process is similar to the formation of SiN/PDACs, as described before (Scheme 1). Briefly, an aqueous solution of lipase was mixed with a heptane solution of silica NPs, followed by emulsification at 20 000 rpm for 2 min. In this case, almost all the enzymes were encapsulated within the SiNDs, achieving a high loading efficiency (no enzyme activity was determined in the residual liquid). Dopamine powder was then added to the emulsion, emulsified at 20 000 rpm for 1 min and incubated at 28 °C and 180 rpm for 24 h, giving rise to a reinforced Pickering emulsion that consisted of lipase-bonding SiN/PDACs.

To confirm the location of lipases in the SiN/PDACs, the lipase molecules were labeled with fluorescein isothiocyanate (FITC) and then used for immobilization following the same procedure mentioned before. For comparison, the FITC-labeled lipases were immobilized along with the formation of SiNDs and SiN/PDACs, respectively. As shown in Figure 3c,d, a distinct green fluorescence was generated in both cases under excitation at 448 nm, identifying the presence of lipases in the colloidosomes. Furthermore, the higher fluorescence intensity on the SiN/PDACs shell indicates that lipases are mainly localized on the colloidosome shell (Figure 3c). However, uniform fluorescence intensity is observed in the core of SiNDs, indicating that the lipases are uniformly distributed within the bulk aqueous phase of colloidosomes.

Additionally, the SiO_2 NPs, PDA-coated SiO_2 NPs, and PDA/lipase-coated SiO_2 NPs were detached from SiNDs, SiN/PDACs, and lipase-SiN/PDACs, respectively, via the centrifugation, ultrasonic, and cleaning treatment with water and ethanol for multiple cycles and then collected for the thermogravimetric analysis (TGA). As shown in Figure S11 (SI), the samples lost ~7% weight when the temperature reached 200 °C due to the evaporation of water. Compared with the SiO_2 NPs alone, the dopamine-coated SiO_2 NPs show an increased weight loss (9.7 vs 13.6% at 525 °C), which should be attributed to the binding of PDA to the silica surface. More weight loss (21.1% at 525 °C) was found in the PDA-lipase coated SiO_2 NPs, suggesting a higher content of organic components (PDA and lipase) derived from the interfacial polymerization and enzyme immobilization. Overall, the CLSM imaging and the TGA data clearly revealed that the lipases were successfully immobilized at the PDA layer in the inner surface of the SiN/PDACs' shells. In this process, the existing polydopamine contains residual quinones that are highly reactive toward the amino groups of the lipases via Michael addition and Schiff base formation,[42−44] leading to covalent bonding of enzymes at the surface of the PDA layer along with its growth at oil/water interfaces (Scheme 1).

Catalytic Efficiency and Stability of the Lipase-SiN/PDACs. The catalytic performance of the immobilized lipases was investigated. For this purpose, two emulsions, denoted as lipase-SiNDs and lipase-SiN/PDACs, respectively, were pre-

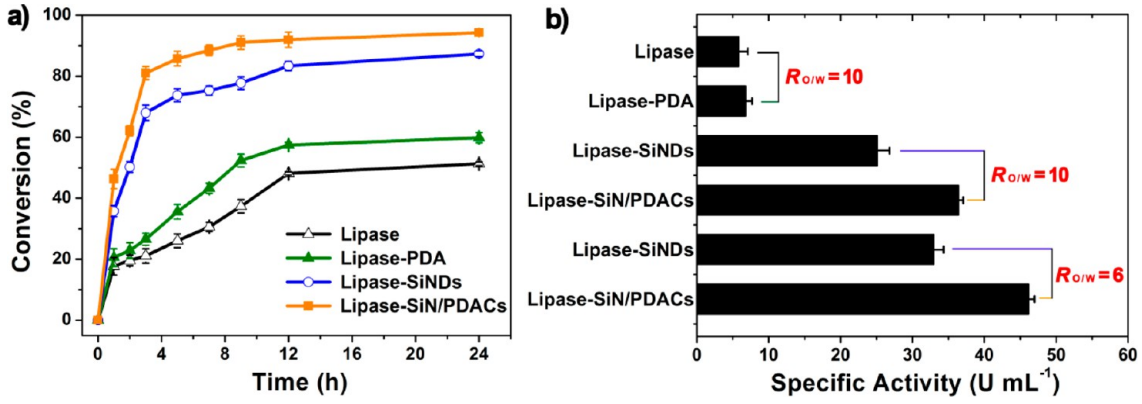


Figure 4. (a) Time courses of esterification of 1-hexanol and hexanoic acid using free lipase, lipase-PDA, lipase-SiNDs, and lipase-SiN/PDACs. (b) The specific activities for the first 1 h of free and immobilized lipases.

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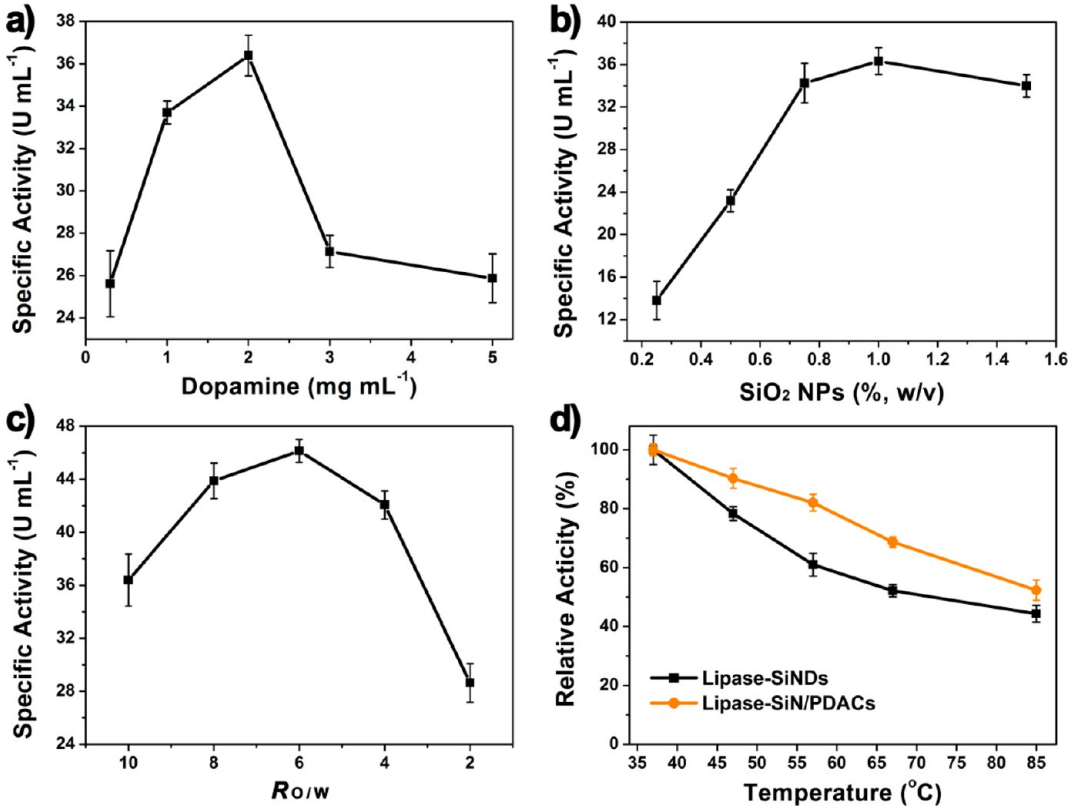

Figure 5. Effect of (a) dopamine concentrations, (b) SiO_2 NPs loading, and (c) the $R_{o/w}$ on the specific activity for lipase-SiN/PDACs. (d) The relative activity of lipase-SiNDs and lipase-SiN/PDACs after heating at different temperatures for 1 h. Relative activity (%) represents the ratio of residual activity to initial activity of each sample at 37 °C.

pared by emulsifying an aqueous solution of lipase with a heptane solution of SiO_2 NPs (lipase-SiNDs), followed by interfacial polymerization of dopamine (lipase-SiN/PDACs). Additionally, two biphasic water/heptane systems containing the same amount of lipases in the absence/presence of dopamine (denoted as lipase, lipase-PDA) were used as the control. The oil-soluble substrates, 1-hexanol and hexanoic acid, were introduced into the heptane phase to initiate the enzymatic reaction. Figure 4a shows the time courses of the conversion of 1-hexanol at $R_{o/w}$ =10 using lipase, lipase-PDA, lipase-SiNDs, and lipase-SiN/PDACs, respectively. The immobilization of lipase in Pickering emulsions significantly improved the catalytic efficiency compared to the free lipases. This enhanced catalytic performance may be attributed to the increased interfacial area created by the colloidosomes, which improved mass transfer and the accessibility of the enzymes because the substrates and product were sufficiently soluble in the organic phase. The specific activity of free lipase at $R_{o/w}$ = 10 for the first 1 h was 5.78 U mL^{-1}, which slightly increased to 6.78 U mL^{-1} in the presence of dopamine (Figure 4b), possibly due to the increased hydrophobicity of the lipase-PDA complex compared to free lipase alone. When the lipases were encapsulated within the core of SiNDs, the specific activity of lipase-SiNDs was 25.12 U mL^{-1}, which is 4.3 times higher than that of free lipase. Recently, we achieved similar improvement in silica JP-stabilized capsules.[19] As expected, the value further increased to 36.43 U mL^{-1} when using lipase-SiN/PDACs, in which lipases were immobilized on the inner surface of colloidosome shell. These results indicated the specific activity of lipases immobilized in the colloidosome shell increased by 45% compared to that of lipases positioned in the aqueous core. Although the PDA layer hinders substrate/product transport through the colloidosome shell, the immobilization of lipases at oil/water interfaces significantly increases the accessibility of enzymes, thus achieving an enhanced catalytic performance. Moreover, with decreasing $R_{o/w}$ to 6, the specific activity of lipase-SiNDs and lipase-SiN/PDACs increased from 32.95 to 46.14 U mL^{-1}. In this case, lipase-SiN/PDACs have 8 and 1.4 times higher specific activity than free lipase and lipase-SiNDs, respectively.

We further investigated the effect of dopamine concentrations, SiO_2 NP loading, and $R_{o/w}$ on the catalytic activity of lipase-SiN/PDACs. As shown in Figure 5a, with increasing dopamine concentrations, the specific activity of lipase-SiN/PDACs increased up to 36.43 U mL^{-1} at a concentration of 2 mg mL^{-1}, while it decreased when more dopamine was used. This observation is likely attributed to the increased amount of lipases localized on the shell but low mass transfer efficiency derived from the more PDA positioned on the shell (Figure 2) with increasing dopamine dosage. Generally, enlarging the area of the oil/water interface, which is highly dependent on SiO_2 NP loading and $R_{o/w}$, is able to improve the catalytic activity of the enzymes in emulsions. As shown in Figure S4c,d (SI), with increasing SiO_2 NP loading and $R_{o/w}$, the average diameter of emulsion droplets decreased, indicative of increased interfacial area. In particular, when $R_{o/w}$ was held constant at 10, excess SiO_2 NPs led to a slight decrease in specific activity (Figure 5b). A similar result was also observed at a high $R_{o/w}$ value when the SiO_2 NP loading was held constant at 1% (Figure 5c). This change is likely attributed to the increased mass transfer limitation derived from the more densely packed NPs on the

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colloidosome shell. In this work, these two parameters were optimized at 1% (w/v) and 6, respectively.

Figure 5d shows the thermal stability of lipase-SiNDs and lipase-SiN/PDACs, which is very important for immobilized enzymes in view of their potential industrial applications. In comparison with lipase-SiNDs, the lipase-SiN/PDACs retained higher specific activity after heating at 37−85 °C for 1 h. For example, the lipase-SiNDs and lipase-SiN/PDACs remained at approximately 61 and 82% of their initial activities after 1 h of heating at 57 °C. These results suggest that the covalent attachment onto the colloidosome shell enhanced thermal stability compared to the free enzymes encapsulated in the core. This finding is also in agreement with the results demonstrated in other immobilization methods.[45] In addition, the effects of the lipase loading and the storage time on the specific activity of lipase-SiN/PDACs were also investigated. As shown in Figure S12a (SI), with increasing content of lipase, the specific activity of lipase-SiN/PDACs increased up to 47.65 U mL^{-1} at a lipase loading of 30 μL. Further increase of the enzyme loading led to a decrease in the specific activity of lipase-SiN/PDACs, which is possibly attributed to the limited mass transfer and/or too much enzymes at the colloidosomes shell. The stability of lipase-SiN/PDACs was also evaluated by storing the catalysts at 4 °C followed by measuring their specific activities. The data are shown in Figure S12b (SI). The lipase-SiN/PDACs retained 80% of its initial activity after storage at 4 °C for 10 days, which is much higher that (5.2%) for the free lipase, indicative of a significantly enhanced storage stability.

Recycling and Reuse of the Lipase-SiN/PDACs. A distinct advantage of water-in-oil Pickering emulsions is the ease of enzyme recycling from organic reaction media containing oil-soluble substrates and products. To demonstrate this point, recycling of lipase-SiN/PDACs for esterification of 1-hexanol and hexanoic acid in a heptane/water emulsion ($R_{o/w}$ = 6) was conducted at 37 °C and 200 rpm for 1 h for each cycle. The lipase-SiN/PDACs were collected by centrifugation after removing organic solvent and washing with fresh solvent, and then reused for new cycles of reaction. As shown in Figure 6, the specific activity of lipase-SiN/PDACs was maintained at 86.6%, even after 15 cycles, suggesting high stability and excellent recyclability. The recycling performance of lipase-SiN/PDACs is better than that of traditional colloidosomes, such as SiO_2 JPs (52% after 8 cycles)[19] and polyethoxysiloxane cross-linkable SiO_2 colloidosomes (70% after 6 cycles).[20] The results indicate that the selective polymerization of dopamine at oil/water interfaces significantly improved the stability of colloidosomes. In views of the enzyme activity, thermal stability, and recyclability of lipase-SiN/PDACs, these results suggest that immobilization of enzymes in the inner surface of colloidosome shell along with the formation of the stable SiN/PDACs is a simple and useful method for the preparation of active and recyclable biocatalysts for practical in vitro biotransformation. Due to the strong adhesion of PDA to diverse substrates, the method demonstrated in this study can be extended by using other colloid particles for the synthesis of new PDA cross-linkable colloidosomes for various applications.

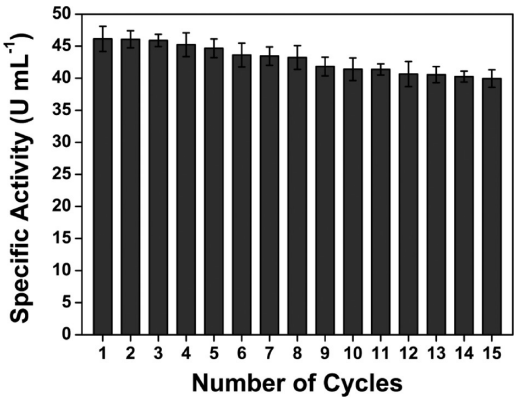

Figure 6. Specific activity of lipase-SiN/PDACs during the recycling and reuse process.

CONCLUSIONS

In summary, we have demonstrated the successful synthesis of cross-linkable and highly stable colloidosomes by the selective polymerization of dopamine at oil/water interfaces in a Pickering emulsion. The addition of dopamine powder, rather than an aqueous solution of dopamine, into a Pickering emulsion led to preferential dopamine polymerization around the SiO_2 NPs localized at oil/water interfaces. This selective interfacial polymerization is possibly attributed to the gradual diffusion of dopamine from the oil phase into the interfaces and the strong hydrogen-bonding between catechol groups and the silica surface. On the basis of this concept, a simple approach for efficient enzyme immobilization by chemical bonding of enzymes to a PDA layer along with the formation of such cross-linkable colloidosomes was developed. The immobilized enzyme exhibited a significantly enhanced specific activity and thermal stability in comparison with the free enzyme and the encapsulated enzyme positioned in the colloidosome core. Moreover, the immobilized enzyme also demonstrated excellent operational stability and recyclability. This excellent catalytic performance was attributed to the high stability of cross-linkable colloidosomes and high surface availability of the enzymes for catalytic reaction at interfaces. In view of the versatile adhesion characteristics of PDA to diverse surfaces, together with the easy linking of biomolecules via a quinone-amino/thiol reaction, we believe this new concept and method can be developed into a versatile technique for the synthesis of stable colloidosomes for various applications.

ASSOCIATED CONTENT

Supporting Information

The zeta potential and the contact angle measurement of the hydrophobic silica NPs; optical micrographs of SiNDs and SiN/PDACs; photographs and the change in the average diameters of SiNDs at different $R_{o/w}$ and SiO_2 NP loadings; SEM images of the SiN/PDACs; EDS analysis of the PDA layer; XPS survey spectra of the detached SiO_2 NPs from SiN/PDACs; SEM images of the SiNDs and SiN/PDAiCs (the dopamine solution was used); schematic illustration of the fluorescent labeling of PDA via the polymerization of dopamine; fluorescence emission spectra of the fluorescent colloidosomes; thermogravimetric data of the mixture of SiO_2 NPs and lipase; the detached SiO_2 NPs, PDA-coated SiO_2 NPs, and PDA/lipase-coated SiO_2 NPs; the effect of lipase loading and storage time on the specific activity. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b03787.

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Notes

The authors declare no competing financial interest.

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