

Polymer Communication

Preparation of poly(β -hydroxybutyrate) and poly(lactide) hollow spheres with controlled wall thickness

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Abstract

In recent years, polymer microcapsules have attracted more and more attention because of their specific properties and applications in encapsulation and drug delivery. Great effort has been made to investigate the preparation methods, structure controls as well as the property designs for the polymer microcapsules. In this work, we reported an effective route for the preparation of poly(β -hydroxybutyrate) (PHB) and poly(lactic acid) (PLA) hollow spheres with controlled wall thickness, which involves the graft polymerization of the biodegradable polymers from the surface of silica spheres followed by removing the template cores. Nuclear magnetic resonance spectroscopy (NMR), thermal gravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscope (TEM) have been used to prove the structure of the hollow sphere and the intermediates. The result reveals that with the increase of reaction time the wall thickness of the hollow sphere will increase gradually.

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Keywords: Poly(β -hydroxybutyrate); Poly(lactic acid); Hollow spheres

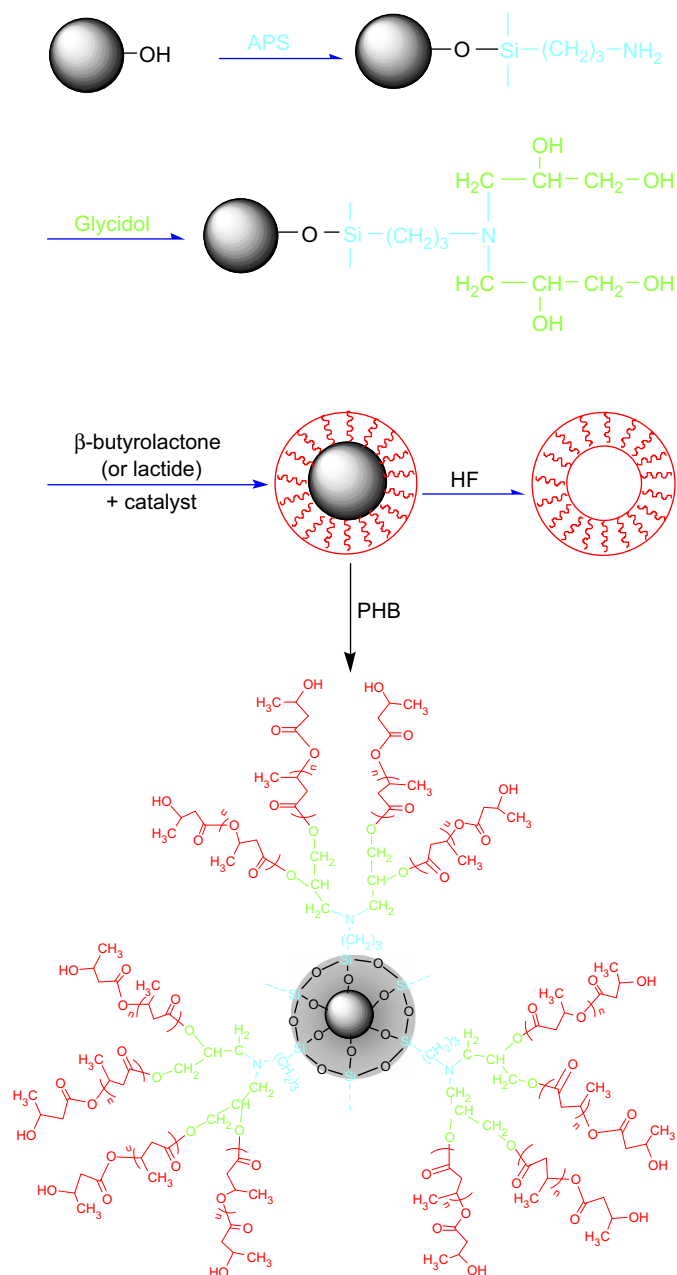
1. Introduction

In recent years, polymer microcapsules [1] and biomaterial [2] have attracted much attention because of their specific properties and applications. For instance, polymer microcapsules can be used as micro reactors for the preparation of nanoparticles or carriers for drugs and DNA [3]. There are two dominant approaches for the preparation of polymer microcapsules, i.e., the self-assembly of block copolymers [4,5] and the template method including the surface-grafting technique [6,7] and layer-by-layer assembly [3,8]. The template method is considered as a facile route to synthesize polymer microcapsules with a given size [6]. Very recently, we have reported a new method based on the silica sphere template to prepare poly(ϵ -caprolactone) hollow spheres [9]. The work presented here has successfully extended the method

to prepare the PHB and PLA hollow spheres. In the method (Scheme 1), the monomers of β -butyrolactone or lactide have been grafted from the surface of silica particles, and then the hollow spheres were prepared by the removal of the template cores. Generally, for the preparation of polymer microcapsules using the silica templates the Si-OH on the silica surface is transformed into two organic hydroxyl groups for subsequent surface grafting. The advantage of our method is that every Si-OH group can be enlarged into four organic hydroxyl groups, which provides more chances to initiate the living ring-opening polymerization of monomers from the particles surface and facilitate the complete functionalization. In addition, it was also found that the wall thickness of the obtained hollow spheres increases with the reaction time.

Although several types of polyester-*g*-silica spheres have been prepared by Choi et al. and Dumas et al. [10,11], the relevant hollow polyester structures were seldom reported. To our knowledge, the poly(β -hydroxybutyrate) and poly(lactic acid) hollow spheres have not been prepared hitherto by the template method as shown in Scheme 1. For the well-known

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Scheme 1. Reaction scheme for the synthesis of poly(β -hydroxybutyrate) and poly(lactic acid) hollow spheres.

good mechanical strength and biodegradability of PHB and PLA, in combination with the hollow structure, the polymer microcapsules obtained in this paper are promising in biomedical applications, especially in controlled drug delivery [12].

2. Experimental part

2.1. Materials

Triethylaluminum (25% w/w in hexane, 95%) was purchased from Alfa Aesar-a Johnson Matthey Company. All the other chemical reagents were purchased from the Aldrich Chemical Co. and used as received unless otherwise stated.

2.2. Instrumentation

¹H NMR measurements are performed on a Varian Mercury plus-400 spectrometer; TMS is used as the internal reference. TGA is carried out on a Perkin–Elmer TGA-7 instrument with a heating rate of 20 °C/min in a nitrogen flow (20 ml min⁻¹). FTIR is conducted on a Perkin–Elmer Paragon 1000 instrument. All samples are prepared as pellets using spectroscopic grade KBr. TEM studies are performed on a JEOL JEM-100CX-II instrument operating at a voltage of 100 kV. Samples are prepared by dropping the sample solutions onto carbon-coated copper grids and air-dried before measurement. The photographic negatives are scanned with a ScanMaker 6800 scanner. Attenuated total reflection (ATR)-FTIR spectra are conducted on Smart Nexus-470 FTIR Spectrometer. Brunauer–Emmett–Teller (BET) surface area measurement is performed on ASAP 2010M+C surface area and porosimetry analyzer. SEM observations were conducted on a Philips Sirion 200 at an accelerating voltage of 10 kV. The specimens for SEM observations were prepared by depositing several drops of the solutions onto silicon wafers and were dried in vacuum at room temperature.

2.2.1. Preparation and surface modification of the SiO₂ particles

The monodisperse silica spheres were prepared by the modified Stöber synthesis [13]. A reaction mixture consisting of 15 ml tetraethylorthosilicate (TEOS), 10 ml deionized water, 8 ml ammonia (25%) and 100 ml alcohol was stirred at room temperature for 48 h, and the obtained silica particles were modified with 3-aminopropyl-triethoxysilane (APS), according to literature [11]. Then, the APS-modified particles (0.50 g) were put into 15 ml xylene, and 0.2 ml glycidol was added into the mixture [14]. The reaction was performed at 60 °C for 5 h under an argon atmosphere. The resulting product was washed with THF for five times until all of the unreacted silane molecules were eliminated and dried at 50 °C under vacuum for 24 h. The attenuated total reflection FTIR (ATR-FTIR) and ¹H NMR were used to characterize the hydroxylating silica spheres (Fig. 1).

2.3. Synthesis of hollow spheres

The polymerization of PHB from the functionalized silica sphere cores was based on the method used to obtain the PHB homopolymers [15]. Functionalized silica particles (0.05 g) were put into 15 ml dried xylene, and then 0.2 ml triethylaluminum (25% w/w) was injected into the mixture. The reaction was carried out at room temperature for 2 h under an argon atmosphere. The product was centrifuged to remove the residual triethylaluminum. Then, 10 ml xylene and 0.5 ml (5.8 × 10⁻³ M) β-butyrolactone were mixed together with the Al-alkoxide functionalized silica spheres under argon. The mixture was reacted at 60 °C for a certain time. The products were purified by ultrasonic dispersion and centrifugation for 5–8 times in ethyl acetate and acetone to ensure the complete removing of resultant

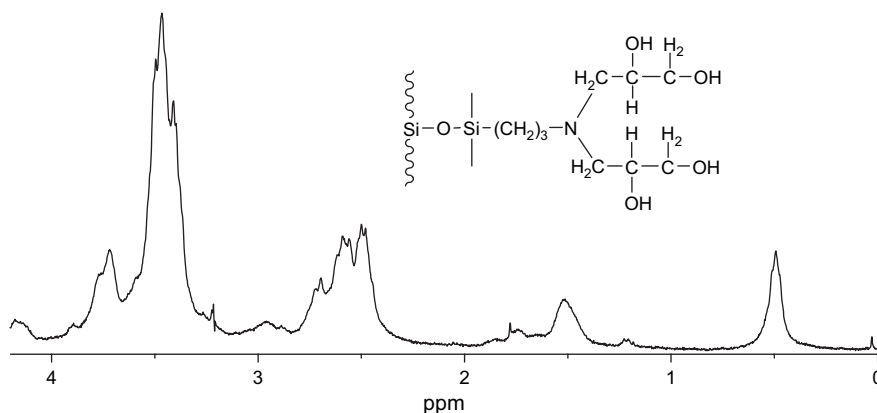


Fig. 1. ^1H NMR spectrum (400 MHz) of functionalized silica particles in D_2O . δ (ppm): 0.4–0.6 (2H, br s, SiCH_2), 1.4–1.6 (2H, br s, SiCH_2CH_2), 2.4–2.8 (6H, br m, NCH_2), 3.3–3.8 (6H, br m, OCH_2 , OCH).

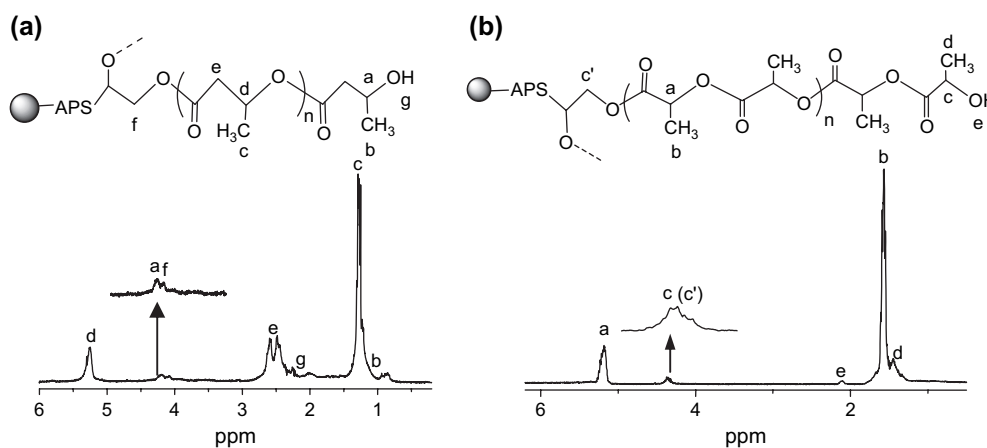


Fig. 2. (a) ^1H NMR spectrum (400 MHz) of PHB-g-silica particles; (b) ^1H NMR spectrum (400 MHz) of PLA-g-silica particles.

homopolymers, and finally the product was dried at 30°C under vacuum for 24 h to obtain the white powder. Evidence for the success of grafting was provided by FTIR and ^1H NMR measurements (Fig. 2).

The hollow polymer spheres were obtained by immersing the polymer-coated silica spheres in a 10% aqueous solution of HF at room temperature for 1 h. The resulting products were purified by dialyzing against deionized water until the pH value of the solution approached pH 7 [16]. The hollow spheres were characterized by TEM and SEM.

For synthesis of PLA-g-silica, the 0.50 g lactide (3.45×10^{-3} M), 0.020 g $\text{Sn}(\text{Oct})_2$ catalyst (0.05 mmol) and 0.05 g hydroxylating silica spheres were mixed together with 10 ml dried xylene (the monomer and the catalyst are superfluous) under argon, and then the reaction was conducted at 110°C for a certain time. The method used here is similar to the literature [17]. The resulted PLA hollow spheres were characterized by TEM and SEM.

3. Results and discussion

3.1. Synthesis and functionalization of silica particles

The monodisperse silica spheres were obtained by Stöber method. The average diameter of the silica spheres is

360 ± 10 nm and the Brunauer–Emmett–Teller (BET) surface area is 4.2659 m^2/g . The silica spheres were modified with 3-aminopropyl-triethoxysilane (APS) and followed with glycidol (Scheme 1), and then the functionalized silica particles with a number of hydroxyl groups were obtained [9]. The ATR-FTIR and ^1H NMR were used to characterize the functionalized silica spheres. In the ATR-FTIR spectrum, the C–H groups from the functionalized organic molecules (stretching vibration at 2947, 2893 and 2837 cm^{-1} and bending vibration at 1470 and 1444 cm^{-1}) can be clearly detected. ^1H NMR measurement (Fig. 1) is much more powerful and has provided the more detailed evidences to indicate that the APS and glycidol have been grafted onto the surface of silica spheres successfully. According to the ratio of peak area integration of H atoms which belong to the APS and glycidol, the yield of grafting glycidol to APS–silica was 98.58% by this method.

3.2. Synthesis of hollow spheres

The functionalized silica spheres combining with catalysts were used to initiate the grafting polymerization of β -butyrolactone and lactide. FTIR, ^1H NMR, TEM and SEM were used to characterize the obtained polymer-g-silica particles. Compared with the hydroxylating silica spheres, the FTIR spectra of the polymer-g-silica particles demonstrate two big changes.

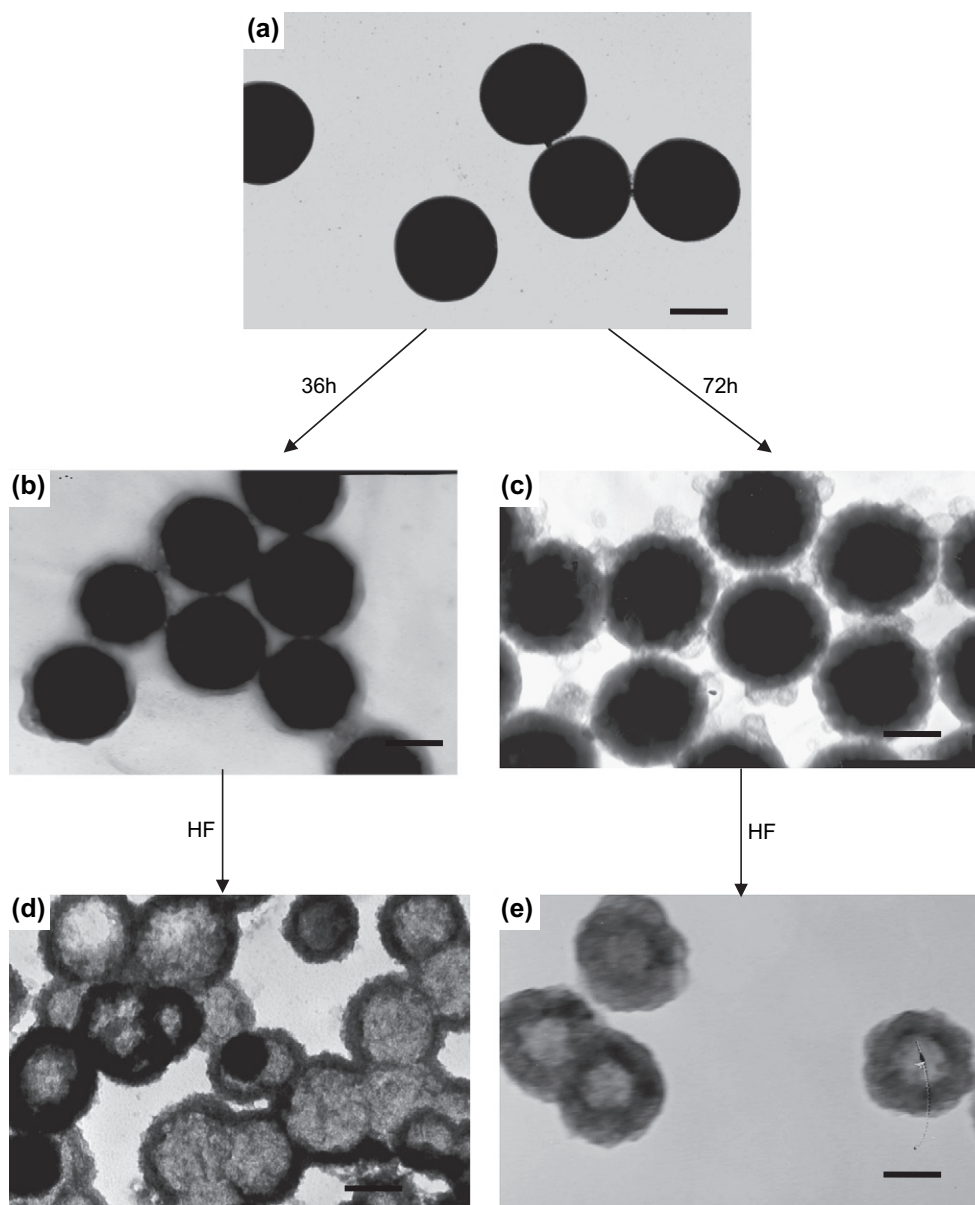


Fig. 3. (a) TEM image of bare silica particles; (b) TEM image of PHB-grafted silica particles after reacting for 36 h; (c) TEM image of PHB-grafted silica particles after reacting for 72 h; (d, e) TEM images of PHB hollow spheres dispersed in water. The scale bar is equal to 200 nm.

First, a new strong peak at 1735 cm^{-1} corresponding to the $\text{C}=\text{O}$ groups in the polyesters occurs; Second, the peaks corresponding to the CH , $\text{C}-\text{C}$ ($1050\text{--}1160\text{ cm}^{-1}$, stretching vibration bands) and $\text{C}-\text{O}$ groups (1270 cm^{-1} , stretching vibration band) become much stronger and clearer. These results agree with the graft of polyesters from the silica spheres very well. The ^1H NMR spectra together with the detailed attributions are shown in Fig. 2, which provides the further evidences to prove the covalent graft process. More attention should be paid to the proton peaks around 4.2 ppm (enlarged insets in Fig. 2). In the ^1H NMR spectrum of PHB-*g*-silica samples (Fig. 2a), the peak at 4.15 ppm (peak f) indicates that the organic hydroxyl groups on the surface of hydroxylating silica spheres have initiated the ring-opening polymerization of the β -butyrolactone monomers to form the

ester bonds [18], which provides the direct evidence for the covalent graft of PHB from silica spheres. The peak around 4.2 ppm for PLA-*g*-silica samples (Fig. 2b) is not so discriminable as that of PHB-*g*-silica, and it became much more complex in spite that the shoulder peaks were still clear. We attributed it to the overlapping peaks from groups c and c'. The TEM photographs of the resultant core-shell particle samples with different reaction times are presented in Figs. 3 and 4, and the SEM images of PHB-*g*-silica spheres and PLA-*g*-silica are shown in Fig. 5 as well. The clear polymer shells around the silica sphere in the TEM images strongly confirmed the successful graft of PHB and PLA from the silica templates as shown in Scheme 1.

It is interesting to note that the thickness of the polymer shells on the silica spheres increases with reaction time. The

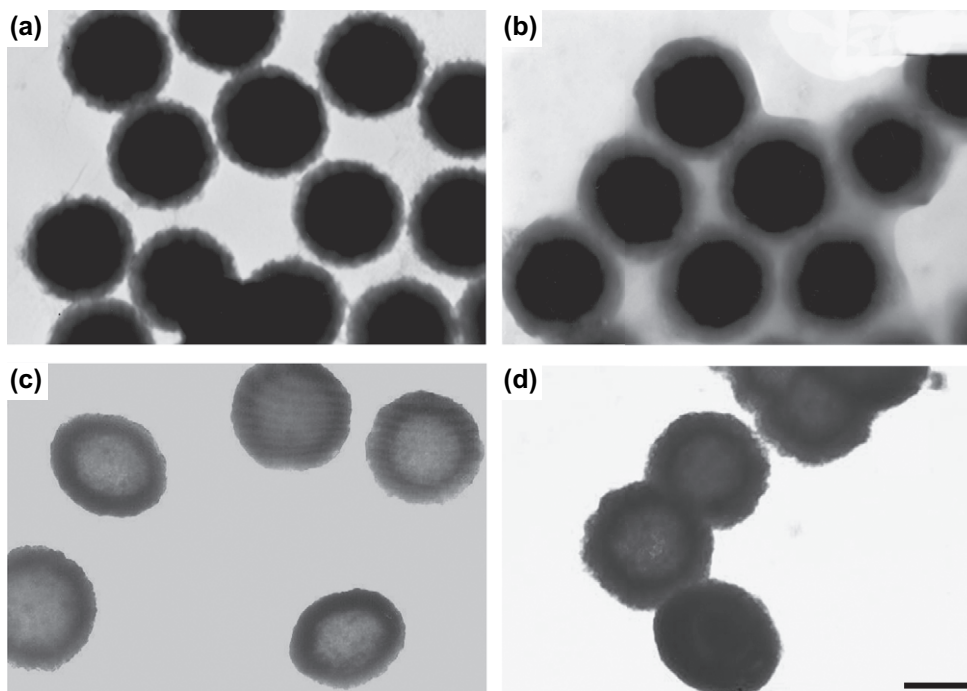


Fig. 4. (a) TEM image of PLA-grafted silica particles after reacting for 24 h; (b) TEM image of PLA-grafted silica particles after reacting for 48 h; (c) TEM image of PLA hollow spheres dispersed in water made from (a); (d) TEM image of PLA hollow spheres dispersed in water made from (b). The scale bar is equal to 200 nm.

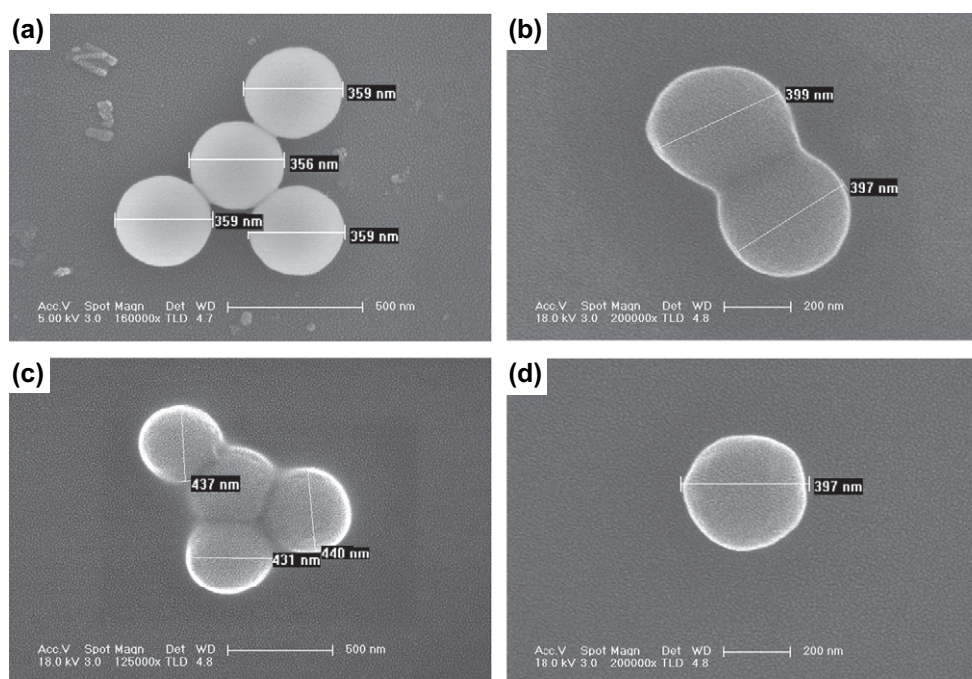


Fig. 5. The SEM images of PHB-g-silica spheres. (a) The SEM image of bare silica spheres; (b) the SEM image of PHB-g-silica spheres after reacting for 36 h; (c) the SEM image of PHB-g-silica spheres after reacting for 72 h; (d) the SEM image of PLA-g-silica spheres after reacting for 24 h.

PHB shell thickness measured from the TEM images is approximately 15 ± 5 nm (Fig. 3b) after reacted for 36 h and 25 ± 5 nm (Fig. 3c) after 72 h, which agrees well with the result of SEM measurements (Fig. 5). PLA shell thickness is near 18 ± 5 nm (Fig. 4a) after 24 h and 30 ± 5 nm (Fig. 4b)

after 48 h. The increase in shell thickness with increasing reaction time was further confirmed by TGA analysis (Fig. 6). The weight loss before 600°C in the TGA curves was attributed to the thermal degradation of polymers grafted from the silica spheres, from which the polymer content in the

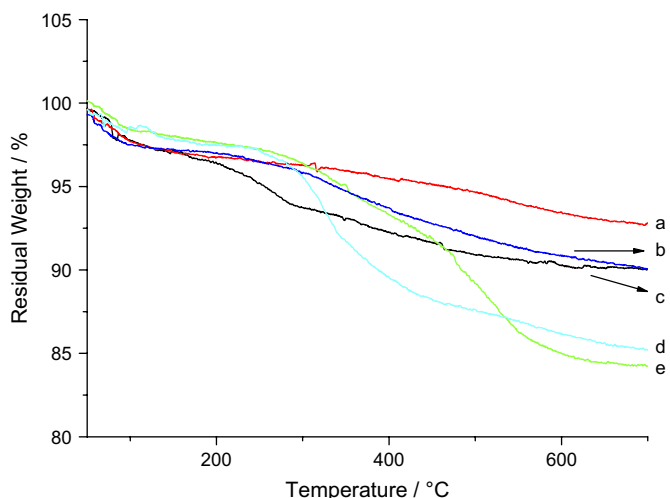


Fig. 6. (a) TGA data of bare silica particles; (b) TGA data of PLA-grafted silica particles after reacting for 24 h; (c) TGA data of PHB-grafted silica particles after reacting for 36 h; (d) TGA data of PHB-grafted silica particles after reacting for 72 h; (e) TGA data of PLA-grafted silica particles after reacting for 48 h.

core-shell particles can be calculated. For example, the PHB content in PHB-*g*-silica particles with a shell thickness of 15 ± 5 nm is 9.3%, while it is 14.1% when the shell thickness is 25 ± 5 nm. Similar result can also be achieved for the PLA-*g*-silica particle samples. Evidently, a thicker polymer shells have been grafted from the silica spheres with a longer reaction time.

The removal of silica cores was carried out through chemical etching with hydrofluoric acid (10%). The TEM image of PHB-*g*-silica particles treated with HF for 5 min is shown in Fig. 7. Compared with the core-shell particles in Fig. 3b, the

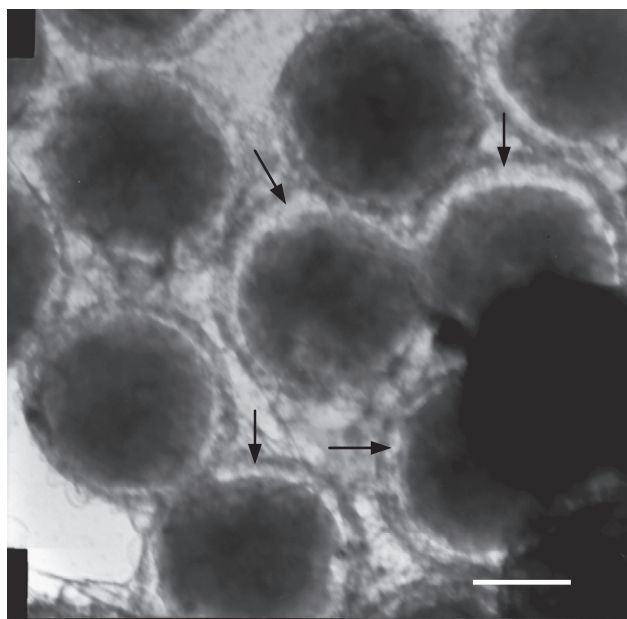


Fig. 7. The TEM image of PHB-*g*-silica particles treated with HF for 5 min. The arrows in the picture show the gaps between the separated polymer shell and silica core. The scale bar is equal to 200 nm.

silica cores became separated from the PHB shell as a result of partial etching and there existed the gap between the polymer shell and the silica core (arrows in Fig. 7). After etching for 1 h, the silica cores were completely removed, leading to the PHB and PLA hollow spheres (Figs. 3d, e and 4c, d). The hollow spheres became rather loose and crumpled due to the disappearance of the limitation of the silica cores after etching.

4. Conclusion

In conclusion, the PHB and PLA hollow spheres have been successfully prepared according to the method described in Scheme 1. A “graft-from” approach by using the silica spheres as the templates was performed to prepare PHB/PLA-*g*-silica core-shell particles, and then the hollow spheres were obtained after chemical etching of silica templates with hydrofluoric acid. The wall thickness of the hollow spheres was controlled by the reaction time. It is the first report of the PHB and PLA hollow spheres by template method, which are promising to further application as carriers.

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