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Formation of poly(L,D-lactide) spheres with controlled size by direct dialysis

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Abstract

In this work, we show that simple dialysis of a poly(DL-lactic acid) (PLA) solution against water resulted in the spontaneous formation of PLA spheres. We observed initial formation of particles with irregular shape and large size, which then were disrupted into uniform and smooth nanospheres. Further, the formation process of PLA spheres was also investigated by dynamic light scattering technique (DLS) *in situ*. Based on these experimental results, it was proposed that the PLA spheres were formed in three steps: (1) aggregation – individual PLA chains got aggregated with each other in solution; (2) formation and disruption of PLA particles; (3) solidification of PLA spheres. In addition, the size of the spheres could be well controlled by the preparation conditions such as the speed of dialysis, initial solvent, initial water content and polymer concentration. The ease with which these spheres could be fabricated and the ability to control the size of spheres should facilitate investigations of their scope for drug delivery.

Keywords: PLA; Dialysis; Particles

1. Introduction

In the past decades, extensive efforts by numerous groups have been made in the development of poly(DL-lactic acid) (PLA), poly(L-lactic acid) and poly(lactide-*co*-glycolide) copolymer micro and nanoparticles for drug delivery. By delivering drug at a controlled rate over a prolonged time, such particles can maintain optimal drug concentrations [1–4], target specific organs and tissues [5], protect and stabilize the drug [6] and aid patient compliance by reducing the frequency of administration. Furthermore, particles with desired size are easily administered by injection. Therefore, the usage of micro and nanoparticles in vivo has attracted considerable interest to achieve these objectives.

The sizes of biodegradable polymer micro and nanoparticles have several critical implications for controlled-release drug delivery. For example, particle size influences allowable routes of administration [7] and the final disposition [8] of the spheres in the body. Importantly, micro and nanoparticle size is a determining factor of drug release rates and controlled manipulation of the size may provide a means to tailor release rate profiles [9]. Finally, the size of particle can be used to passively target the delivery vehicles for uptake by specific types of cells, such as professional antigen-presenting cells [10], or to target specific tissues [11]. For all of these reasons, it is necessary to develop a system that can control the particle

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size which will provide an efficient route to clinical implementation.

Various techniques such as emulsion method [12,13], phase separation [14], spraying [15], and precipitation [16,17] were used in the preparation of micro and nanoparticles. However, the application of these methods was greatly limited by many problems, such as requiring large amounts of emulsifiers, low yield of the particles, higher energy consumption (ultrasonic generator, homogenizer and so on) or working with toxic solvents. Recently, direct dialysis method was developed to prepare polymer particles [18–23]. Dialysis method is an acceptable simple and effective preparation method for surfactant-free micro and nanoparticles. In a dialysis technique, the active ingredient is dissolved in a solution of the polymer in a suitable water-miscible organic solvent; the solution is then introduced into a dialysis tube which is put into water. As the dialysis proceeds, the water diffuses into the organic solvent and the organic solvent also diffuses into the water, the polymer precipitates into micro and nanoparticles, meanwhile, the active ingredient is encapsulated into the particles.

However, a systematic and in-depth evaluation of the effects of dialysis conditions on the size of particles is still not available in literature. In this study, we have developed dialysis method for preparing PLA spheres with controlled size. The mechanisms of the formation of PLA spheres are also suggested by investigating the dynamic process of particle formation during dialysis. The effect of preparation conditions on the size of the spheres is systematically studied and the rationales behind the results are revealed. Finally, in order to evaluate the potential of these PLA spheres as drug delivery carriers, epirubicin, as a model drug, is encapsulated into PLA spheres, and the properties of the drug-loaded spheres are studied.

2. Materials and methods

2.1. Materials

Poly(DL-lactic acid) (PLA) with different molecular weights (Mw) (5 kDa, 10 kDa and 50 kDa) was purchased from the Institute of Medical Instrument of Shandong Province (PR China). Epirubicin hydrochloride (EPI) was purchased from Zhejiang Hisun Pharmaceutical Company (PR China). Solvents and all other chemicals were obtained from commercial sources and used as received unless otherwise noted. Dialysis tube with a molecular weight cut-off of 8000–15,000 g/mol was purchased from Green Bird Science and Technology Development, Shanghai, China.

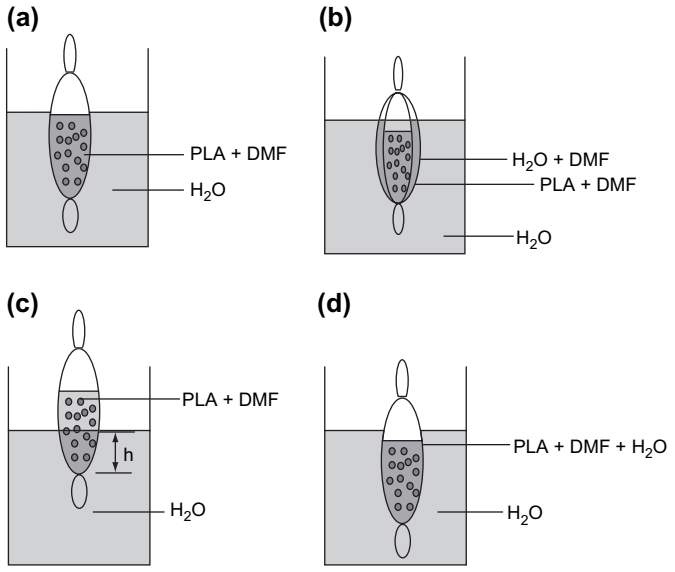
2.2. Preparation of PLA microspheres

PLA spheres were prepared by dialysis. First, PLA (Mw = 5 kDa) was dissolved in dimethylformamide (DMF) or other solvents, such as dimethylacetamide (DMAC), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), acetone and 1,4-dioxane. Second, the PLA solution was introduced into a dialysis tube and dialyzed against deionized water for 12 h at room temperature (Scheme 1a). The water was replaced every 3 h. Then, the sample solution in the tube was collected and centrifuged at 10,000 rpm for 20 min; the precipitant was redispersed in water and freeze-dried for one day to get the powder. This simple dialysis way was used to prepare spheres unless otherwise noted.

For investigating the effect of dialysis speed on the size of spheres, two dialysis ways were used to control the dialysis speed: (1) two-layer membrane dialysis way: PLA solution was introduced into a dialysis tube with small diameter and the dialysis tube was put into another dialysis tube with larger diameter which was filled with different concentration of DMF/water mixture, then the large tube was put into water (Scheme 1b); (2) various areas dialysis way: PLA solution was introduced into dialysis tube and then different area of dialysis tube was put into water. The area of dialysis was controlled by varying the height of dialysis tube (h) in water (Scheme 1c).

In addition, in order to investigate the effect of initial water content on the size of spheres, different amounts of deionized water were added to PLA solvent before dialysis (Scheme 1d), which we called initial water dialysis way.

EPI-loaded PLA spheres were prepared as follows: EPI·HCl was dissolved in DMF and then triethylamine (TEA) was added to this solution with stirring. The EPI solution and the PLA in DMF solution were mixed at room temperature, and deionized



Scheme 1. Schematic diagrams showing four different dialysis ways. (a) The simple dialysis way: PLA solution was introduced into a dialysis tube and dialyzed against water; (b) two-layer membrane dialysis way: PLA solution was introduced into a dialysis tube with a small diameter and this dialysis tube loaded with the solution was put into another dialysis tube with larger diameter which was filled with DMF and water mixture in different concentrations, then the larger dialysis tube was put into water; (c) various areas dialysis way: PLA solution was introduced into dialysis tube and then different areas of dialysis tube were put into water, the area of dialysis was controlled by varying the height of dialysis tube (h) in water; (d) initial water dialysis way: different amounts of water were added to PLA solvent before dialysis.

water was added. The polymer solution was then dialyzed for 12 h at room temperature using dialysis tube. The water was replaced every 3 h. Then, the sample solution in the tube was collected; the products were isolated and subsequently washed with deionized water via centrifugation–redispersion cycles. Each successive supernatant was decanted and replaced with deionized water. Finally, the precipitant was lyophilized.

2.3. Morphology of spheres

The morphology of the spheres was observed by environmental scanning electron microscope (SEM, Phillips XL30). One drop of the sample suspension was placed on a silicon surface. After drying, the sample was coated with gold by using Ion Sputter (JEOL, JFC-1100). Coating was provided at 20 mA for 25 s. Observation was performed at 20 kV.

2.4. Dynamic light scattering

The size and size distribution of spheres were measured by a dynamic light scattering technique (DLS, ZetaPALS Brookhaven Instruments Corporation). The samples were suspended in deionized water and sonicated before measurement. The obtained homogeneous suspension was determined for the mean size and polydispersity. The samples were observed for 30 s under DLS. The determination of three different batches of each sphere formulation was detected to give mean particle size and standard deviation for diameter of sphere.

2.5. Determining the water content

PLA (20 mg) was dissolved in 3 ml DMF or other solvents, such as dimethylacetamide (DMAC), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), acetone and 1,4-dioxane. The solution was dialyzed. At predetermined times, samples were collected from the dialysis tube, and were diluted to 10 ml to precipitate all PLA by adding deionized water. The resulting mixture was filtered to remove precipitant. The content of water in the mixture was then determined by Abbe Refractometer (WAY-2S, Shanghai, China) thermostatted at 20 °C.

2.6. Physical status and distribution of EPI in spheres

The physical status of EPI encapsulated in the spheres was analyzed by using a differential scanning calorimetry (DSC, Netzsch DSC 204). A total of 5–10 mg of sample was weighed and placed into a sealed aluminum pan. The sample was purged with 40 ml/min of dry nitrogen and heated from 0 °C to 250 °C at a rate of 10 °C/min. Indium was used as the standard reference material for temperature and energy scale calibration.

The EPI distribution inside PLA spheres was observed with confocal laser scanning fluorescence microscope (CLSFM, Leica TCS SP2 SE). One drop of the sphere suspension was placed on a glass slide. After drying, EPI in the PLA spheres was excited with a 488 nm argon laser and emission lines were collected after passage through a DD 488/543 filter in a spectral window ranging from 515 nm to 600 nm. The fluorescence distribution in the spheres was observed every 50 nm in *Z*-direction from the uppermost surface to the deepest one. All the *Z* section images were obtained under the same resolution.

2.7. Drug loading

The amount of epirubicin in the spheres was determined by dissolving approximately 5 mg of freeze-dried spheres in 10 ml of DMF. The mixture was stirred for complete dissolution of PLA. The drug content of each sample was measured by UV-2500 spectrophotometer (Shimadzu, Japan) at 480 nm, using a standard calibration curve experimentally obtained with EPI/DMF solutions. The drug content of spheres was calculated using the formula:

$$\text{Drug content } (\%) = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100$$

2.8. In vitro release studies

Drug-loaded PLA spheres (10 mg) were dispersed into 2 ml of PBS (pH 7.4), which was put into a dialysis tube and the tube was immersed into vial containing 10 ml PBS. Then, the drug release tests were performed at 37 °C. At predetermined time intervals, medium was withdrawn and replaced by fresh PBS. The concentration of the released EPI in the samples was determined by UV absorbance at 480 nm.

3. Results and discussion

3.1. The formation process of PLA spheres

The PLA particles were prepared by the simple dialysis way at the initial PLA concentration of 6.7 mg/ml. As the dialysis time prolonged, the water content in the dialysis tube further increased and PLA chains could finally precipitate to micro and nanospheres in the dispersions. The variation of particle morphology was observed by SEM (Fig. 1), the size of particles and the water content in dialysis tube during dialysis process were investigated by DLS (Fig. 2) and refractometer (Fig. 3) *in situ*, respectively. The samples were sonicated before measuring by DLS and the testing time was 30 s, in this case, aggregation of PLA spheres was not easy, and data observed in this way were close to the true result. In addition, we also prolonged the testing time, and the size became larger. It was because PLA suspension in water was not stable and thus the measured size obtained by DLS was bigger than the actual one.

At the initial stage of dialysis (0–2 min), the speed of dialysis was very fast due to the higher concentration gradient across the membrane. There was about 0–10% water content in the PLA/DMF/H_2O mixture inside dialysis tube (Fig. 3), and the intensity of particle size was not obtained by DLS. It was considered that there wasn't any intermolecular association of PLA chains in the DMF/water system because the

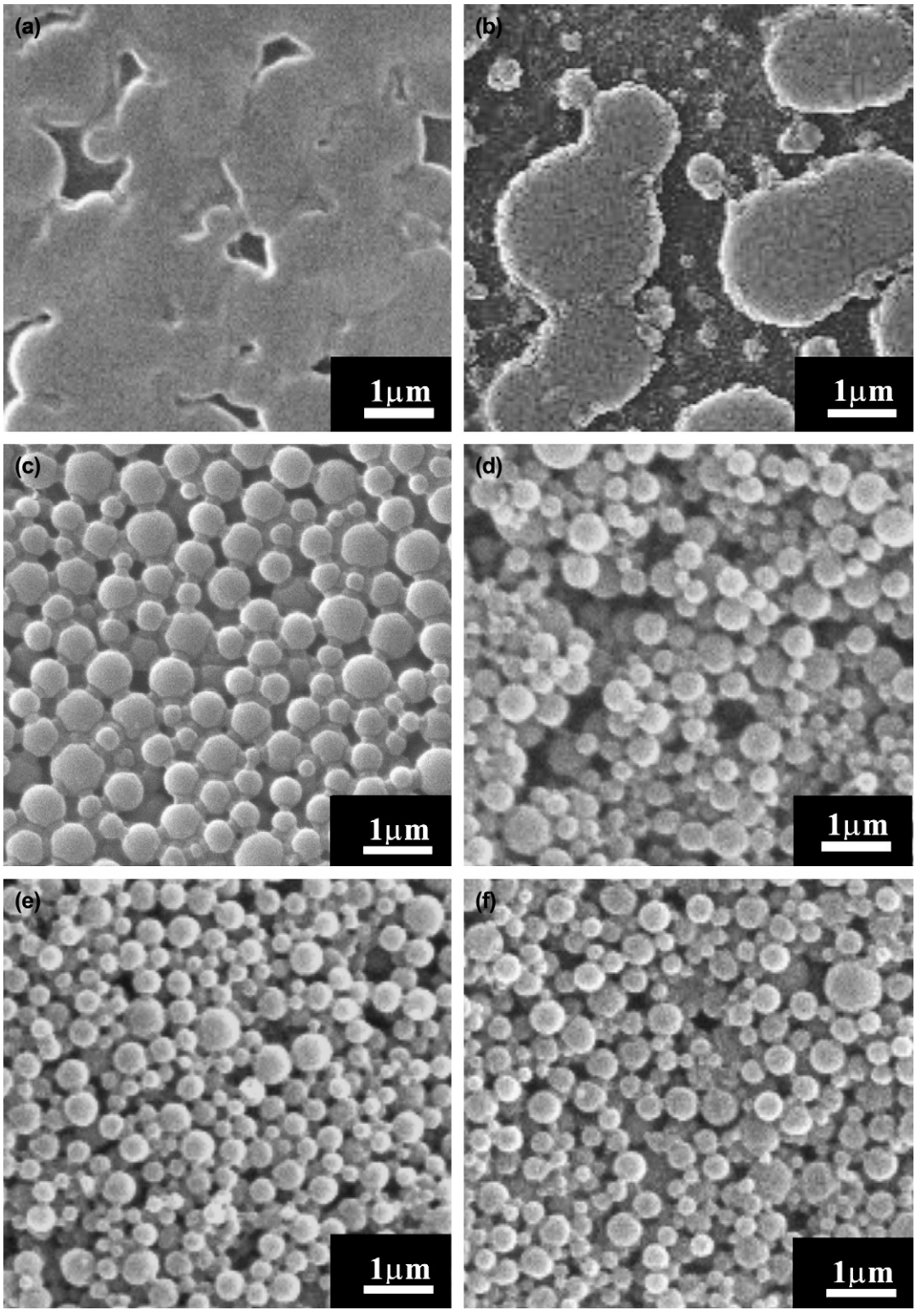


Fig. 1. SEM images monitoring the growth process of PLA spheres prepared by simple dialysis way: (a) 5 min; (b) 10 min; (c) 20 min; (d) 1 h; (e) 2 h; (f) 6 h. [Initial water content: 0, initial PLA concentration: 6.7 mg/ml, initial solvent: DMF.]

hydrophobic interactions of PLA chains were too weak to form particles [24,25]. When the dialysis was carried out for about 5–10 min, the water content in the dialysis tube was about 20–40%, the particle showed irregular shape and tended to disrupt into small particles (Fig. 1a and b); the size of particles was dramatically decreased as shown both in SEM and DLS. In this period, the hydrophobic interactions of PLA were increased due to the decreased solubility in DMF/water mixtures; in this case, PLA chains exhibited a tendency to associate to form a structure with minimum

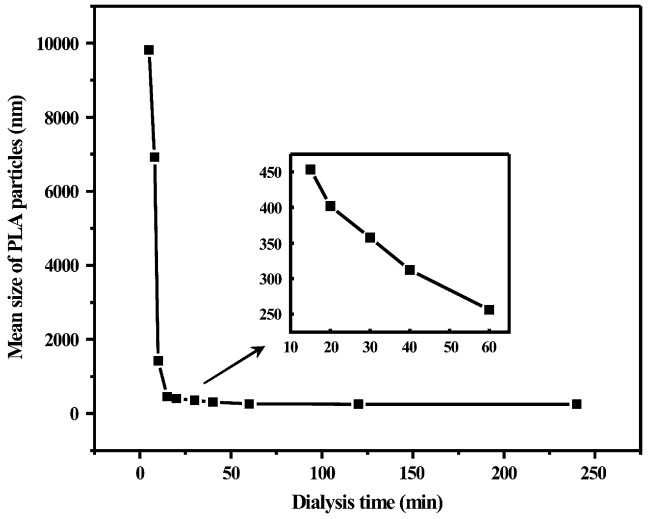


Fig. 2. Effect of dialysis time on the size of PLA spheres (determined by DLS). Inset: enlargement of the same curve from 10 min to 60 min. [Initial water content: 0, initial PLA concentration: 6.7 mg/ml, initial solvent: DMF.]

surface energy, while liquid viscosity had the stabilizing effect and was prone to oppose any change in liquid geometry. So the particles with irregular shape and fraction dimensionality inclined to disrupt into small particles with spherical morphology. It was a very important time point for the morphology change that connected spheres were obtained (Fig. 1c) when the dialysis time was 20 min. At this moment, spherical PLA particle with slack PLA chains was formed; after that, PLA spheres could not be disrupted again. The borderline part of connected particles was the trace of disruption. This was a transition state and important evidence that large particle disrupted into small one. When the dialysis was carried out for about 40 min or more, the water content in the dialysis tube was more than 40%, well-separated spherical particles were obtained (Fig. 1d, f and e). The mean size of spheres showed a gradual decrease as observed by DLS.

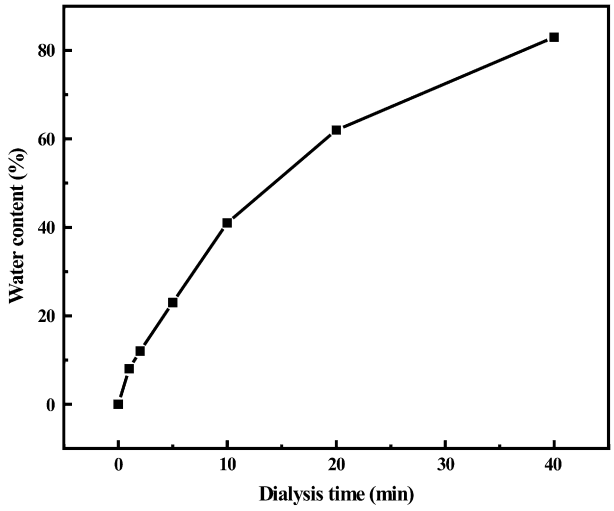
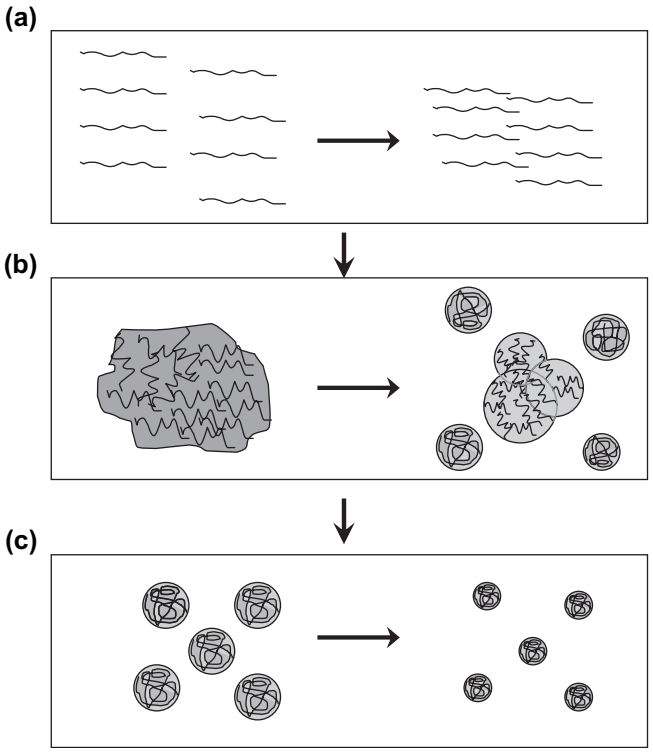


Fig. 3. The relationship between water content and dialysis time. [PLA concentration: 6.7 mg/ml, initial solvent: DMF, initial water content: 0.]

When the water content in dialysis tube was >40%, the particles could not be disrupted into small particles owing to the equilibrium state of disruptive forces and the internal cohesive forces (such as surface tension and liquid viscosity), however, the size of spheres could be slowly decreased with increase of water content in virtue of the spheres turning from swollen state to densely packed state. The formation of this densely packed state was due to the mobility of PLA chains, which was the inner force for the formation of sphere [26]. Nevertheless, the change of the sphere size could not be seen from SEM images when the dialysis time was 20 min or more, it could be ascribed to the drying process of samples before SEM observation [27], which substituted dialysis process, led to the solidification of spheres and as a result the size of PLA spheres showed no change with the prolonged time from SEM images.

For further confirming the formation process of PLA spheres, we also monitored the sphere growth process using high molecular weight PLA (10 kDa, 50 kDa) samples (data not shown), the same result was obtained regardless of the molecular weight of PLA, which indicated that the driving force for the formation of the spheres is the solvent quality. At the same time, the sphere growth process at different PLA concentrations (1.7–26.7 mg/ml) was monitored, similar trend was obtained.

On the basis of particle shape, size, and growth process observed above, the formation of PLA sphere probably consists of three steps: (1) aggregation – individual PLA chains got



Scheme 2. Schematic illustration of the mechanism proposed to account for the formation of PLA spheres during dialysis: (a) aggregation – individual PLA chains got aggregated with each other in solution; (b) formation and disruption of PLA particles; (c) solidification – PLA spheres' transition from swollen state to densely packed state.

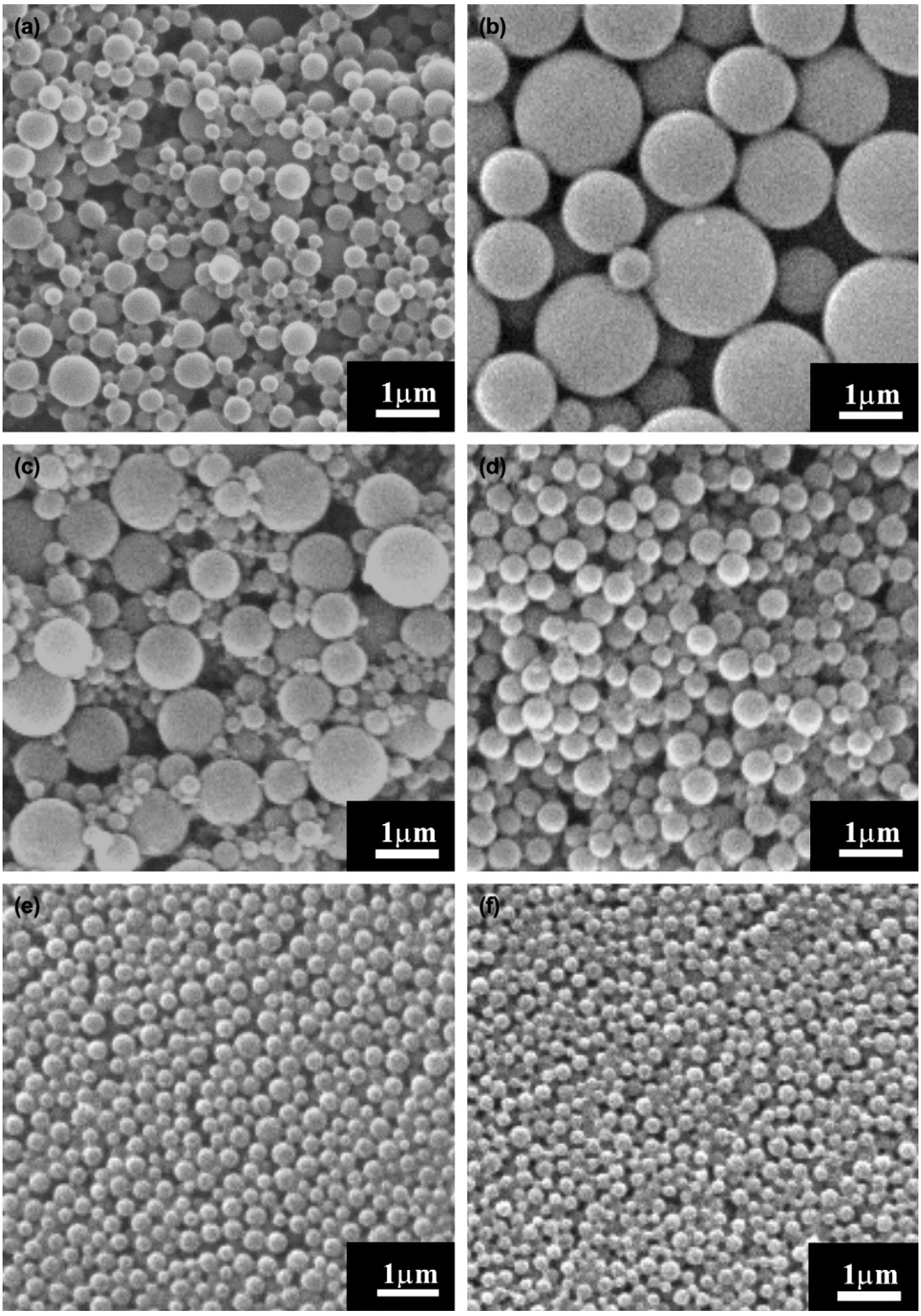


Fig. 4. SEM images of PLA spheres prepared with different initial water contents: (a) 0%; (b) 5%; (c) 10%; (d) 20%; (e) 40%; (f) 80%. [Initial PLA concentration: 6.7 mg/ml, initial solvent: DMF, dialysis time: 12 h.]

aggregated with each other in solution (Scheme 2a); (2) formation and disruption of PLA particles – particles with irregular shape were formed due to the association of PLA chains, meanwhile, the particles tended to disrupt into small particles to obtain small surface energy as shown in Scheme 2b, at the end of this period, PLA spheres with slack chains were obtained (Fig. 1c); (3) solidification – PLA chains turned from slack state to compact state which was corresponding to PLA spheres' transition from swollen state to densely packed state (Scheme 2c).

3.2. Effect of preparation conditions on the size of PLA spheres

3.2.1. Initial water content

In order to observe the effect of initial water content on the size of PLA spheres, 0–80% water was added into PLA solution with stirring, then the dialysis for preparing PLA spheres was carried out for 12 h to completely remove DMF in PLA solution, and the water was replaced every 3 h. Then each sample was centrifuged and freeze-dried. The morphology and size of the PLA spheres were observed by SEM. The mean size of PLA spheres after dialysis was measured by DLS. The relationship between water concentration and dialysis time was investigated by refractometer. The results are shown in Fig. 4, Table 1 and Fig. 5. When the initial water content was 0%, the dialysis speed was very fast (Fig. 5), the mean size of PLA spheres was 221.3 nm, however the size distribution was not uniform. Similar results were also reported by other groups with other polymers [28]. On the other hand, when the dialysis process started with the sample solution containing 20% or higher initial water content, the dialysis speed was relatively slow (Fig. 5), the size of PLA spheres became smaller and the size distribution was more uniform compared with those obtained from the simple dialysis way. In addition, the maximum size of PLA spheres after dialysis was obtained at 5% initial water content. The probable reason for the difference was that PLA chains adopt different conformations in various water/DMF mixtures. At the initial 5–10% water content, PLA chains and water/DMF mixtures formed large droplet; as the water increased in the PLA/water/DMF mixtures by dialysis, disruption of large PLA particles to small particles was difficult due to the slower speed of dialysis, as a result, the size of PLA spheres obtained after dialysis was relatively larger. When water content in PLA solution was over 20%, stable and uniform PLA particles were formed, as the dialysis was further carried out, the slow speed of dialysis only led the PLA chains to more compact, so in this case PLA spheres were relatively uniform. All these results mean that the initial water content is an important factor in controlling the size of PLA spheres.

3.2.2. Concentration of PLA

The PLA concentration here refers to the PLA concentration in DMF before adding water. Fig. 6 and Table 2 show the morphology and size of PLA spheres prepared from different concentrations of PLA using DMF as initial solvent, with 20% initial water content followed by dialysis. With other conditions fixed, as the PLA concentration increased, the mean size of spheres increased; meanwhile, the distribution of spheres became wider. By increasing the initial concentration of PLA from 1.7 mg/ml to 26.7 mg/ml, the mean diameter of spheres increased from 141 nm to 927 nm. The results were in agreement with a general observation found in preparation of polymer particles that increasing the concentration of polymer caused an increase of the particle size [29]. These indicated that the polymer concentration was an important factor influencing the size of spheres. In our system, this could be explained by two reasons: (1) an increase in the polymer concentration, which means high viscosity of the polymer solution, may increase the difficulty in disruption of large particle into small particles; (2) higher concentration of PLA results in the formation of larger particles in initial stage of the second step (Scheme 2b), which corresponds to larger PLA spheres.

Table 1
Effect of initial solvent on the mean size and polydispersity of PLA spheres (determined by DLS)

Water content (%)	Diameter (nm)	Polydispersity
0	325	0.141
5	1088	0.155
10	517	0.138
20	281	0.085
40	230	0.068
80	198.6	0.062

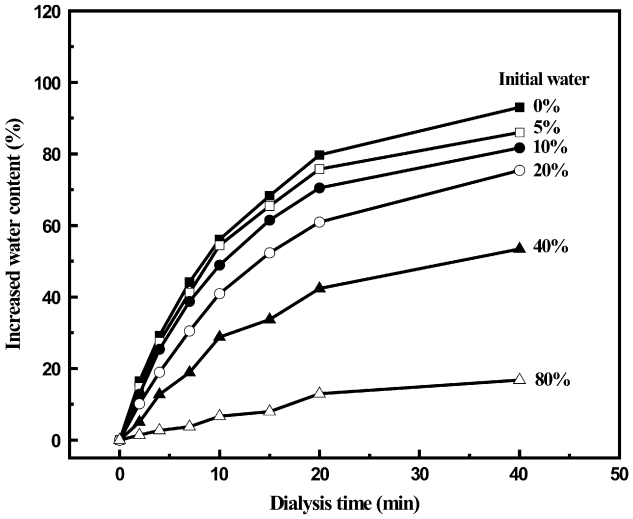


Fig. 5. The relationship between increased water content and dialysis time during dialysis at different initial water contents. [Initial solvent: DMF, PLA concentration: 6.7 mg/ml.]

3.2.3. Initial solvent

Fig. 7 and Table 3 show the morphologies and size of PLA particles prepared by simple dialysis method using different initial solvents (acetone, THF, 1,4-dioxane, DMF, DMAC and DMSO) at the initial PLA concentration of 6.7 mg/ml, respectively. As shown in Fig. 7, no matter what kind of initial solvent was used, spherical PLA particles can be obtained. However, the size of PLA spheres showed great dependence on the initial solvents. When DMF, DMSO and DMAC were used as the initial solvents, particle sizes were relatively smaller than those of acetone, THF and 1,4-dioxane. The sphere sizes measured with DLS (Table 3) were consistent with the particle sizes observed in SEM (Fig. 7). In addition, the polydispersity tended to increase

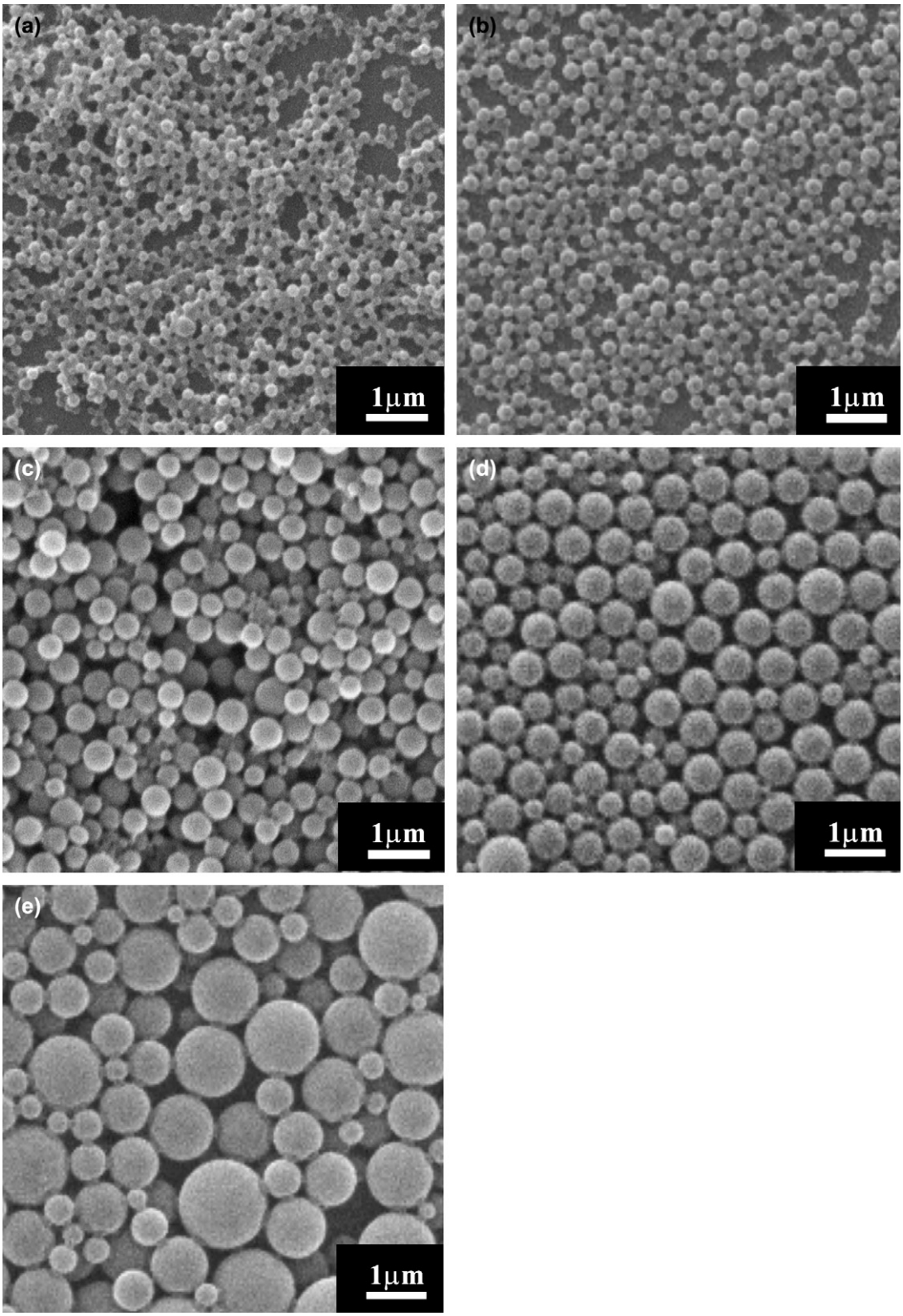


Fig. 6. SEM images of PLA spheres prepared with different initial PLA concentrations: (a) 1.7 mg/ml; (b) 3.4 mg/ml; (c) 6.8 mg/ml; (d) 13.3 mg/ml; (e) 26.7 mg/ml. [Initial water content: 20%, initial solvent: DMF, dialysis time: 12 h.]

when the mean size of spheres was increased. As the size of PLA spheres could be influenced by dialysis speed, we observed the effect of different solvents on the dialysis speed. However, there wasn't any significant difference between dialysis speeds in different solvents (Fig. 8). The different sizes of PLA spheres prepared from different initial solvents might be caused by the differences in physicochemical properties between polymer and solvents such as solubility and

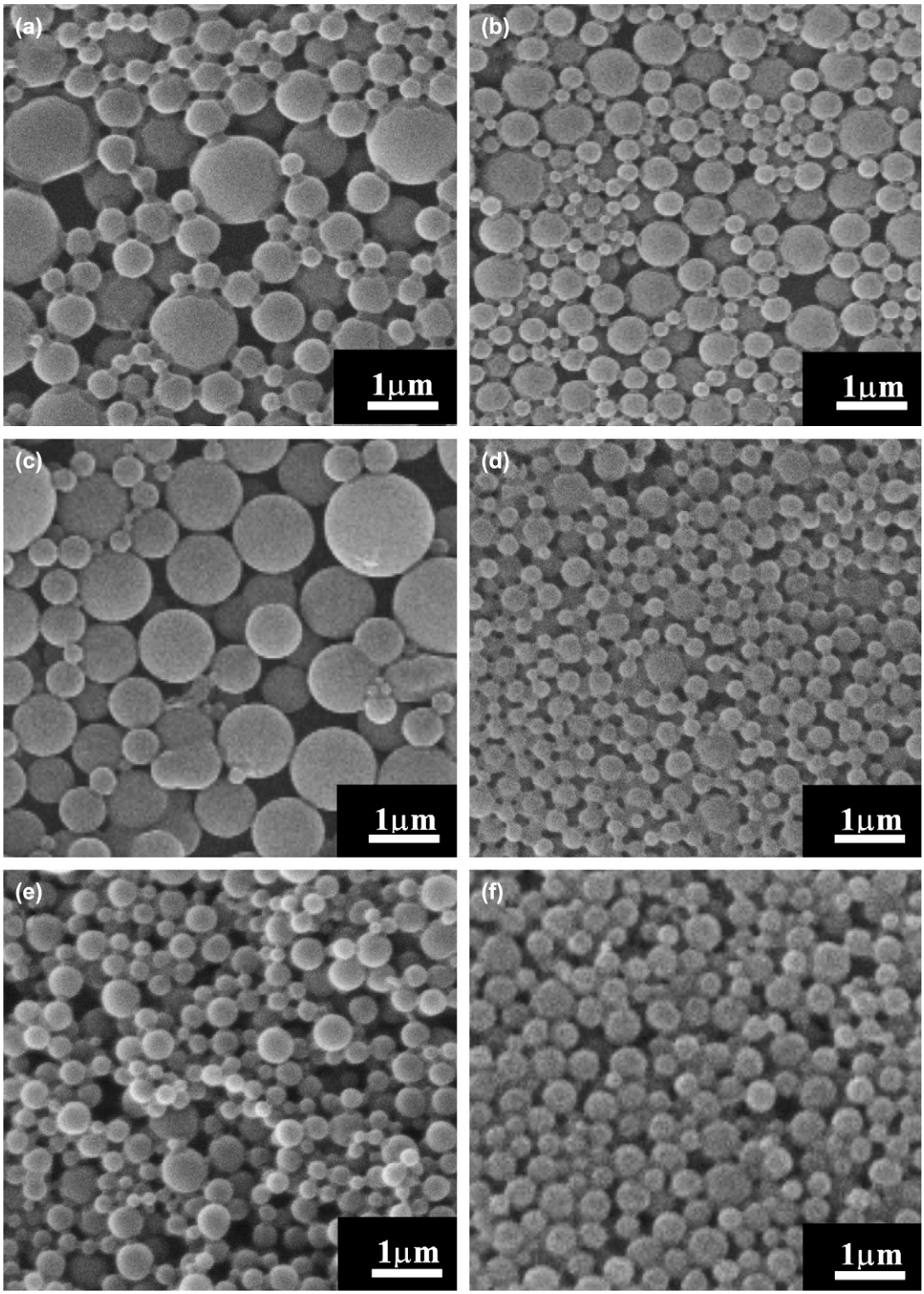


Fig. 7. SEM images of PLA spheres prepared by simple dialysis way from different initial solvents: (a) acetone; (b) THF; (c) 1,4-dioxane; (d) DMF; (e) DMAC; (f) DMSO. [Initial water content: 0, initial PLA concentration: 6.7 mg/ml, dialysis time: 12 h.]

viscosity of polymer to the solvent and miscibility of solvent and water [18].

3.2.4. The speed of dialysis

Dialysis is the simplest application of the solution-diffusion model because only concentration gradients are involved [30]. In dialysis, a membrane separates two solutions of different compositions. The concentration gradient across the membrane causes a flow of solute and solvent from one side of the membrane to the other.

Following the general procedure described above, equating Fick's law [31]:

Table 2
Effect of PLA concentration on the mean size and polydispersity of PLA spheres (determined by DLS)

PLA concentration (mg/ml)	Diameter (nm)	Polydispersity
1.7	128	0.053
3.4	226	0.061
6.7	308	0.068
13.3	498	0.087
26.7	851	0.123

$$J = D \times \Delta c/\Delta x = \Delta m/(\Delta t \times A)$$

$$\Delta m/\Delta t = D \times A \times \Delta c/\Delta x$$

where $\Delta m/\Delta t$ is the dialysis speed at time t; D is the diffusion coefficient; A is the surface area of dialysis tube; $\Delta c/\Delta x$ is the concentration gradient. It is clearly seen that the dialysis rate can be controlled by A and $\Delta c/\Delta x$.

In our system, dialysis speed was also controlled by varying the surface area of dialysis tube in water and the concentration gradient in the following ways: (1) various areas dialysis way: PLA solution was introduced into dialysis tube and then different areas of dialysis tube were put into water (Scheme 1c); (2) two-layer membrane dialysis way: PLA solution was introduced into a dialysis tube with small diameter and then this dialysis tube was put into another dialysis tube with larger diameter which was filled with different concentrations of DMF/water blend (Scheme 1b). In the first way, the size of spheres increased with the decrease of dialysis area (Fig. 9a, b and c). Meanwhile, the decreased dialysis area led to larger size distribution. These results could be ascribed to the huge distinction of dialysis speed in different areas of dialysis tube. In the second way, as other conditions were fixed, increasing the DMF content in the dialysis tube with larger diameter resulted in the increasing size of spheres (Fig. 9d, e, and f). It was clear that for both dialysis ways the sphere size increased with decreasing dialysis speed. The reason for this result was also probably because PLA chains adopted different conformations in various DMF/water mixtures. When the dialysis speed was fast, PLA chains could only associate in a small area during the aggregation step, and then particles with small size were formed, while PLA chains could associate in a large area at slow dialysis speed, and larger particles were formed in the initial stage of the second step in the formation of spheres. In addition, disruption of the particle into small one by fast dialysis during the disruption process was easier.

Table 3
Effect of different solvents on the mean size and polydispersity of PLA spheres (determined by DLS)

Solvent	Diameter (nm)	Polydispersity
Acetone	521	1.69
THF	495	1.58
1,4-Dioxane	654	1.64
DMF	321	1.06
DMAC	354	0.98
DMSO	465	1.12

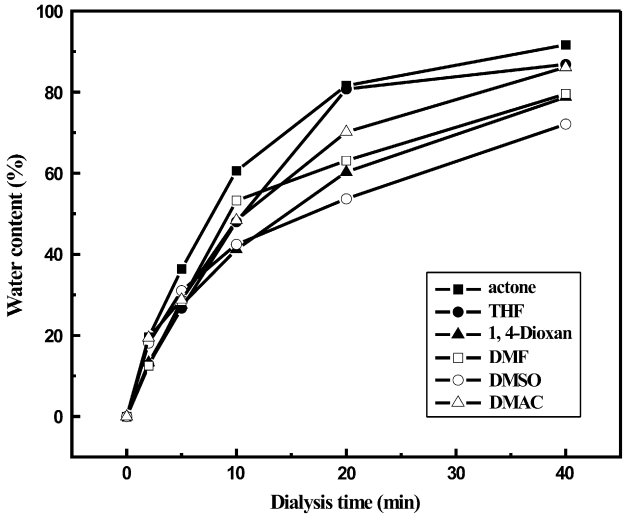


Fig. 8. The relationship between water content and dialysis time during dialysis with different initial solvents. [Initial water content: 0, initial PLA concentration: 6.7 mg/ml, dialysis time: 12 h.]

3.3. Properties of EPI-loaded PLA spheres

3.3.1. Epirubicin loading studies

Epirubicin loading capacity (drug content) of PLA spheres was evaluated by testing drug content in drug-loaded nanospheres, and the spheres were prepared as described above.

To obtain a high amount of drug content, the hydrophilic EPI·HCl was transformed into a hydrophobic one by adding TEA to the organic solvent before the dialysis procedure [32]. TEA from 1.0–6.0 times equivalent to the EPI·HCl quantity was thereby added. The results are shown in Fig. 10. When the ratio of TEA/EPI was 0–4, there was a linear increase in the loading capacity of EPI in PLA spheres to a maximum of 18.6% at an EPI/PLA ratio of 0.6 and at four-fold equivalent of TEA against EPI. However, with increase of the added TEA, the pH value of solution will increase which would influence the stability of EPI [33]. So the pH value should be controlled to <9, and the concentration of TEA in DMF solution will be <2.5 μl/ml. In addition, drug content was also gradually increased according to increasing ratio of EPI/PLA (Fig. 11).

3.3.2. Morphology and size of EPI-loaded spheres

The morphology and size of EPI-loaded spheres were examined by SEM and DLS, respectively. EPI-loaded spheres showed spherical shape and a relatively smooth surface, which were similar to those of blank PLA spheres. Under the same preparation conditions, the EPI-loaded spheres exhibited larger size compared with blank PLA spheres. The size of EPI-loaded spheres found from the SEM images tallied with DLS results.

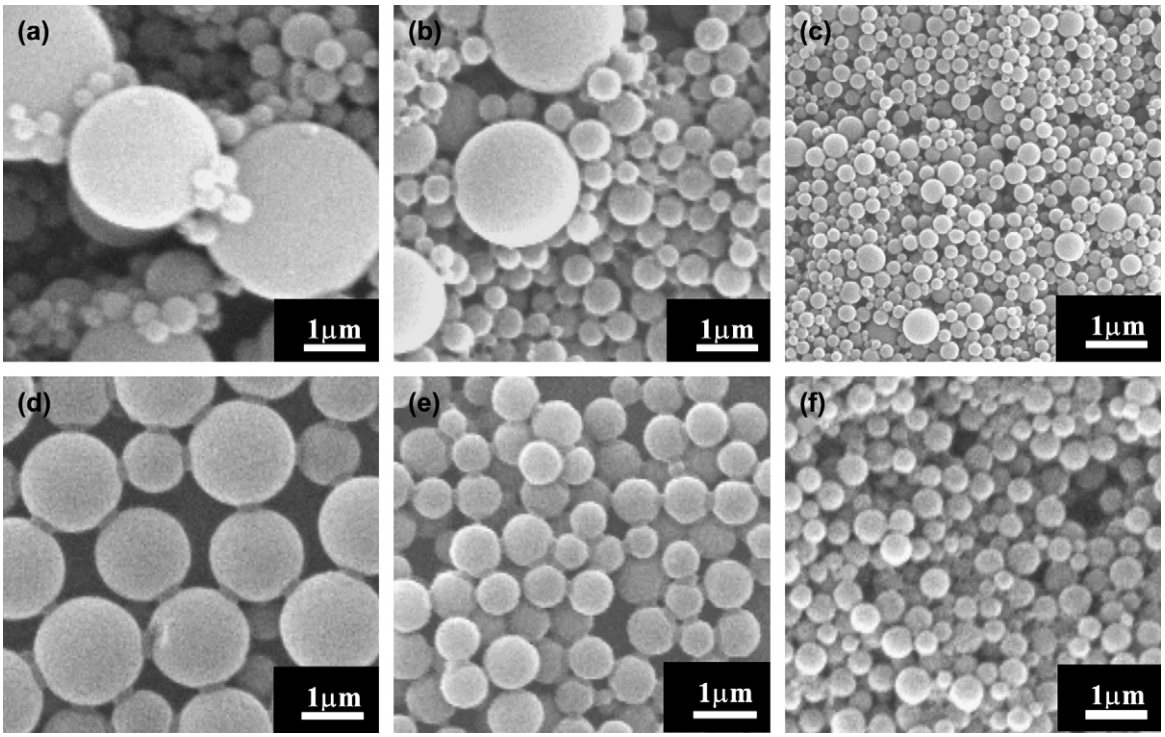


Fig. 9. SEM images of PLA spheres prepared by various areas dialysis way (a–c) and two-layer membrane dialysis way (d–f): (a) dialysis with 1/3 area of dialysis tube; (b) dialysis with 2/3 area of dialysis tube; (c) dialysis with all areas of dialysis tube; (d) dialysis by two different diameter tubes, dialysis tube with larger diameter was filled with DMF; (e) dialysis by two different diameter tubes, dialysis tube with larger diameter was filled with mixture of DMF/water = 1:1 by volume; (f) dialysis by two different diameter tubes, dialysis tube with larger diameter was filled with water. [Initial water content: 0, initial PLA concentration: 6.7 mg/ml, initial solvent: DMF, dialysis time: 12 h.]

3.3.3. Physical status and distribution of EPI in spheres

The physical status of EPI formulated in PLA spheres was investigated by DSC and the result is shown in Fig. 12. Pure EPI showed a melting exothermic peak at 200.31 °C, whereas no peak was detected from 100 °C to 250 °C for the EPI-loaded spheres. These results demonstrated that EPI formulated in PLA spheres existed in an amorphous or disordered crystalline phase. The peak at 59 °C for EPI was suspected as coming from the sample history, therefore we purified EPI by high performance liquid chromatography (HPLC). However, only one peak was observed in the chromatogram. The DSC of EPI was also carried out by five repeated cycles from 20 °C to 100 °C again and the peak at 59 °C was also observed. The peak at 59 °C cannot be explained clearly at this

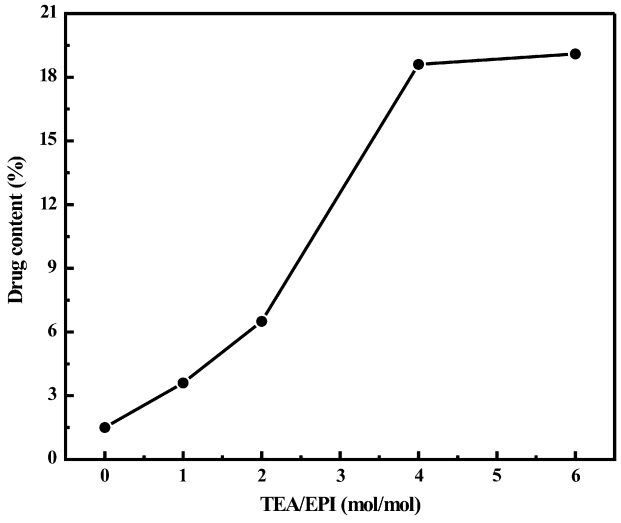


Fig. 10. Influence of the ratio of TEA/EPI on the drug content. [EPI/PLA = 3:5, initial water content: 20%, PLA concentration: 3.3 mg/ml, initial solvent: DMF, dialysis time: 12 h.]

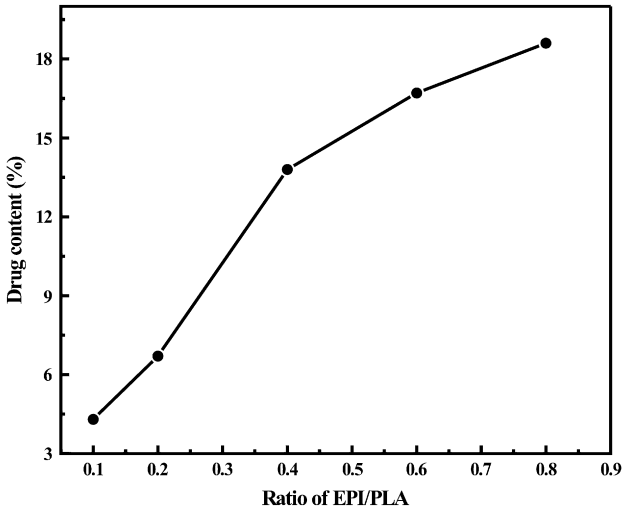


Fig. 11. Influence of the ratio of EPI/PLA on the drug content. [TEA/EPI (mol/mol) = 4, initial water content: 20%, PLA concentration: 3.3 mg/ml, initial solvent: DMF, dialysis time: 12 h.]

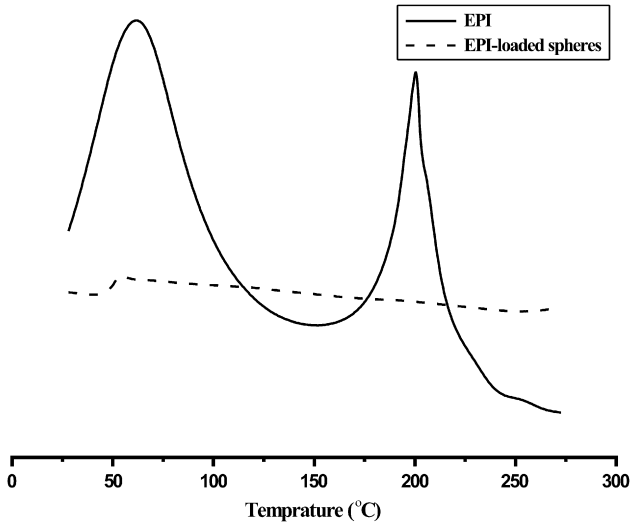


Fig. 12. DSC thermograms of EPI and EPI-loaded spheres.

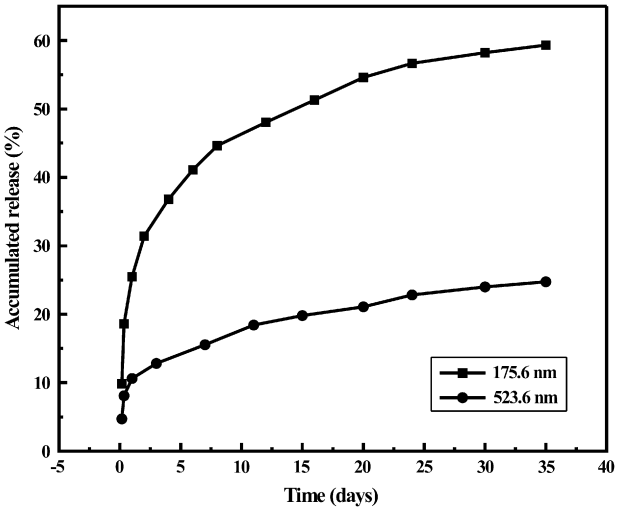


Fig. 14. Release profiles of EPI from PLA spheres with different sizes in phosphate buffer solutions.

stage. However it does not affect our result: EPI formulated in PLA spheres existed in an amorphous or disordered crystalline phase.

The distribution of EPI in PLA spheres is presented in Fig. 13. The CLSFM images were obtained at intervals of 200 nm in the *Z*-direction from the topmost layer to the deepest layer of a PLA sphere. Since PLA is not fluorescent, the bright colored area qualitatively represents EPI. It was clear that the distribution of EPI in whole spheres was very uniform.

3.3.4. In vitro release

Fig. 14 shows the release of EPI from spheres with different sizes. PLA spheres with mean size of 523.6 nm exhibited a small initial release rate. The initial burst effect was observed due to the diffusion of EPI close to the surface of spheres [34]. However, when the mean size of PLA spheres was 175.6 nm, EPI demonstrated a significant release amount (>30%) from PLA spheres in the initial 0–2 days. Then the sustained release was kept for 33 days by drug diffusion from inside of PLA spheres or due to PLA degradation. It was obvious that the initial release rates decreased with increasing sphere diameter. This was expected due to the decrease in surface area/volume ratio with increasing size. The result was in accordance with another report [35], which strongly suggested that drug release can be controlled by the size of spheres.

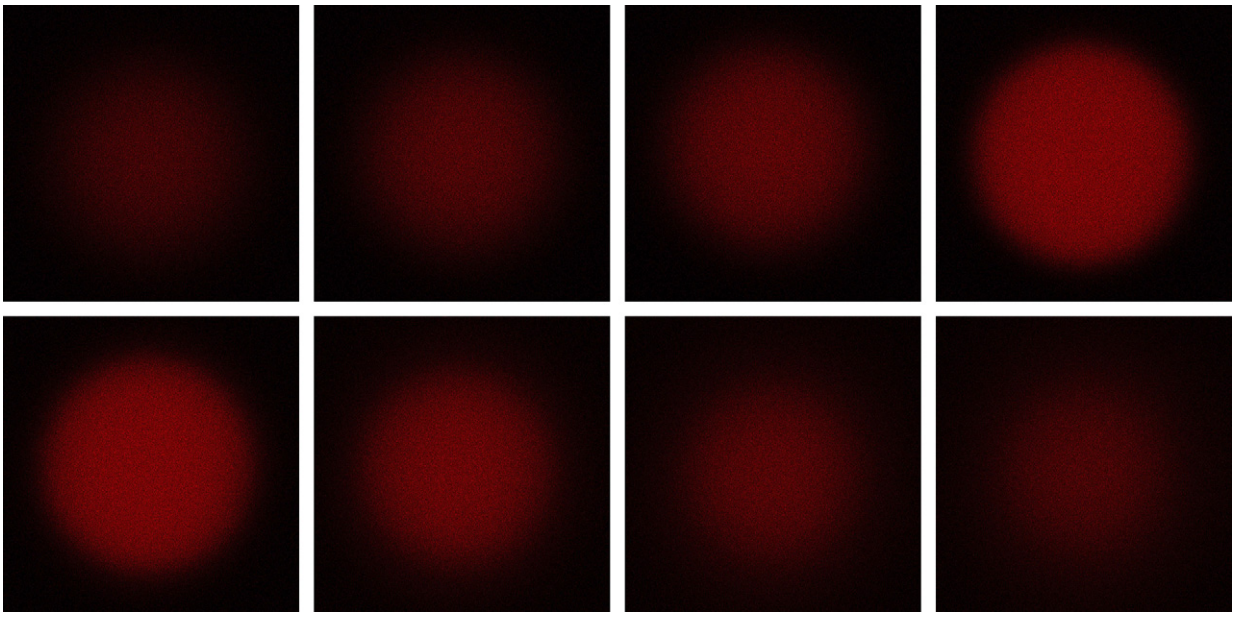


Fig. 13. Subset of a series of confocal optical sections of EPI/PLA spheres captured by CLSFM. The upper left image of both images comes from the topmost layer of PLA spheres whereas the bottom right image from the deepest layer. The distance between sections in the subset is about 200 nm. [Initial water content: 5%, initial PLA concentration: 13.3 mg/ml, initial solvent: DMF, EPI concentration: 0.67 mg/ml, TEA/EPI (mol/mol) = 4, dialysis time: 12 h.]

4. Conclusion

Biodegradable PLA spheres with uniform size in the range of nanometers to micrometers were obtained by direct dialysis method. The size of PLA spheres can be well controlled by initial solvent, PLA concentration, initial water content and the speed of dialysis. Besides, in order to investigate the mechanism of PLA formation, the changes of morphology and size of PLA particles during dialysis process were observed by SEM and DLS. All those results strongly suggested that the formation of PLA spheres could be divided into three steps: (1) aggregation – individual PLA chains got aggregated with each other in solution; (2) formation and disruption of PLA particles; (3) solidification of PLA spheres.

In addition, EPI-loaded PLA spheres were also successfully obtained by direct dialysis. The properties of the drug-loaded spheres were investigated by SEM, DLS, CLSM, UV and DSC. All the experimental observations indicate that the PLA spheres prepared by dialysis could be a good candidate as drug carrier for controlled release of the drug.

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References

[1] Chiba M, Hanes J, Langer R. Biomaterials 1997;18:893–901.
[2] Mathiowitz E, Kline D, Langer R. Scanning Microsc 1990;4:329–40.
[3] Mathiowitz E, Saltzman WM, Domb A, Langer R. J Appl Polym Sci 1998;35:755–74.
[4] Tamada J, Langer R. J Biomater Sci Polym Ed 1992;3:315–53.
[5] Truong VL, Williams JR, Hildreth JEK, Leong KW. Drug Deliv 1995;2:166–74.
[6] Castellanos IJ, Cuadrado WO, Griebenow K. J Pharm Pharmacol 2001;53:1099–107.
[7] Smith PF. IDrugs 2003;6:1173–7.
[8] Nomura T, Koreeda N, Yamashita F, Takakura Y, Hashida M. Pharm Res 1998;15:128–32.
[9] Waterman KC, Sutton SC. J Controlled Release 2003;86:293–304.
[10] Thiele L, Rothen-Rutishauser B, Jilek S, Wunderli-Allenspach H, Merkle HP, Walter E. J Controlled Release 2001;76:59–71.
[11] Tinsley-Bown AM, Fretwell R, Dowsett AB, Davis SL, Farrar GH. J Controlled Release 2000;66:229–41.
[12] Luck M, Pistel KF, Li YX, Blunk T, Muller RH, Kissel T. J Controlled Release 1998;55:107–20.
[13] Chang CJ, Kao PC. Polymer 2006;47:591–601.
[14] Wang FJ, Wang CH. J Biomater Sci Polym Ed 2003;14:157–83.
[15] Zhao CS, Liu XD, Nomizu M, Nishi N. J Colloid Interface Sci 2004;275(2):470–6.
[16] Young TJ, Johnston KP, Mishima K, Tanaka H. J Pharm Sci 1999;88:640–50.
[17] Yoshioka Y, Asao K, Yamamoto K, Tachi H. Polymer 2007;48:2214–20.
[18] Jeon HJ, Jeong YI, Jang MK, Park YH, Nah JW. Int J Pharm 2000;207:99–108.
[19] Zhang ZP, Feng SS. Biomacromolecules 2006;7:1139–46.
[20] Kim JH, Kim YS, Kim S, Park JH, Kim K, Choi K, et al. J Controlled Release 2006;111:228–34.
[21] Jeong YI, Shim YH, Song KC, Park YG, Ryu HW, Nah JW. Bull Korean Chem Soc 2002;23:1579–84.
[22] Ataman-Onal Y, Munier S, Ganée A, Terrat C, Durand PY, Battail N, et al. J Controlled Release 2006;112:175–85.
[23] Jeong YI, Cho CS, Kim SH, Ko KS, Kim SI, Shim YH, et al. J Appl Polym Sci 2001;80:2228–36.
[24] Lo CL, Lin KM, Hsiue GH. J Controlled Release 2005;104:477–88.
[25] Deng YH, Li YB, Wang XG. Macromolecules 2006;39:6590–8.
[26] Pillin I, Montrelay N, Grohens Y. Polymer 2006;47:4676–82.
[27] Tang M, Dou H, Sun K. Polymer 2006;47:728–34.
[28] Gadzinowski M, Sosnowski S, Slomkowski S. e-Polymers 2005;084.
[29] Hu J, Tang Z, Pang X, Yang Y, Chen X, Jing X. Biomacromolecules 2005;6:2843–50.
[30] Mathias U. Polymer 2006;472:217–26.
[31] Baker RW. Membrane technology and applications. 2nd ed. New York: J. Wiley; 2004.
[32] Kohori F, Yokoyama M, Sakai K, Okano T. J Controlled Release 2002;78:155–63.
[33] Pasut G, Scaramuzza S, Schiavon O, Mendichi R, Veronese FM. J Bioact Compat Polym 2005;20:213–23.
[34] Wu Y, Gao J, Yang W, Wang C, Fu S. Polymer 2006;47:5287–94.
[35] Berkland C, Kim K, Pack DW. J Controlled Release 2001;73:59–74.