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ORIGINAL CONTRIBUTION

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Polylactide hollow spheres fabricated by interfacial polymerization in an oil-in-water emulsion system

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Abstract A one-step pathway has been adopted to fabricate biodegradable polylactide (PLA) hollow spheres by interfacial polymerization in an oil-in-water emulsion system. The mechanism of sphere formation is suggested with respect to interfacial cross-linking polymerization and subsequent precipitation of the unreacted PLA macromonomers onto the preformed shells. Their hollow nature and morphology were verified by transmission electron microscopy and scanning electron microscope characterizations.

Keywords Biodegradable · Hollow spheres · Interfacial polymerization

Introduction

Organic hollow nanoparticles have a wide variety of applications [1], particularly in drug delivery systems and protection of biologically active materials, e.g., enzymes, proteins, and DNAs. Comparing with their solid counterparts, hollow particles also offer a number of immediate advantages due to their relatively low density and consumption [2]. To date, various chemical approaches for hollow sphere fabrication are broadly classified by Murthy and coworkers into (1) sacrificial core method and (2) interfacial synthesis [3]. In the former method, inorganic or organic shells are formed around solid or gel cores which are called "template" [4–7]. This approach involves multiple steps or severe synthesis conditions in the formation of the shells and in subsequent removal of the template, although the shell shape and void size can be readily controlled. By comparison, the latter approach is more simple and flexible because, in principle, liquid cores are used.

Great efforts have been attempted to construct hollow spheres on a liquid interface in various ways, including condensation polymerization and assembly of polymers [8, 9]. The interfacial polymerization is also adopted to fabricate hollow spheres with encapsulation of nonsolvent [10] based on a principle of emulsion polymerization driven by the differences of interfacial tension and phase separation process. Due to the existence of polymer/hydrocarbon or water/polymer interface, however, several configurations of particles are obtained in various engulfing degrees of water and oil phases under different synthesis conditions [11]. As an extension of this approach, polymer capsules have also been produced by polymerization of hydrophobic monomers which are solubilized within vesicle bilayers of amphiphilic copolymers [12]. By this approach, two types of morphology are reported, i.e., closed spherical polymer shells and so-called parachute-like hollow particles [13]. To obtain hollow spheres with a stable structure, a cross-linking polymerization can be performed within the vesicles [14]. Due to the cross-linked structure, the nanospheres are shape-persistent even after they are isolated from aqueous solution.

Therefore, fabrication of stable hollow spheres via a more simple way would be of both scientific and technological importance. We describe here the production of submicron-sized biodegradable hollow spheres by cross-linking polymerization of polylactide macromonomers (PLAM). Taking volatile droplets as cores, nano-scale polymeric shells are formed, which consist of the cross-linked copolymers and the unreacted PLAM precipitated from the oil phase. For this purpose, only an oil-in-water (O/W) system is required instead of the frequently employed multiphase emulsion [10, 11]. To the best of our knowledge, this is a rather facile way to fabricate stable

hollow spheres in the range of nanometers so far. These PLA capsules may thus find wide applications as new colloidal structures in fields such as medicine, drug delivery, and, for example, catalysis.

Experimental

Synthesis of vinyl-ended PLA macromonomers

Polylactide macromonomers were synthesized by ring open polymerization of lactide catalyzed by stannous chloride [15] in the presence of allyloxylethanol (AOE) as initiators. This can introduce vinyl terminal groups to the PLA oligomers. In a typical experiment, lactide and AOE with a mole ratio of 21:1 were mixed in a glass tube with 0.8‰ stannous chloride (wt./wt. of PLAM) as catalyzer. After the glass tube was sealed under reduced pressure, it was immersed in an oil bath at 140°C for 12 h. The resultant polymers were purified by dissolution in acetone, followed by precipitation in excess distilled water, and finally dried under reduced pressure at 40°C for 3 days. PLAM with a number-average molecular weight of 2,100 was obtained.

Preparation of PLA hollow spheres

In a typical fabrication process, 0.3 g PLAM ($\overline{M}_n$ =2,100) was dissolved in 12 ml chloroform, supplemented with 30 μl (27.4 mg) divinylbenzene (DVB). The mixture was then poured into a three-necked flask containing 61 mg methacrylic acid (MAA) with an oil/water volume ratio of 1:5. After being stirred (600 rpm) for 30 min, the interfacial polymerization was initiated by injection of an aqueous solution of $K_2S_2O_8$ (KPS) and $NaHSO_3$ with a weight ratio of 1:1 (4 wt% of PLAM) under a nitrogen atmosphere at 40°C. After being reacted for 8 h, the organic solvent was evaporated. The particles were washed with deionized water three times and then separated by centrifugation. For the polymerization systems with a foreign stabilizer, poly (vinyl alcohol) (PVA) with a concentration of 2 mg ml^{-1} was added into the aqueous phase.

Characterizations

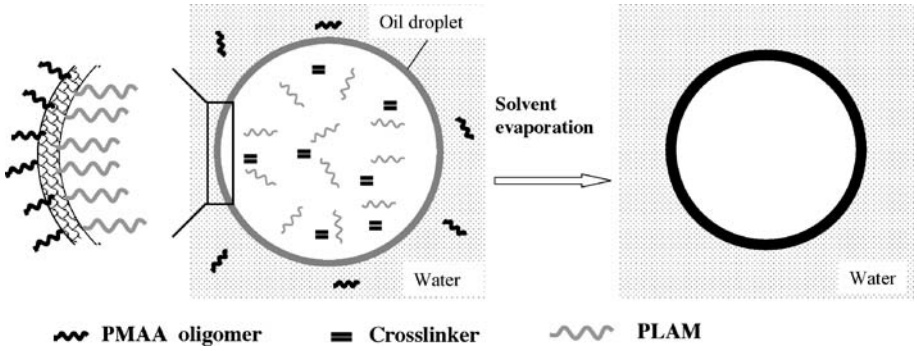
Average molecular weight ($\overline{M}_n$ and $\overline{M}_w$) of the PLAM was determined by gel permeation chromatography (GPC) (Waters 515) with tetrahydrofuran (THF) as an eluent by referring to polystyrene standard. ^{1}H-nuclear magnetic resonance (NMR) and IR spectra were recorded on an NMR spectrometer (ANAVCE DMX500) with chloroform-d_1 as solvent working at 500 MHz and a Fourier-transform infrared (FTIR) spectrometer (BRUKER VECOTR22), respectively. Element analysis was performed on an element analyzer (Flash EA-1112).

The obtained spheres were freeze-dried and sputtered using a gold target then observed under a scanning electron microscope (SEM, SIRION) working at 5 kV electron beam accelerating voltage. Transmission electron microscopy (TEM) images were taken on a JEM 200CX system at a 100-kV electron beam accelerating voltage. The samples were loaded on a 200-mesh copper grid after being stained by 1% phosphotungstic acid (PTA). The zeta potential and size of the particles were determined by dynamic light scattering (DLS, Zetasizer 2000) at 25°C. All the samples were washed with deionized water by ultrafiltration three times before characterizations.

Online observation of the morphology change of the hollow spheres was performed on a Zeiss Axiovert 200 fluorescent microscope. The hollow spheres were incubated in tetramethyl rhodamine isothiocyanate (TRITC) solution for 1 h followed by six washings to remove the excess dye molecules. A drop of the hollow sphere suspension was applied on an amino-silanized glass slide [16]. After the sphere was settled down and the water was evaporated, THF was added, and fluorescent images were recorded as a function of time.

Results and discussion

Scheme 1 illustrates the basic mechanism for fabrication of the hollow PLA sphere by interfacial polymerization in an O/W emulsion system. The process involves polymerization of PLAM, MAA, and DVB, which takes place mainly at the interface of the droplet, and successive precipitation of the unreacted PLAM onto the preformed sphere shell. For a system without a foreign stabilizer, an oil droplet is firstly formed under mechanical agitation. The free radicals are initially generated in the water phase and most possibly initiate polymerization of MAA [17]. These resultant oligomeric radicals may then diffuse to the exterior of the droplet to initiate cross-linking polymerization of PLAM and DVB [18]. Of course, the initial radicals can also diffuse into the oil interior to initiate copolymerization directly [19]. Along with the polymerization, phase separation can be expected because the cross-linked copolymers are not soluble in both water and organic solvent, thus resulting in a shell structure at the interface. This shell will dramatically reduce the diffusion of the newly formed radicals into the oil interior. Therefore, at this moment, the polymerization will mainly proceed in a thin layer between the oil and the water phases. The formed shell will reduce polymerization rate in the oil droplet as well; thus, a considerable amount of the monomers cannot be polymerized [18, 20]. Further, the shell serves as a locus for precipitation of the unreacted PLAM along with solvent evaporation to form a stable sphere. The thin shell thus formed should possess a cross-linked network with hydrophobic PLA chains inside the oil phase and hydrophilic



Scheme 1 Schematic illustration to show the formation mechanism of PLA hollow sphere at oil droplet/water interface. Under agitation, cross-linking polymerization takes place to form a thin layer of copolymers at the interface (middle). The copolymers in the layer possess amphiphilic polymer network (*left*). The unreacted PLAM is subsequently precipitated onto the layer to yield the hollow sphere after solvent evaporation (*right*)

short chains of PMAA exposing to aqueous phase, as shown in the left of Scheme 1.

To synthesize the vinyl-ended PLAMs, AOE was introduced as an initiator to initiate the polymerization of lactide. The resultant PLAM has a number-average molecular weight ($\overline{M}_n$) of 2,100, with $\overline{M}_n/\overline{M}_w$ /=1.46 as detected by GPC. ^{1}H-NMR peaks (Fig. 1) at 5.2 (a) and 1.6 ppm (b) are assigned to hydrogen of methine and methyl groups of a PLA unit, respectively, while those at 5.9 (c), 5.2 (d), 4.0 (e), and 3.7 ppm (f) are assigned to vinyl and methylene groups of an AOE unit, respectively. This has proven that the vinyl group surely exists in the PLA macromolecule.

Polymerization of the PLAM in the presence of DVB and MAA, with $CHCl_3$ as oil phase, hollow PLA microspheres (designated as PLA-I) were successfully fabricated, as evidenced by TEM and SEM observations (Fig. 2). TEM images (Fig. 2a,b) show clearly that the spheres possess a hollow structure. They have a wall thickness of several tens of nanometers, with a size distribution of 0.8–1.6 μm. Dynamic light scattering measured, however, a hydrodynamic diameter of 2.5±0.1 μm, with a polydispersity of 0.83 (Table 1). Star-like shadows are found in the middle of most microspheres, as clearly shown in Fig. 2b. SEM observation (Fig. 2c) reveals that the microspheres have collapsed as a result of the evaporation of water content and lower mechanical strength of the wall, demonstrating again the hollow nature of the microspheres. Corresponding to the star-like shadows in TEM micrographs, considerable high ridges can also be found in the middle of the microspheres (Fig. 2c).

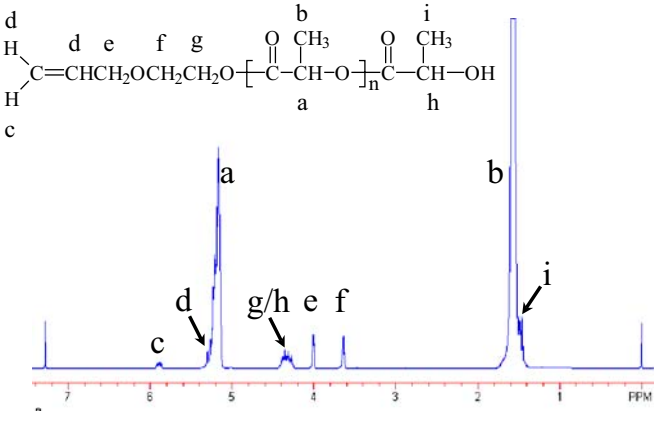


Fig. 1 ^{1}H-NMR spectrum of PLAM

Although hollow microspheres can be fabricated at a PLAM/MAA ratio of 5:1 (weight) as shown in Fig. 2, we observed, however, macroscopic precipitation took place if the ratio was higher than 7:1. Since the in-situ-formed amphiphilic MAA-PLAM copolymers serve as an emulsifier or stabilizer in the reaction system, an insufficient amount of MAA will certainly yield less hydrophilic copolymers with poorer stabilization efficiency. Consequently, the coalescence of the particles may occur above the critical point. On the other hand, at an extremely high content of MAA (for example, PLAM/MAA=3:1), solid spheres were also produced besides hollow microspheres, which probably attributes to the PMAA particles. These results imply that the window for fabrication of the hollow spheres is rather narrow without the addition of a foreign stabilizer.

To further tune the microstructure of the hollow spheres in a more controllable manner, we thus presupplemented the reaction system with a foreign stabilizer, exemplified here with PVA. TEM and SEM observations (Fig. 3) reveal again the hollow nature of the resultant spheres (designated as PLA-II), which were fabricated at exactly the same conditions as PLA-I except for the addition of 2 mg ml^{-1} PVA. Figure 3a also shows that the resultant spheres are more homogenously distributed, with diameters in the range of 0.3–0.5 μm, which are only one third of the PLA-I. DLS measured a very similar diameter of 0.49±0.03 μm, with a quite narrow polydispersity of 0.16 (Table 1). The addition of PVA would be helpful to decrease interfacial energy, resulting in smaller droplets corresponding to an

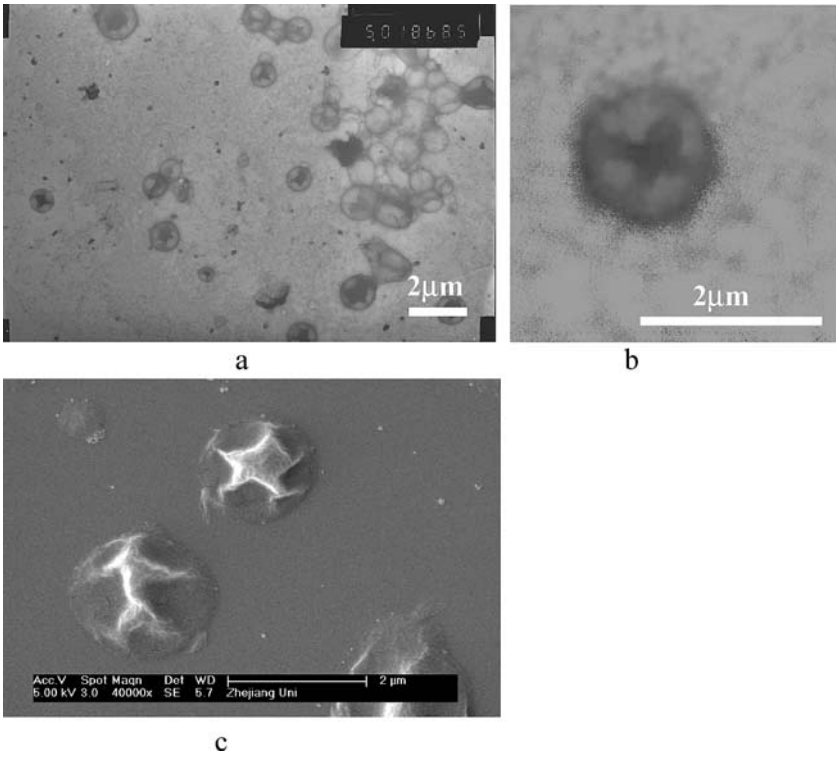


Fig. 2 TEM (**a**, **b**) and SEM (**c**) images of PLA-I spheres fabricated from PLAM, DVB, and MAA. The ratio between PLAM and MAA is 5:1

overall larger surface area in the emulsion system. The wall thickness is estimated as approximately 30 nm (Fig. 3b), which is basically thinner than that of PLA-I. No star-like shadows were observed in these spheres. Observation under SEM also detected collapsed sphere morphology. It is worth noting that the sphere size can be readily tuned by variation of the MAA content. With a higher amount of MAA, a smaller sphere size and a narrower size distribution are obtained. This is understood as the result of introduction of the in-situ-formed MAA-PLAM copolymers, which can improve the stability of the system.

To identify the chemical structure of the shells, the spheres were extracted with THF to separate soluble and insoluble polymers. The soluble components contribute roughly 70 and 75% (calculated from the yield of the cross-linked copolymers on Table 2) to the total mass of the spheres for PLA-I and PLA-II, respectively. FTIR characterization found that the soluble extractants from PLA-I (Fig. 4c) and PLA-II (Fig. 4e) have almost the same spectrum as PLAM (Fig. 4a), indicating the existence of PLAM in the hollow spheres. GPC characterization of the soluble extractant measured exactly the same molecular weight as PLAM for both PLA-I and PLA-II, except for a slight broadening of the polydispersity. Hence, one can conclude that the soluble component should be mainly PLAM.

Table 1 Zeta potential, particle size, and size distribution of PLA-I and PLA-II spheres

Particle	δ-Potential (mV)	Particle diameter (nm)	Polydispersity index
PLA-I	−17.6±0.8	2,470±60	0.829
PLA-II	1.7±0.8	490±30	0.160

The FTIR spectra of the insoluble components (known as cross-linked copolymers) of both PLA-I (Fig. 4b) and PLA-II (Fig. 4d) show apparent carboxylic absorbance at 3,350 cm^{-1}, demonstrating the incorporation of PMAA in the spheres. The vinyl absorbance at 1,610 cm^{-1} (indicated by the arrows) and the phenyl multiplet near 800 cm^{-1} confirm the involvement of DVB. These spectra also demonstrate that at least partial vinyl groups of DVB have not been polymerized. The strongest absorbance of carbonyl at 1,758 cm^{-1} reveals that the PLAM should be certainly existent in the spheres. Therefore, these cross-linked components are composed of MAA–DVB–PLA copolymers. Moreover, from the relative stronger intensity of carboxylic groups at 3,350 cm^{-1} and vinyl groups at 1,610 cm^{-1} (Fig. 4b), one can conclude that a larger percentage of PMAA and DVB are incorporated in the cross-linked copolymers of PLA-I than PLA-II. In conclusion, the wall of the hollow sphere is surely composed of a smaller amount of cross-linked MAA–DVB–PLA copolymers and a larger amount of precipitated PLAM.

Elemental analysis of PLAM and the cross-linked copolymers was further conducted to assess the exact components involved in the cross-linked part of the spheres (Table 2). A control sample of PLA-II* microspheres was prepared without the addition of MAA in the initial feed.

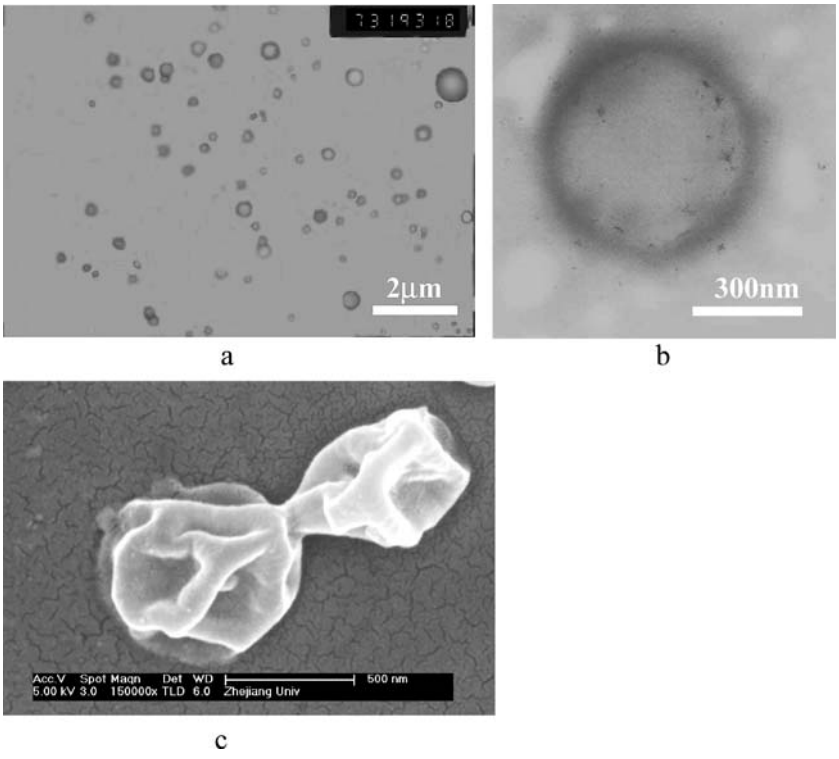


Fig. 3 TEM (**a**, **b**) and SEM (**c**) images of PLA-II spheres fabricated from PLAM, DVB, and MAA with the addition of PVA. The ratio between PLAM and MAA is 5:1

Taking into account its yield (20.5 wt%) and the DVB ratio (38.5 wt%), one can know that the involved DVB percentage, 8 wt%, matches exactly to its feeding percentage. This would also mean that all the DVB molecules have been incorporated into the cross-linked part of the spheres. Therefore, by assuming that the ratio of PLAM/DVB is constant regardless of the addition of MAA and/or PVA, percentages of PLAM, MAA, and DVB involved in the cross-linked part of PLA-I and PLA-II spheres are calculated and summarized in Table 2. Again, a relatively larger amount of MAA (36.5 wt%) was found in the cross-linked part of the PLA-I spheres than that (25.1 wt%) of the PLA-II spheres. This quantitative result is consistent with the IR analysis. The result conveys a hint that the PVA molecules might have a function to retard the diffusion of MAA from the water phase to the oil phase.

To check the stability of the resultant spheres against solvent etching and the possible location of the uncross-linked PLAM, the structure change of the spheres was observed under a fluorescent microscope. For the convenience of optical observation, a quite-large amount of PLAM (1.2 g) was used in the initial feed. At this case, micron-sized particles were obtained with a broader size distribution, and more unpolymerized PLAM could be

Table 2 Elemental analysis of PLAM and cross-linked copolymers from PLA-I and PLA-II spheres

Sample	Percentage of the cross-linked part (wt%)[a]	Element percentage (wt%)[b]			Unit percentage (wt%)[c]		
		C	H	C/H (wt.)	PLAM	DVB	MAA
PLAM	–	47.84±0.02	5.67±0.02	8.44	100	–	–
DVB	–	92.26	7.74	11.92	–	100	–
MAA	–	55.80	7.02	7.95	–	–	100
PLA-I	30.1±1.9	53.13±0.07	5.83±0.04	9.11	39.1	24.4	36.5
PLA-II	24.7±2.1	54.87±0.19	5.89±0.04	9.32	46.1	28.8	25.1
PLA-II[d]	20.5±2.7	55.36±0.04	5.66±0.08	9.78	61.5	38.5	–

[a]Defined as the weight ratio (%) of the cross-linked copolymers to the total weight of the spheres
[b]The C and H percentages of DVB and MAA are calculated from their molecular formulas
[c]The percentage of pure PLAM, DVB and MAA is assigned as 100%. The percentage of PLAM and DVB in PLA-II[d] spheres is calculated from the C/H ratio by referring to the C/H ratios of the pure PLAM and DVB. By fixing the PLAM/DVB ratio in PLA-I and PLA-II spheres, the percentage of PLAM, DVB, and MAA in the PLA-I and PLA-II spheres is calculated accordingly
[d]Prepared at the same conditions as PLA-II except for the absence of MAA. The spheres were washed in 50°C water eight times to thoroughly remove the PVA molecules

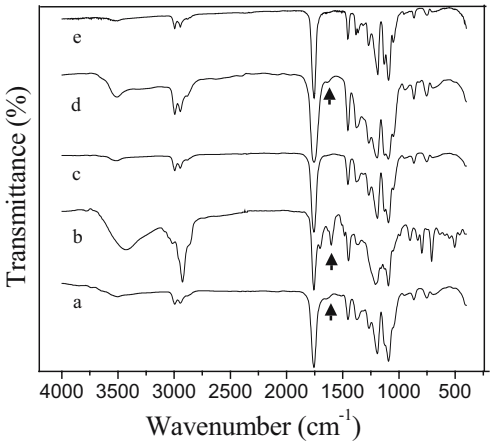


Fig. 4 FTIR spectra of PLAM monomer and (co)polymers separated from the resultant microspheres. **a** PLAM; **b** cross-linked and **c** soluble parts of PLA-I microspheres; **d** cross-linked and **e** soluble parts of PLA-II microspheres

incorporated into the spheres (Fig. 5a). Since TRITC is rather hydrophobic, it can permeate and stain the PLA spheres to show high fluorescence intensity. After incubation in THF for several tens of seconds (Fig. 5b,c), the seemingly filled spheres became hollow with the remaining bright circles. Along with the prolongation of the incubation time, fluorescence from the sphere interiors weakened gradually and finally reached the same level as the bulk solution (Fig. 5c–e). During this process, no apparent variation of sphere size was observed. Thirty minutes later, the spheres possessed a thinner shell and a larger interior space (Fig. 5e) than that of the initial. These results demonstrate that the PLA spheres are generally in a cross-linked state so that they can withstand THF etching to keep macroscopic topology. From the fact that the interiors of the spheres had the same fluorescence intensity as the bulk, one can conclude that the inner uncross-linked PLAM had been extracted, and the cross-linking most possibly took place in the exteriors of the spheres.

We have proposed in Scheme 1 that the in situ synthesized amphiphilic copolymers should be dominantly existent on the exterior of the particle. This has been partially verified by zeta-potential measurement. Note that the zeta potential of a colloidal particle is mainly decided by its surface charge. For the PLA-I without addition of PVA, negative zeta potential (−17.6m±0.8V) was recorded (Table 1), which is exclusively attributed to the carboxylic groups. Together with the IR spectra and the THF etching results, one can deduct that the cross-linked copolymers should mainly locate in the external surface of the spheres. These hydrophilic-segment-contained copolymers should be responsible for stabilization of the particles during polymerization. For the PLA-II with the addition of PVA, a neutral zeta potential (1.7±0.8 mV) was recorded regardless of the existence of MAA in the polymerization system. This would imply that the sphere surface should be covered

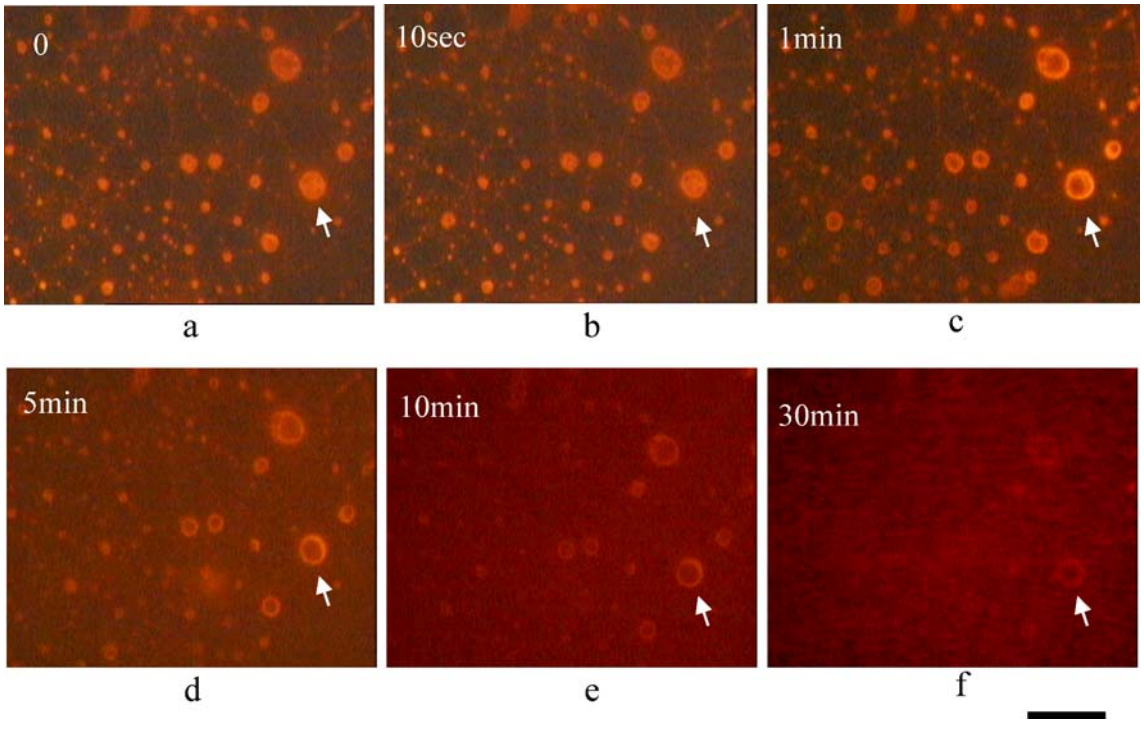


Fig. 5 Fluorescent images to show the structure variation of the PLA spheres during THF etching process. The PLA spheres were prepared by using a larger amount of PLAM and stained with TRITC previously. The *arrows* indicate a typical sphere during this changing process. The *scale bar* is 10 μm

by PVA molecules, which might be stabilized by physical entrapment and/or hydrogen binding with the carboxylic groups. In this case, the exact location of the carboxylic groups cannot be identified, although the carboxylic groups are most possibly in the exterior layer of the sphere.

Conclusions

We have proposed a facile pathway to fabricate biodegradable hollow spheres using a method of O/W emulsion polymerization. The process involves interfacial cross-linking polymerization and subsequent precipitation of the unreacted PLA macromonomers onto the preformed shells. For this purpose, vinyl-ended PLAMs are synthesized by the introduction of AOE as a coinitiator for lactide polymerization. PLA hollow spheres (PLA-I) are thus fabricated from a polymerization system containing PLAM, hydrophilic MAA, and cross-linker DVB using chloroform as an oil phase. The resultant hollow spheres have a submicron diameter and a nano-scale wall thickness as identified by TEM and SEM observations. By the addition of foreign stabilizer PVA (PLA-II), both the sphere diameter and wall thickness are effectively decreased with more homogenous size distribution. Solvent extraction and FTIR spectra detect that the PLAM contributes 70% of the total mass of the spheres, while the cross-linked copolymers are originated from MAA, PLAM, and DVB.

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