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Facile Method To Prepare Microcapsules Inspired by Polyphenol Chemistry for Efficient Enzyme Immobilization

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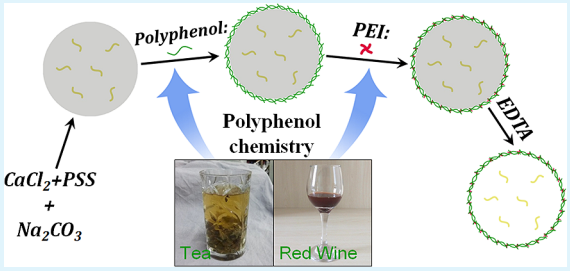
Supporting Information

ABSTRACT: In this study, a method inspired by polyphenol chemistry is developed for the facile preparation of microcapsules under mild conditions. Specifically, the preparation process includes four steps: formation of the sacrificial template, generation of the polyphenol coating on the template surface, cross-linking of the polyphenol coating by cationic polymers, and removal of the template. Tannic acid (TA) is chosen as a representative polyphenol coating precursor for the preparation of microcapsules. The strong interfacial affinity of TA contributes to the formation of polyphenol coating through oxidative oligomerization, while the high reactivity of TA is in charge of reacting/cross-linking with cationic polymer polyethylenimine (PEI) through Schiff base/Michael addition reaction. The chemical/topological structures of the resultant microcapsules are simultaneously characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier Transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), *etc.* The wall thickness of the microcapsules could be tailored from 257 ± 20 nm to 486 ± 46 nm through changing the TA concentration. The microcapsules are then utilized for encapsulating glucose oxidase (GOD), and the immobilized enzyme exhibits desired catalytic activity and enhanced pH and thermal stabilities. Owing to the structural diversity and functional versatility of polyphenols, this study may offer a facile and generic method to prepare microcapsules and other kinds of functional porous materials.



KEYWORDS: *polyphenol chemistry, microcapsules, tannic acid, enzyme immobilization, glucose oxidase*

1. INTRODUCTION

As a unique hollow structured material, microcapsules possess several excellent properties, such as functional lumen, protective shell, low density, and so on. These properties have made microcapsules particularly attractive and widely used in catalysis, drug delivery, sensors, *etc.*[1−4] In general, the preparation methods of microcapsules could be categorized into three types (i.e., hard-templating, soft-templating, and self/no-templating methods) based on the formation process of the capsule lumen. Among them, the hard-templating method is most frequently adopted, owing to the following two reasons: (1) the preparation conditions could be flexibly manipulated and (2) the resultant microcapsules could basically inherit the uniform particle size from the templates. For this method, the preparation process can be generally divided into three steps: (1) fabrication of the hard template, (2) formation of the coating on the template surface, and (3) removal of the template. The second step is often regarded as a key step that directly dominates the properties of the resultant microcapsules. The diversity and functionality of microcapsules are greatly expanded to meet the different application requirements by regulating the coating process.

To date, various coating methods, including bioinspired adhesion, biomimetic mineralization, layer-by-layer assembly, interfacial polymerization, *etc.*,[5−10] have been explored for the preparation of microcapsules. Particularly, in recent years, bioinspired methods have attracted considerable attention for the preparation of microcapsules, owing to their environmental-benignity, high efficiency, controllability and universality.[11,12] Undoubtedly, one of the most representative examples is bioinspired adhesion, also called mussel-inspired chemistry.[13,14] Based on mussel-inspired chemistry, polycatecholamine microcapsules can be prepared by simply immersing the sacrificial templates into catecholamine (primary dopamine) aqueous solution to form polycatecholamine coatings, followed by the template removal.[5,6,15] A variety of templates with different composition, such as polystyrene latex microspheres, silica microspheres, calcium carbonate ($CaCO_3$) microspheres, *etc.*,[15−17] have been adopted for the preparation of microcapsules. The structure and formation mechanism of

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polycatecholamine coatings have been extensively investigated.[18−20] Particularly, in a recent study, Lee and co-workers proposed that the covalent polymerization and noncovalent self-assembly cocontributed to the formation of a polycatecholamine coating.[19] Meanwhile, owing to the high reactivity of catechol groups in polycatecholamine that could coordinate with metal ions and chemically conjugate with amine-/thiol-containing molecules, multifunctional microcapsules for different application purposes have also been prepared.[21,22] Currently, the coating precursors for mussel-inspired chemistry were primarily limited to dopamine, DOPA, and norepinephrine.[23] Facile and mild preparation of microcapsules derived from coating precursors with broad availability and structural diversity is highly desired and urgently required.

In this regard, plant polyphenols with high content of catechol/pyrogallol groups have received enormous attention due to their similarities in structure and property to catecholamine. The concept of "polyphenol chemistry" has therefore emerged, inspired by which multifunctional coatings have been successfully fabricated. Interestingly, this coating not only retained the common merits of polycatecholamine derived from catechol/pyrogallol groups, but also exhibited unique properties of polyphenols, such as antibacterial and antioxidant properties, *etc.*[24] The formation of such coatings should be ascribed to the oxidative oligomerization of polyphenol precursors as reported by Messersmith and co-workers.[24] Furthermore, to expand the molecular diversity of the coating precursors, eight natural or synthetic polyphenol precursors were identified and verified to possess the coating-forming capability.[25] Considering the low-cost, easy availability, and structural diversity of the polyphenol precursors,[26] this method may provide a novel polyphenol-based platform for the preparation of multifunctional coatings. However, to the best of our knowledge, the preparation of microcapsules based on polyphenol chemistry has not yet been reported thus far.

Herein, we explored the feasibility of utilizing low-cost polyphenols as precursors for the preparation of microcapsules. Tannic acid (TA) was chosen as a typical plant polyphenol precursor to illustrate the preparation and structural manipulation of the microcapsules. Briefly, PSS-doped $CaCO_3$ microspheres were dispersed in Tris-HCl buffer *(50 mM, pH 8.0)* containing TA and stirred at room temperature for 2 h. During this period, TA would undergo oxidative oligomerization and then deposit on the template surface. Subsequently, the as-formed coating was covalently cross-linked by PEI through Schiff base/Michael addition reaction between amine groups of PEI and catechol/pyrogallol groups of TA. TA/PEI microcapsules were finally obtained after removing the $CaCO_3$ templates through ethylenediaminetetraacetic acid (EDTA) treatment. The influence of TA concentration, PEI concentration, and pH values during the oxidative oligomerization of TA on the structure of TA/PEI microcapsules was specifically investigated. Potential applications of TA/PEI microcapsules as a bioreactor were also illustrated by using glucose oxidase (GOD) as a model enzyme. This method may offer several distinct/unique advantages. First, microcapsules are prepared under mild conditions. Second, it is applicable to prepare a variety of microcapsules with different composition, owing to the structural diversity of polyphenols and polymeric cross-linkers. Third, the resultant microcapsules may show great potential in some specific applications, such as enzyme catalysis.

2. EXPERIMENTAL SECTION

2.1. Materials. Polyethylenimine (PEI; M_w ca. 1800 Da; branched), poly(sodium 4-styrenesulfonate) (PSS, M_w ca. 70000), tris(hydroxymethyl) aminomethane (Tris), glucose oxidase (GOD, E.C.1.1.3.4, 100−250 U mg^{-1}), and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich Chemical Co. (−)-Epigallocatechin gallate (EGCG) was purchased from Dalian MeilunBiotech Co., Ltd. (Dalian, China). Oligomeric proanthocyanidins (OPC) were obtained from Hebei Bonherb technology Co., Ltd. (Hebei, China). Horseradish peroxidase (HRP, E.C. 1.11.1.7, ≥300 U mg^{-1}) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Tannic acid (TA), sodium carbonate (Na_2CO_3), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) and ethylenediaminetetraacetic acid (EDTA) were obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Water used in all experiments was treated by Millipore Milli-Q purification system. The pH values of solutions were measured with a FE20-K pH-meter (Mettler-Toledo Instruments, FE20-K, Shanghai, China) and adjusted by addition of NaOH solution (100 mM) or HCl solution (100 mM).

2.2. Preparation of microcapsules with TA, EGCG, and OPC. First, PSS-doped $CaCO_3$ microspheres (diameter: 4−5 μm) were prepared and utilized as the templates for the preparation of microcapsules. Specifically, 10 mL of 0.33 M Na_2CO_3 solution was rapidly added into 10 mL of 0.33 M $CaCl_2$ solution containing 30 mg of PSS under vigorous stirring for 30 s. After standing for 10 min, PSS-doped $CaCO_3$ microspheres were acquired through centrifugation and water washing.

The PSS-doped $CaCO_3$ microspheres as mentioned above were dispersed in 20 mL of Tris-HCl buffer *(50 mM, pH 8.0)* containing 0.5 mg mL^{-1} TA (EGCG or OPC). After gently stirring at room temperature for 2 h, the microspheres were collected by centrifugation and washed with water for three times. The acquired microspheres were redispersed in 20 mL of Tris-HCl buffer *(50 mM, pH 8.0)* with 1.0 mg mL^{-1} PEI, which was stirred at room temperature for 0.5 h. Then, the microspheres were centrifuged and washed with water for three times. Microcapsules were obtained after removing the templates through EDTA *(50 mM, pH 5.0)* treatment.

2.3. Preparation of microcapsules from tea and red wine. Oolong tea was immersed in hot water for 10 min. Then, the tea infusion was transferred to beaker and cooled to room temperature. The as-prepared PSS-doped $CaCO_3$ microspheres were added into 20 mL of tea infusion and stirred at room temperature for 2 h. After centrifugation and water washing, the particles were redispersed in 20 mL of Tris-HCl buffer *(50 mM, pH 8.0)* with 1.0 mg mL^{-1} PEI, which was stirred at room temperature for 0.5 h. Subsequently, the particles were added into EDTA aqueous solution *(50 mM, pH 5.0)* to remove the $CaCO_3$ templates. Microcapsules from tea were finally obtained after washing with water. Likewise, microcapsules from red wine were also prepared by adding PSS-doped $CaCO_3$ microspheres into 20 mL of red wine and stirred at room temperature for 2 h.

2.4. Characterizations. SEM (Nanosem 430) and the attached energy dispersive spectroscope (EDS) were performed to measure the morphology, elemental composition and wall thickness of the microcapsules, respectively. TEM (JEM-100CX II) were conducted to evaluate the hollow structure, wall thickness and surface roughness of the microcapsules. FTIR spectra of the microcapsules and TA powders were measured by Nicolet-6700 spectrometer. UV−vis spectra of TA oligomer were measured by Hitachi U-3010 spectrometer. XPS of the microcapsules were measured by PHI 1600 ESCA using the monochromatic Mg Kα source. Circular dichroism (CD) spectra of GOD were measured at 25 °C through Jasco J-810 spectropolarimeter.

2.5. Enzyme immobilization and general procedure for assaying enzyme activity. GOD was chosen as a model enzyme that was entrapped in the $CaCO_3$ microspheres during the coprecipitation process.[21,22] After formation of the capsule wall and dissolution of the templates, GOD was immobilized in TA/PEI microcapsules.

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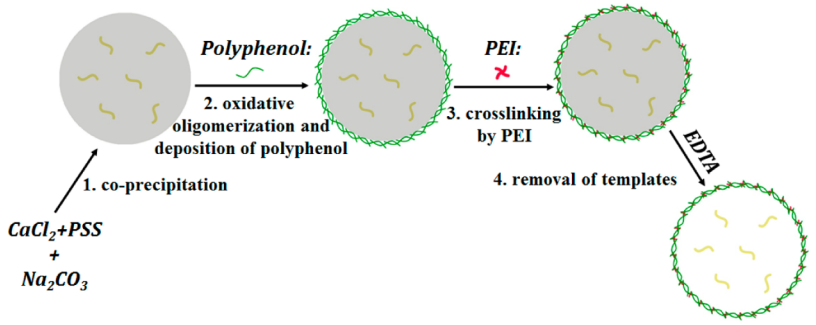

Figure 1. Schematic preparation process of polyphenol/PEI microcapsules: (1) formation of PSS-doped $CaCO_3$ microspheres through coprecipitation of PSS-containing $CaCl_2$ and Na_2CO_3; (2) formation of polyphenol-coated $CaCO_3$ microspheres through oxidative oligomerization and deposition of polyphenol; (3) formation of polyphenol/PEI-coated $CaCO_3$ microspheres through cross-linking the polyphenol coating by PEI; and (4) formation of polyphenol/PEI microcapsules after removing $CaCO_3$ microsphere via EDTA treatment. Hereafter, "polyphenol" in "polyphenol/PEI microcapsules", "polyphenol-coated $CaCO_3$ microspheres", "polyphenol/PEI-coated $CaCO_3$ microspheres", and "polyphenol-coating" represents "polyphenol oligomer".

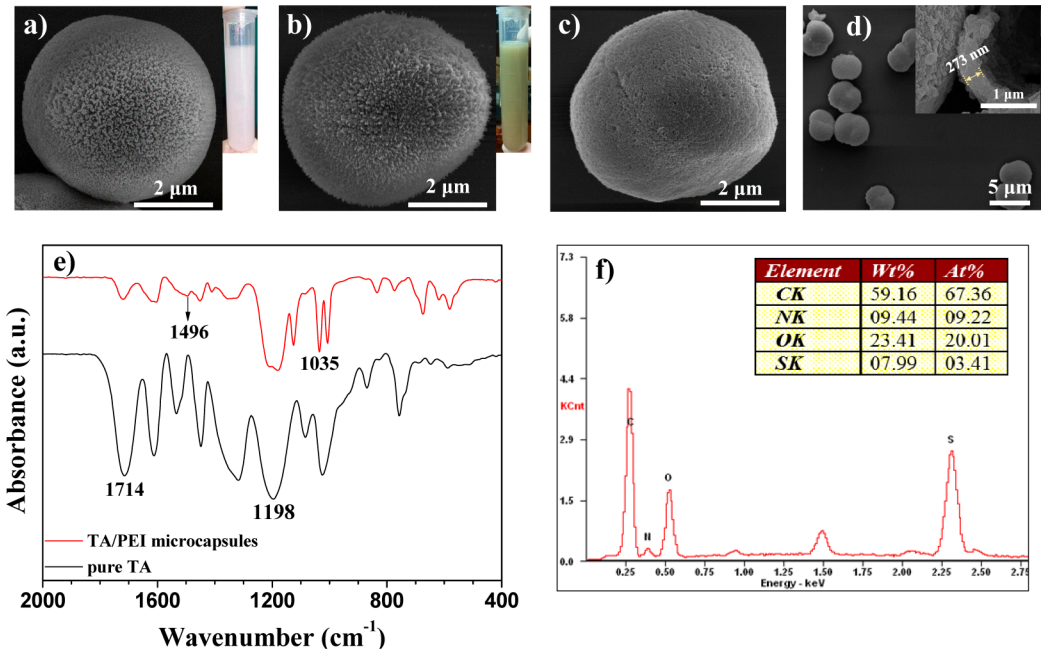

Figure 2. SEM images of (a) $CaCO_3$ microspheres, (b) TA-coated $CaCO_3$ microspheres, (c) TA/PEI-coated $CaCO_3$ microspheres, and (d) TA/PEI microcapsules. The insets in (a) and (b) were, respectively, the suspension of $CaCO_3$ microspheres and TA-coated $CaCO_3$ microspheres. The inset in (d) was the cross-sectional SEM image of TA/PEI microcapsules. (e) FTIR spectra of TA/PEI microcapsules and pure TA powders. (f) EDS spectrum of TA/PEI microcapsules.

The enzyme loading capacity (μg mg^{-1} (microcapsules)) of TA/PEI microcapsules was determined according to eq 1:

$$\text{Loading capacity}_{GOD} = M_{GOD,immobilized}/M_{microcapsules} \quad (1)$$

where $M_{GOD,\ immobilized}$ was the weight of the immobilized GOD (μg) and $M_{microcapsules}$ was the weight of the freeze-dried capsules (mg). $M_{GOD,\ immobilized}$ was calculated through measuring the GOD concentration of the supernatant before and after immobilization at 595 nm by the Coomassie Blue staining method using a UV−vis spectrophotometer. The loading capacities of GOD for TA/PEI microcapsules prepared with different TA concentrations (*0.5, 1.0, and 2.0 mg mL*$^{-1}$) were manipulated through tailoring the amount of GOD during the immobilization process and finally fixed at an equal value of 89.0 μg mg^{-1} (microcapsules) for subsequent enzyme catalysis.

Herein, a colorimetric method was used to evaluate the activity of free and immobilized GOD.[27,28] Color rendering principle was shown as eq 2 and 3:

$$\text{glucose} + O_2 \xrightarrow{GOD} \text{gluconic acid} + H_2O_2 \quad (2)$$

$$H_2O_2 + \text{TMB} \xrightarrow{HRP} H_2O + \text{oxTMB} \quad (3)$$

First, β-D-glucose was converted to gluconic acid and hydrogen peroxide (H_2O_2) catalyzed by GOD. The generated H_2O_2 was then involved in the oxidation of TMB catalyzed by HRP, leading to the color change from clear to blue. The absorbance at 652 nm of the blue solution was measured by UV−vis spectrometer and the concentration of H_2O_2 could be obtained by the standard calibration curve.

2.6. Standard calibration curve. 200 μL of 4 mM TMB, 100 μL of 1.0 mg mL^{-1} HRP and 200 μL of H_2O_2 solution with different concentrations were added into 1.5 mL of HAc-NaAc *(100 mM, pH 5.0)* buffer. After incubating at 25 °C for 10 min, the absorbance of solutions was measured.

2.7. Enzyme activity assay. The activity of free and immobilized GOD was measured by the absorbance change at 652 nm with a UV−vis spectrophotometer. Specifically, 50 μL of 1.0 mg mL^{-1} free GOD and 100 μL of 50 mM glucose was added into 850 μL of PBS buffer *(100 mM, pH 7.0)* and incubated at room temperature for 1 min. Then, 20 μL of the reaction solution were added into 980 μL of HAc-

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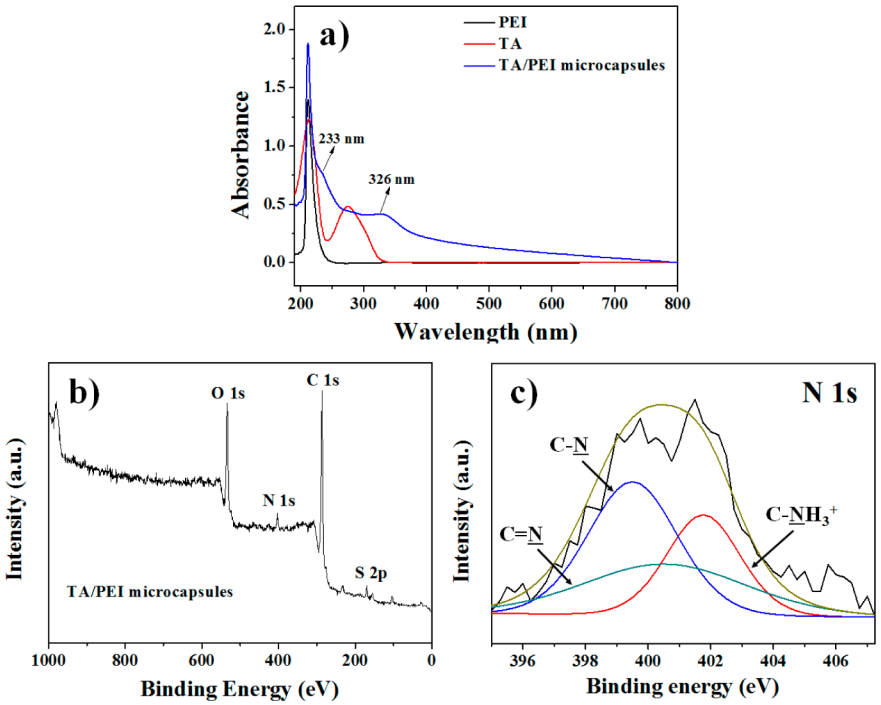

Figure 3. (a) UV−vis spectra of TA, PEI, and TA/PEI microcapsules. (b) XPS spectrum of TA/PEI microcapsules, and (c) the corresponding N 1s spectrum.

NaAc *(100 mM, pH 5.0)* buffer containing 0.44 mM TMB and 0.05 mg of HRP. After incubating at 25 °C for 1 min, the absorbance of solutions was measured.

5.0 mg of immobilized GOD and 100 μL of 50 mM glucose was added into 800 μL of PBS buffer *(100 mM, pH 7.0)* and incubated at room temperature for 40 min. Next, the reaction solution was centrifuged and 100 μL of supernatant was added into 900 μL of HAc-NaAc *(100 mM, pH 5.0)* buffer containing 0.44 mM TMB and 0.05 mg of HRP. After incubating at 25 °C for 10 min, the absorbance of solutions was measured.

2.8. Stabilities. The pH and thermal stabilities: the activity of free and immobilized GOD were measured after incubating them at different pH values *(4−9)* or temperatures *(30−70 °C)* for 2 h. The pH and thermal stability were expressed by the ratio of residual activity after incubation to their highest residual activity.

The recycling stability: GOD-encapsulated microcapsules was collected by centrifugation after each reaction batch, washed with PBS buffer *(100 mM, pH 7.0)* and used for the next reaction cycle. The recycling stability was expressed by the ratio of the activity of immobilized GOD after recycling to its initial activity.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterizations of polyphenol/PEI microcapsules. Figure 1 presented the schematic preparation process of polyphenol/PEI microcapsules inspired by polyphenol chemistry, which can be divided into four steps: (1) generation of PSS-doped $CaCO_3$ microspheres through a coprecipitation process;[29] (2) oxidative oligomerization and deposition of polyphenol on the surface of the microspheres; (3) further cross-linking of the as-formed polyphenol coating with PEI through Schiff base/Michael addition reaction between the amine groups of PEI and the catechol/pyrogallol groups of polyphenol; and (4) removal of the template through EDTA treatment. To demonstrate the feasibility of polyphenol chemistry for the preparation of microcapsules, a natural polyphenol TA was chosen as a low-cost and easily available precursor. As a kind of representative hydrolyzable tannins, TA was composed of a central glucose that was surrounded by several gallic acid residuals (Figure S1, Supporting Information). The pyrogallol groups of TA can be easily oxidized to reactive quinones[30] that could connected with each other through oxidative coupling reactions, then forming the oligomers (Figure S2, Supporting Information).[31,32] Considering the strong interfacial affinity of TA,[24] the oligomers could be subsequently deposited on the surface of templates. As shown in Figure 2a and 2b, after treatment in Tris-HCl buffer *(50 mM, pH 8.0)* with 0.5 mg mL^{-1} TA, nanoparticles and pores on the surface of $CaCO_3$ microspheres disappeared and a rougher surface with "massif-like" humps could be observed. This indicated that TA oligomers were successfully deposited on the template, which could also be evidenced by colorimetric change of the suspension from milky white to dark green (insets of Figure 2a and 2b). The rougher surface was probably as a result of the porous surface of $CaCO_3$ microspheres and irregular stacking of TA oligomers. However, owing to the weak interaction between TA oligomers (hydrogen bonds and π-stacking interactions),[30] the TA coating would dissemble, which led to the unavailability of microcapsules after treating TA-coated $CaCO_3$ templates with EDTA (Figure S3, Supporting Information). To strengthen the mechanical stability of the TA coating, PEI was introduced to chemically cross-link TA oligomers. As shown in Figure 2c, a much smoother and compacter surface was obtained after PEI cross-linking, which indicated that the interaction between TA oligomers was strengthened by PEI. Finally, the as-coated particles were treated with EDTA aqueous solution to remove the templates and TA/PEI microcapsules were finally obtained. The hollow nature was demonstrated by the disappearance of calcium element and by the TEM image of the microcapsules (Figure S4, Supporting Information). As shown in Figure 2d, nearly all of the microcapsules maintained a spherical shape after drying and a wall thickness of ~270 nm was observed,

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suggesting an excellent mechanical stability. FTIR and EDS were implemented to investigate the chemical/elemental composition of TA/PEI microcapsules. Figure 2e presented the FTIR spectra of pure TA powders and TA/PEI microcapsules. The absorption bands at 1714 and 1198 cm^{-1} were, respectively, ascribed to the stretching vibration of C═O *(ester group)* and Ar−OH in TA molecules.[33] The newly appeared bands for TA/PEI microcapsules at 1496 cm^{-1} were attributed to the bending vibration of N−H, indicating the successful introduction of PEI. The incorporation of PEI in the microcapsules could be further proved by the N element detected from the EDS spectrum of TA/PEI microcapsules (Figure 2f). Besides, the characteristic band of PSS molecules at 1035 cm^{-1} was also found, which resulted from the symmetric stretching vibration of the sulfonic group.[34] This indicated that PSS molecules were left in the microcapsules after the dissolution of $CaCO_3$ templates. Seen from the EDS result (Figure 2f), S element and N element, respectively, came from PSS and PEI, while C element was from the TA oligomers, PEI and PSS. Since the atomic ratios of C/N and C/S in PEI and PSS molecules were, respectively, 2:1 and 8:1, 32 at % of C element should come from TA oligomers, upon calculation.

To specifically elucidate the interaction between PEI and TA oligomers, UV−vis spectroscopy and X-ray photoelectron spectroscopy (XPS) were conducted. As shown in Figure 3a, two new absorption peaks at 233 and 326 nm were observed for TA/PEI microcapsules, which should be ascribed to the phenolate form of TA.[35] The absorption peaks of C═N bonds in Schiff base at 340 nm were not observed, which might be covered by the strong absorption of TA at 326 nm.[36] Besides, a wide peak at around 500 nm was observed, which proved the occurrence of a Michael addition reaction.[37,38] Furthermore, the XPS spectrum of TA/PEI microcapsules was also conducted to verify the formation of Schiff bases (Figure 3b and 3c). When focusing on the N 1s spectra of the microcapsules, three peaks at 399.5, 400.5, and 401.7 eV were obtained, which were assigned to C−N, C═N, and C−NH_3^+, respectively.[39,40] The appearance of the C═N component confirmed the formation of a Schiff base. Collectively, it can be conjectured that, under weak alkaline conditions, the residual pyrogallol groups in TA oligomers can be easily oxidized to quinone groups and cross-linked by the primary amine groups of PEI through a Schiff base/Michael addition reaction.[41]

Subsequently, (−)-epigallocatechin gallate (EGCG), oligomeric proanthocyanidins (OPC), as well as polyphenol-rich substances derived from tea and red wine were also employed to prepare microcapsules for further expanding the molecular diversity of polyphenol precursors. As shown in Figure 4, microcapsules with different morphologies were observed, illustrating the versatility of our approach for the preparation of microcapsules. Detailed information about the chemical/element composition of the four microcapsules could also be found in Figures S5 and S6 of the Supporting Information.

3.2. Structure manipulation of polyphenol/PEI microcapsules. Obviously, TA oligomers had a vital role in the formation process of TA/PEI microcapsules. The structure of TA/PEI microcapsules might be regulated by changing the TA concentration. As shown in Figure 5, TA/PEI microcapsules prepared with different TA concentrations all maintained spherical shape after drying, which indicated the excellent mechanical properties of the microcapsules. The increase of wall thickness from 257 ± 20 nm to 486 ± 46 nm was observed

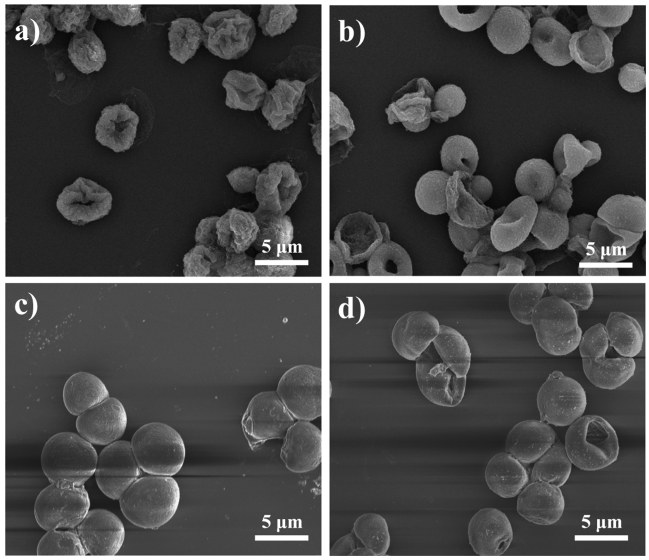

Figure 4. SEM images of the microcapsules prepared with (a) tea, (b) red wine, (c) (−)-epigallocatechin gallate (EGCG), and (d) oligomeric proanthocyanidins (OPC).

in TEM and cross-sectional SEM images as the TA concentration increased from 0.5 mg mL^{-1} to 2.0 mg mL^{-1} (Figure 5d and Figure S7, Supporting Information). Such a phenomenon can be explained as follows: under a higher TA concentration, a higher amount of TA oligomers was formed and deposited on the template surface, so as to generate a thicker capsule wall. This could be supported by the increased intensity of the characteristic band of TA in the FTIR spectra (Figure S8, Supporting Information). Besides, a rougher capsule surface was also observed with the increase of TA concentration, which might be ascribed to the increased oxidative oligomerization rates of TA molecules. This would then lead to the intricate and loose packing of TA oligomers on the particle surface and finally the formation of a rougher capsule wall after PEI cross-linking. Additionally, TA solution with a much lower concentration, such as 0.2 mg mL^{-1}, was adopted to prepare TA/PEI microcapsules, where most of the microcapsules got broken after drying (Figure S9, Supporting Information). Besides TA concentration, pH values for the oxidation of TA and PEI concentration were also changed to manipulate the structure of the microcapsules. Microcapsules with different morphologies and wall thickness were obtained, and detailed information could be found in the Supporting Information.

To further clarify the structure evolution mechanism of the microcapsules, TA-coated $CaCO_3$ microspheres were treated with EDTA aqueous solution *(50 mM, pH 5.0)* to remove the templates. As shown in Figure 5e, UV−vis absorption of 20-fold dilution of the above-mentioned solution was measured. Compared with EDTA solution that only contained TA, the typical peak of TA at 275 nm was slightly shifted to 273 nm for TA-coated $CaCO_3$ microspheres treated with EDTA. This should be attributed to the formation of dipyrogallol through oxidative coupling between TA molecules.[42] When increasing the TA concentration, the peak intensity at 273 nm was elevated. This means that more TA oligomers were deposited onto the template surface, which was consistent with the FTIR result as illustrated above. Besides, a characteristic peak of quinone groups at 349 nm was also observed.[37]

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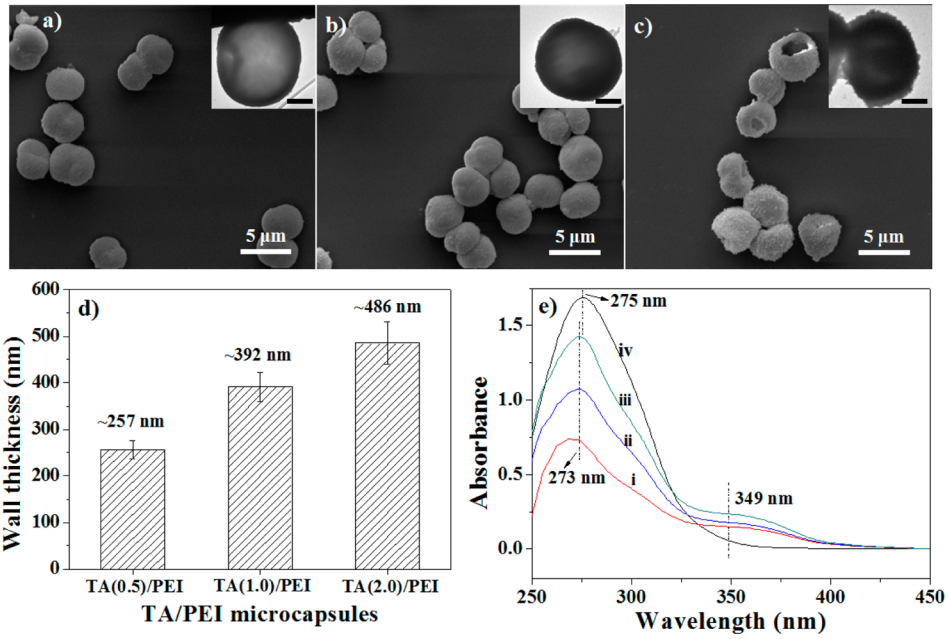

Figure 5. SEM and TEM (inset, bar: 1 μm) images of TA/PEI microcapsules prepared with different TA concentrations of (a) 0.5 mg mL^{-1}, (b) 1.0 mg mL^{-1}, and (c) 2.0 mg mL^{-1} and PEI concentration of 1.0 mg mL^{-1}. Accordingly, the three microcapsules were donated as TA(0.5)/PEI microcapsules, TA(1.0)/PEI microcapsules, and TA(2.0)/PEI microcapsules. (d) Wall thickness of the as-prepared microcapsules calculated from the cross-sectional SEM images of the microcapsules (Figure S7, Supporting Information). More than 20 microcapsules were measured, and the average thickness was calculated. (e) UV−vis spectra of the TA-coated $CaCO_3$ microspheres prepared with a TA concentration of (i) 0.5 mg mL^{-1}, (ii) 1.0 mg mL^{-1}, and (iii) 2.0 mg mL^{-1} dissolved in EDTA solution and (iv) pure TA dissolved in EDTA solution. Herein, TA-coated $CaCO_3$ microspheres were treated with EDTA solution (50 mM, pH 5.0) to remove $CaCO_3$, and the obtained solution was diluted 20-fold with EDTA solution for the subsequent measurement by UV−vis spectrophotometry.

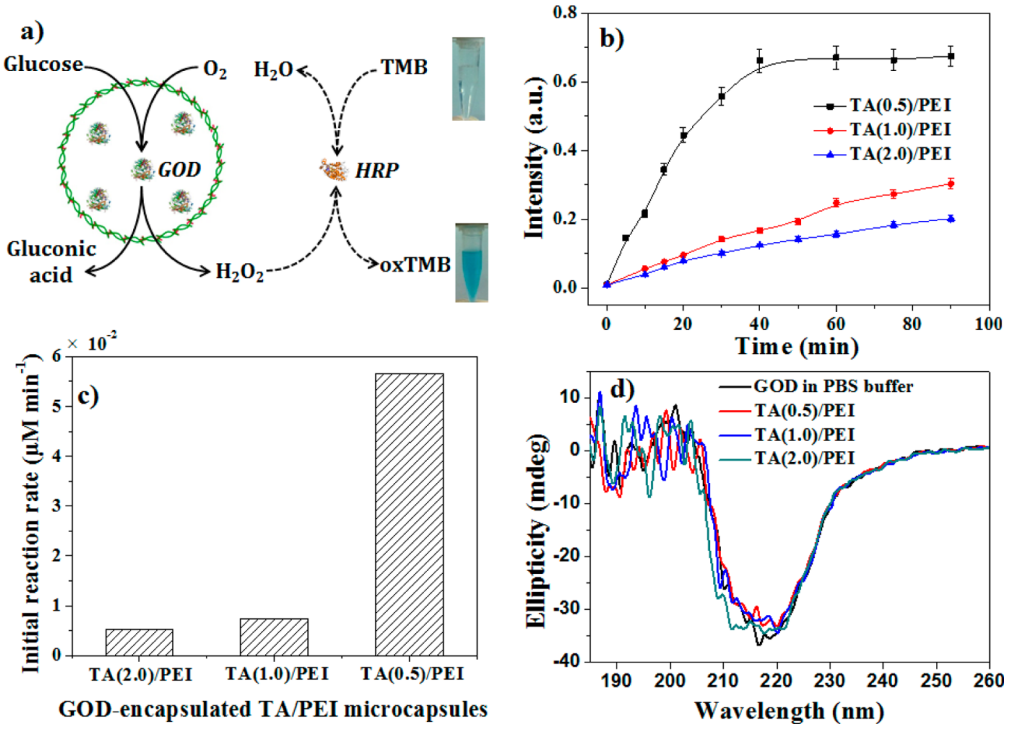

Figure 6. (a) Schematic illustration of the enzymatic conversion of glucose and colorimetric detection of the as-generated H_2O_2; (b) UV−vis absorbance at 652 nm as a function of the reaction time; (c) Initial reaction rate of the GOD-encapsulated TA/PEI microcapsules prepared with different TA concentrations; (d) CD spectra of 0.2 mg mL^{-1} GOD in 20 mM, pH 7.0 PBS buffer treated or untreated with the fragments of TA/PEI microcapsules.

3.3. Application of polyphenol/PEI microcapsules for enzyme immobilization. Further attempts at applying polyphenol/PEI microcapsules in the field of enzyme immobilization and enzyme catalysis were conducted. Briefly,

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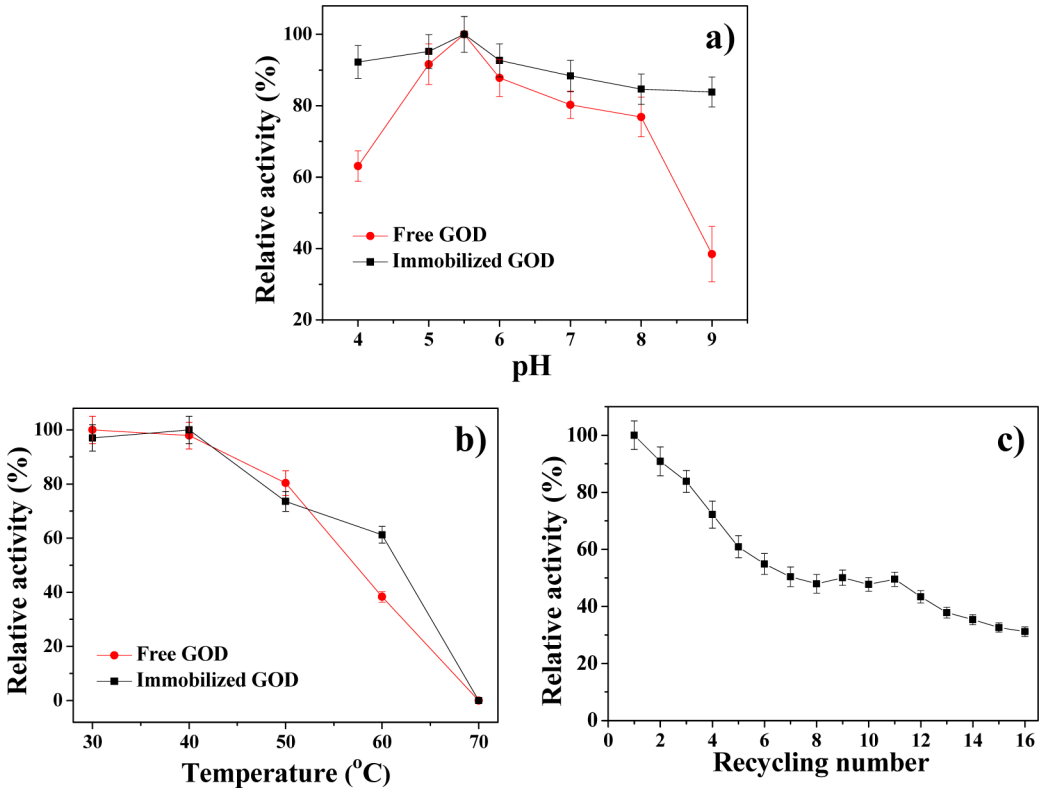

Figure 7. (a) pH and (b) thermal stability of the free GOD and the GOD-encapsulated TA(0.5)/PEI microcapsules; (c) Recycling stability of the GOD-encapsulated TA(0.5)/PEI microcapsules.

microcapsules prepared with TA, EGCG, and OPC were utilized to encapsulate glucose oxidase (GOD) for the enzymatic conversion of glucose. A colorimetric method was used to evaluate the activity of free and immobilized GOD.[27,28,43] Specifically, β-D-glucose would first permeate into the interior of microcapsules and be oxidized by GOD forming gluconic acid and H_2O_2. The as-generated H_2O_2 was subsequently involved in the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) catalyzed by horseradish peroxidase (HRP), which resulted in the color change from transparent to blue. A minor difference in bioactivity was observed for GOD-encapsulated TA/PEI, EGCG/PEI, and OPC/PEI microcapsules (Figure S13, Supporting Information). Considering the low cost and easy availability of TA, TA/PEI microcapsules were chosen as the carrier for enzyme immobilization and subsequent investigation.

To illustrate the potential application for enzymatic conversion of glucose, TA/PEI microcapsules prepared with different TA concentrations, as described above, were used to encapsulate GOD. As shown in Figure 6b, the reaction reached equilibrium at ca. 40 min for TA(0.5)/PEI microcapsules, while TA(1.0)/PEI and TA(2.0)/PEI microcapsules could not reach equilibrium even after 90 min. The UV−vis absorbance for the color solution treated with TA(0.5)/PEI microcapsules was much higher than that treated with the other two microcapsules *(0.675 vs 0.303 and 0.202)* at a reaction time of 90 min. To quantitatively calculate the concentration of H_2O_2, different concentrations of H_2O_2 were added into HAc-NaAc buffer containing HRP and TMB. With the increase of H_2O_2 concentration, the color of the solution was gradually deepened from transparent to dark blue and, accordingly, the UV−vis absorbances between 500 and 750 nm were gradually increased (Figure S14, Supporting Information). A linear relationship between absorbance at 652 nm (A_{652}) and H_2O_2 concentration *(0−60 μM)* was obtained (R^2 = *0.996*). Furthermore, the reaction rate based on the initial 15-min reaction period was calculated and TA(0.5)/PEI microcapsules exhibited the highest initial reaction rate in comparison to other two microcapsules (*5.7* × 10^{-2} *μmol* min^{-1} *vs 7.4* × 10^{-3} *and 5.2* × 10^{-3} *μmol* min^{-1}, Figure 6c). The difference in the catalytic activity for the three microcapsules may be due to the following two aspects. First, higher wall thickness with similar TA/PEI material composition may cause higher transfer resistance of substrate/product, thus leading to decreased activity. Second, higher amount of TA that possesses the protein-precipitation capability in TA/PEI microcapsules may have larger negative influence on the secondary structure of enzymes, thus resulting in decreased activity. In order to identify the primary reason, the secondary structures of the free GOD and GOD treated by grounded TA/PEI microcapsules were characterized by circular dichroism (CD). Specifically, the three microcapsules were ground to fragments and incubated in GOD solution *(PBS buffer, 20 mM, pH 7.0)* at 4 °C for 24 h. As shown in Figure 6d, similar peaks in the CD spectra were observed for the free GOD and GOD treated by the fragments of three TA/PEI microcapsules, which suggested that TA in all the three TA/PEI microcapsules could not affect the secondary structure of GOD. Therefore, it can be concluded that the decreased activity for TA(1.0)/PEI and TA(2.0)/PEI microcapsules could be due to the increased mass transfer resistance resulting from the thicker capsule wall in comparison to TA(0.5)/PEI microcapsules.

From the viewpoint of practical applications, enzyme stability was quite important and worthy of investigation. Herein, TA(0.5)/PEI microcapsules were adopted as the typical carriers

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for enzyme immobilization and stability evaluation. Considering the extreme conditions that may suffer in practical applications, the pH and thermal stabilities of the immobilized GOD were first evaluated. As shown in Figure 7a, the immobilized GOD exhibited better relative activities than the free GOD in the range of pH 4.0−9.0, and both of them got the highest activity at pH 5.5. Specifically, when the pH value reached 9.0, the immobilized GOD retained a relative activity of 84% while only 46% was preserved for the free GOD. The remarkably enhanced pH stability should be mainly ascribed to the buffer function of TA/PEI microcapsules. In detail, the PEI molecules and residues of TA are, respectively, rich in amine groups and hydroxyl groups, which could exchange protons with the solutions and then provide a buffer function.[44] For the thermal stability, both the free and immobilized GOD were inactivated after incubating at 70 °C. However, the immobilized GOD exhibited much better relative activity than the free GOD at 60 °C *(61% vs 38%)*. As enzyme was generally expensive, operational stability was also a key parameter. The GOD-encapsulated TA/PEI microcapsules were collected through centrifugation after each reaction batch and then utilized for the next reaction cycle. After recycling for 16 times, *ca.* 30% of the initial activity was preserved. As part of the microcapsules were broken after utilization for 16 times (Figure S15, Supporting Information), we suspect that the breaking of the microcapsules and the loss of immobilized enzyme during the centrifugation accounted for the reduction of activity.

4. CONCLUSIONS

In summary, we have developed a facile and efficient approach inspired by polyphenol chemistry for the preparation of microcapsules under mild conditions. TA was chosen as a representative polyphenol precursor to illustrate the formation process and mechanism of the microcapsules. The structure of microcapsules can be manipulated through changing several parameters, including TA concentration, PEI concentration, and pH values for oxidative oligomerization of TA. The microcapsules were then utilized to encapsulate GOD for the conversion of *β*-D-glucose. Higher activity could be acquired for microcapsules with thinner capsule wall by decreasing the concentration of TA during the preparation process. Besides, the enhanced pH and thermal stabilities were also obtained for the immobilized enzymes. Furthermore, other plant polyphenols, such as EGCG, OPC, and polyphenol-rich substances derived from tea and red wine, were also utilized as the precursors for the preparation of the microcapsules. Hopefully, our study could provide a facile method for the preparation of microcapsules and other porous materials for diverse applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b03823.

Chemical structure of TA; proposed mechanism of oxidative coupling reactions between gallic acid residues; dissembling behavior of TA coating; template removal process; element/chemical composition of the microcapsules prepared with other plant polyphenols; cross-sectional SEM images of TA/PEI microcapsules; FTIR spectra of TA/PEI microcapsules; SEM and TEM images of TA/PEI microcapsules; N_2 adsorption−desorption isotherm and the pore size distribution of TA(0.5)/PEI microcapsules; the catalytic performance of GOD-encapsulated TA/PEI, EGCG/PEI, and OPC/PEI microcapsules; plot of UV−vis absorbance at 652 nm versus H_2O_2 concentrations *(standard calibration curve)*, and SEM images of TA(0.5)/PEI microcapsules before and after utilization. (PDF)

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Notes

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