

不同溶剂制备的聚乳酸多孔微球的形成机理^{*}

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摘 要 利用改进的双乳液溶剂挥发法制备了多孔聚乳酸(PLA)微球.通过采用具有不同沸点和水溶性的有机溶剂制备得到不同多孔结构的 PLA 微球.结果发现以二氯甲烷、氯仿和甲苯为溶剂制备的微球具有相似的均匀多孔结构,而以乙酸乙酯制备的微球却具有中空结构和多孔的壳层.通过进一步的实验研究了溶剂种类对于微球多孔结构的影响.结果表明溶剂的移出速率对于微球的多孔结构起到决定作用.最后在以上实验基础上探讨了以不同溶剂制备多孔微球的形成机理.

关键词 聚乳酸, 多孔微球, 溶剂移出速率

FORMATION MECHANISM OF SOLVENT-INDUCED POROUS PLA MICROSPHERES

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Abstract Porous polylactide (PLA) microspheres were prepared by means of the modified double emulsion solvent evaporation. The organic solvents with different boiling points and aqueous solubility were used to prepare PLA microspheres with different porous structures. Microspheres prepared with methylene chloride, chloroform and toluene as solvents had the similar homogeneous porous structure, while those microspheres with ethyl acetate as solvent showed a hollow structure with a porous thin shell. The effects of solvent types on porous structure of microspheres were discussed. It was concluded that the removal rate of solvent plays the decisive role in determining the porous structure of microspheres. Accordingly, a formation mechanism of porous microspheres fabricated with different solvents was proposed.

Keywords Polylactide, Porous microspheres, Solvent removal rate

1 INTRODUCTION

Microspheres fabricated by biodegradable polymers have great biomedical potentials in the field of tissue engineering. They can act as microcarriers or scaffolds to help the growth of cells and to recover the defective tissues^[1,2]. It has been known that the porous structure of microspheres is of great significance to the growth behavior of cells^[3]. The interconnected porous structure could enlarge the

microspheres surface for cell attachment, facilitate the nutrient diffusion or cell excretion, and assist the new tissue ingrowths^[4-12]. Therefore, fabrication of polymer microspheres with versatile porous structure is of great importance for the application in tissue engineering.

Among various preparation methods, the modified emulsion solvent evaporation is one of the often used methods to prepare porous microspheres of biodegradable polylactide (PLA)^[9, 13, 14]. In this

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method, microspheres with well interconnected pores were prepared through the simple addition of effervescent salt-ammonium bicarbonate (NH_4HCO_3) in the inner water phase. Many experiment parameters of the emulsion solvent evaporation method were used to control the final properties of microspheres^[15-21]. Among them, organic solvents were reported to play an important role in the fabrication of microspheres^[19, 22-24].

Our previous work reported the fabrication of PLA microspheres with versatile porous structure by the modified emulsion solvent evaporation method through the changing of fabrication parameters. It also interestingly revealed that organic solvent had great influence on the porous structure of PLA microspheres^[25]. However, the formation mechanism of these different porous structures is still unknown. Due to the great effects of organic solvents on the porous structure and final application of microspheres, it is necessary to know how the microspheres form the different porous structure when different solvents were used.

In this work, four kinds of organic solvents were used to fabricate porous PLA microspheres through the modified double emulsion solvent evaporation method. The influence of organic solvents on the solvent removal process and the resultant porous structure of PLA microspheres was discussed. A formation mechanism of porous microspheres during solvent the removal process was proposed to explain the role of organic solvents in controlling the porous structure of microspheres.

2 MATERIALS AND METHODS

2.1 Materials

Poly(DL-lactide) ($M_w = 7.5 \times 10^4 \sim 1.2 \times 10^5$)

and poly(vinyl alcohol) (87% ~ 89% hydrolyzed, $M_w = 8.5 \times 10^4 \sim 1.46 \times 10^5$) (PVA) were purchased from Aldrich. Ammonium bicarbonate (NH_4HCO_3) with analytical grade was from Shanghai Sihewei Chemical Co. Ltd. All the organic solvents used in this work were of analytical grades and purchased from Beijing Chemical Co. Ltd. These chemicals were used as received.

2.2 Preparation of Microspheres

PLA microspheres were prepared by the modified emulsion solvent evaporation technique^[25]. Briefly, NH_4HCO_3 was added to 1.25 mL water with a certain concentration. This aqueous solution (W_1) was added into 4 mL organic solvent which contained 0.125 g PLA (O), and emulsified to form the primary emulsion (W_1/O). Then, this primary emulsion was poured immediately into 150 mL 1 g/L PVA aqueous solution and stirred to produce a double emulsion ($W_1/O/W_2$). The organic solvents were evaporated from the PLA/organic solvent microdroplets during the stirring process. The stirring continued at least for 4 h to permit the complete evaporation of organic solvent. Finally the PLA microspheres were washed three times with deionized water and lyophilized for further use.

In this work, four kinds of organic solvents with different boiling point (b. p.) and solubility were chosen to prepare porous PLA microspheres. The major physical properties of these solvents are listed in Table 1^[26].

2.3 Characterization of Microspheres

The surface and cross-section of PLA microspheres were observed by using a scanning electron microscope (JEOL JSM-6700F, Japan). PLA microspheres and their slices cut by a blade were mounted on metal stubs and coated with platinum.

Table 1 Some physical properties of organic solvents^[26]

Solvent	b. p. (°C)	Density at 20°C (g/mL)	Solubility (wt%) at 20 ~ 25°C	
			Solvent in water	Water in solvent
Methylene chloride	39.8	1.3255	1.32	0.2
Chloroform	61.7	1.4924	0.8	0.2
Toluene	110.6	0.8683	0.06 (30°C)	0.05 (20°C)
Ethyl acetate	76.7	0.9018	8.7	3.3

The size of microspheres was determined using an optical microscope (Leica DMLP, Germany), 150 microspheres were counted to calculate the average diameter and size distribution of microspheres.

2.4 Shrinkage of Microdroplets

To determinate the shrinkage of microdroplets, the dispersion of microdroplets was observed under an optical microscope (Carl Zeiss JENA, Germany) equipped with a CCD camera (Beijing Daheng Co., HV1301UC, China). Pictures were taken immediately following the collection of a small amount of the large volume water phase. This was performed for each preparation at 10, 20, 30, 40, and 50 min after pouring the primary emulsion into the outer PVA solution.

2.5 Stability of the Primary Emulsion

The stability of primary emulsion could be detected through the time when the macroscopic phase separation happened. The primary emulsion was prepared as reported in the above preparation section and stored in 10ml assay tubes fitted with a plastic septum at room temperature.

3 RESULTS AND DISCUSSION

3.1 Morphology and Structure of Microspheres

Figure 1 shows the morphology of porous PLA microspheres prepared with different organic solvents. It was found that the microspheres prepared with methylene chloride, chloroform and toluene showed a similar porous structure (as seen in Fig. 1(a1) ~ (c1)), while the microspheres prepared with ethyl acetate showed a very different morphology (as seen in Fig. 1(d1)). There were many pores on the surface of microspheres when methylene chloride, chloroform and toluene were used as solvents, while a relatively smooth surface was found for the microspheres prepared with ethyl acetate as solvent. Correspondingly, the cross-sections of microspheres (as seen in Fig. 1(a2) ~ (d2)) show that the microspheres prepared with methylene chloride, chloroform and toluene as solvents had the similar homogeneous porous structure, while the microspheres prepared with ethyl acetate had a hollow structure with a porous

thin shell.

Figure 2 shows the average size and size distribution of PLA microspheres fabricated with different solvents. The mean diameters of microspheres prepared with methylene chloride, chloroform, toluene and ethyl acetate are 325, 191, 115 and 225 μm , respectively. The microspheres prepared with ethyl acetate show the widest size distribution, while those microspheres prepared with toluene show the most narrow size distribution.

Based on the above morphological observation and size statistics of PLA microspheres, it was conceivable that the great differences in the morphology, structure and size of porous PLA microspheres were attributed to the different organic solvents. Due to the great effects of porous structure of microspheres on cell growth behavior, it is necessary to reveal the mechanism of how the organic solvents influenced the formation of porous PLA microspheres.

3.2 Formation Mechanism of Microspheres

The stability of the primary emulsion (W_1/O) has a decisive effect on the internal morphology of PLA microspheres. When the aggregate and coalescence of inner aqueous droplets (W_1) become easily, the double emulsion could form several representative precursor structures of matrix microspheres, porous microcapsules, multivesicular microparticles and microcapsules^[15, 27, 28]. In the modified emulsion solvent evaporation method, NH_4HCO_3 was supposed to act as the surfactant and foaming agent, which may contribute to the stability of primary emulsion. In this study, to figure out the function of NH_4HCO_3 , PLA microspheres whose inner aqueous phase was only water were prepared. Ethyl acetate and methylene chloride were chosen as the typical solvents to prepare PLA microspheres.

Figure 3 shows the SEM results of microspheres and their cross-sections. Compared with Fig. 1, it is found that the microspheres with methylene chloride as solvent show the multivesicular and even microcapsules structure (as seen in Fig. 1(b2) and Fig. (3b)). While, the microspheres with ethyl acetate as solvent show the similar hollow structure

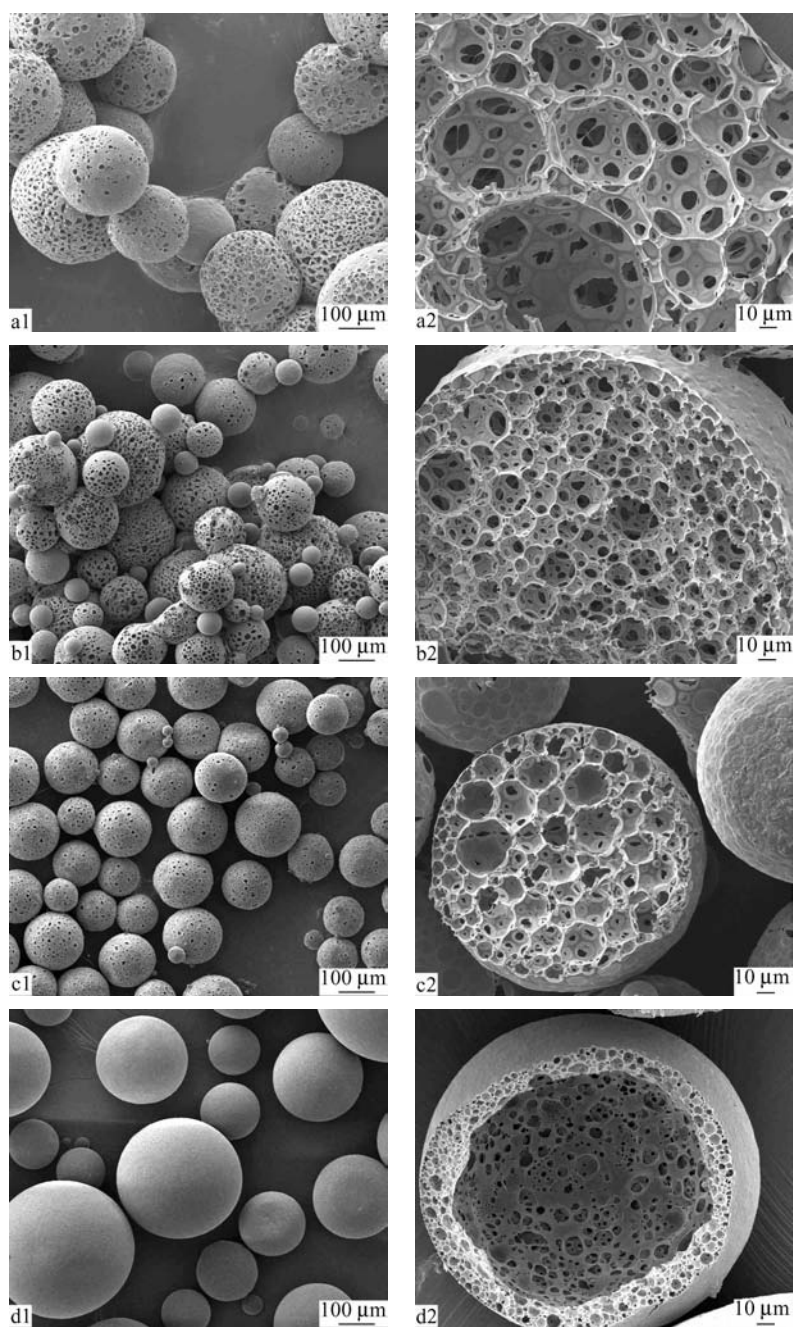


Fig. 1 SEM photographs of PLA microspheres prepared with different organic solvents, methylene chloride (a), chloroform (b), toluene (c) and ethyl acetate (d)

(a1) ~ (d1) show the surface morphology, and (a2) ~ (d2) show the cross-section of corresponding microspheres.

(as seen in Fig. 1 (d) and Fig. (3d)). Furthermore, due to the lack of NH_4HCO_3 , microspheres whose inner aqueous phase is water have less pores than microspheres whose inner aqueous phase is NH_4HCO_3 solution. The results in Fig. 1 and Fig. 3 showed that the decomposed products of NH_4HCO_3 , *i. e.* carbon dioxide and ammonia gas, could not only stabilize the primary

emulsion, but also create open porous morphology throughout the PLA microspheres.

To find the reason of the difference between hollow structure and multivesicular structure of microspheres fabricated by different solvents, the stability of primary emulsion (W_1/O) was detected through the time when the macroscopic phase separation of primary emulsion happened. Figure 4

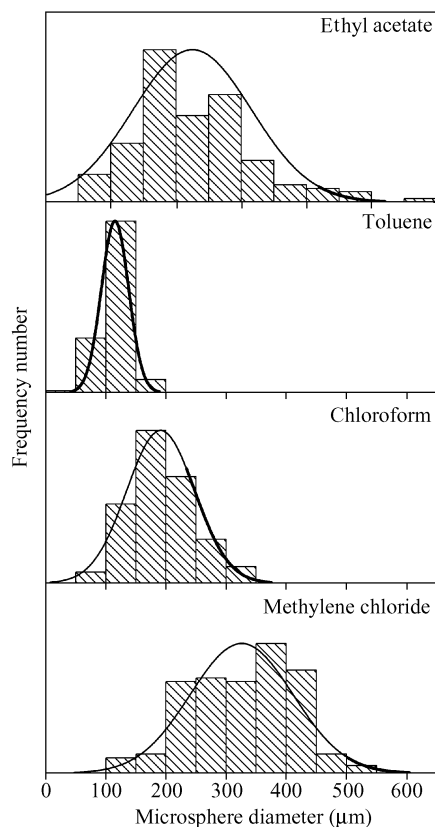


Fig. 2 Average size and size distribution of PLA microspheres prepared with different solvents

showed that after a time period of 1 h an obviously macroscopic phase separation was observed in the emulsion prepared with methylene chloride, while the emulsion prepared with ethyl acetate as solvent still kept homogenous. Due to the higher water solubility of ethyl acetate, the interfacial tension between ethyl acetate phase and inner water phase is lower than that in the case of methylene chloride, and leads to a higher stability of the primary emulsion prepared with ethyl acetate. Therefore, under the same preparation parameters, the different structure between PLA microspheres prepared with ethyl acetate and other organic solvents should be ascribed to the factors caused from the outside of the primary emulsion.

The rate of solvent removal and polymer precipitation which affect the extent of primary emulsion coalescence were measured from the size shrinkage of PLA/organic solvent microdroplets ($W_1/O/W_2$ microdroplets). Figure 5 shows the representative optical microscopy photographs of microdroplets prepared with different organic

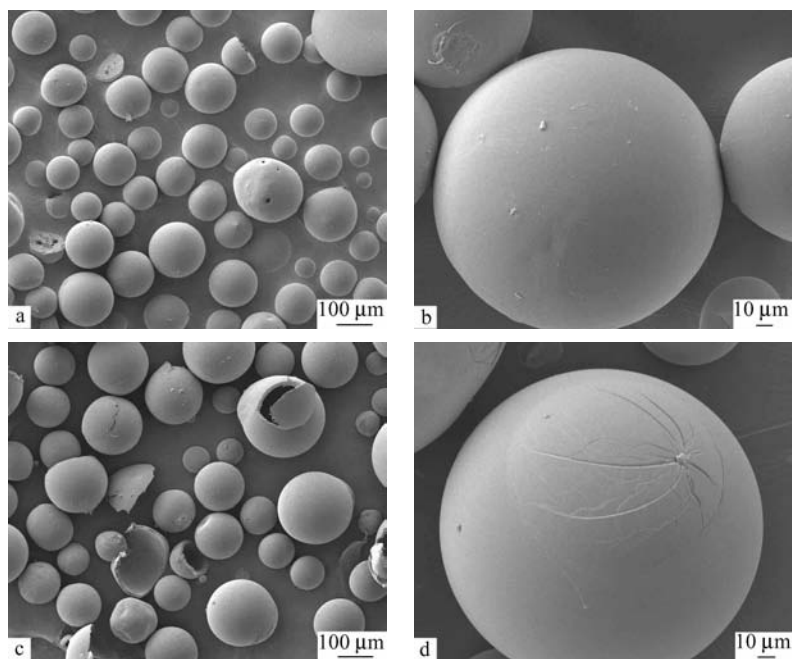


Fig. 3 PLA microspheres prepared with methylene chloride (a, b) and ethyl acetate (c, d) as solvent
The inner aqueous phase is only water without addition of NH_4HCO_3 .

solvents, which were taken at 10, 30 and 50 min after pouring the primary emulsion (W_1/O) into the

outer aqueous phase (W_2). It was found that the microdroplets prepared with methylene chloride,

chloroform and toluene appeared opaque, while those prepared with ethyl acetate looked more transparent. As revealed by SEM results in Fig. 1,

the transparency of the microdroplets with ethyl acetate as solvent is because of the hollow structure of microspheres.

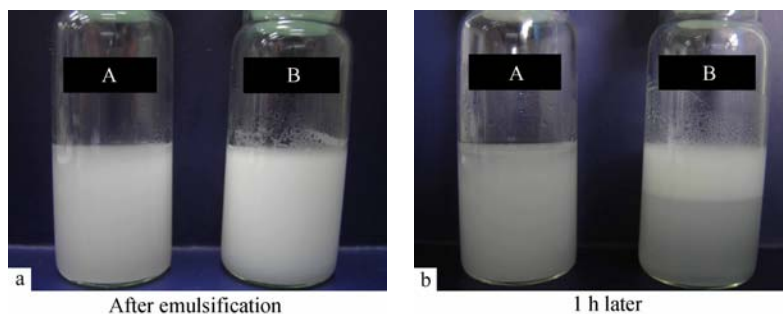


Fig. 4 Photos of primary emulsion with ethyl acetate (A) and methylene chloride (B) as organic solvents

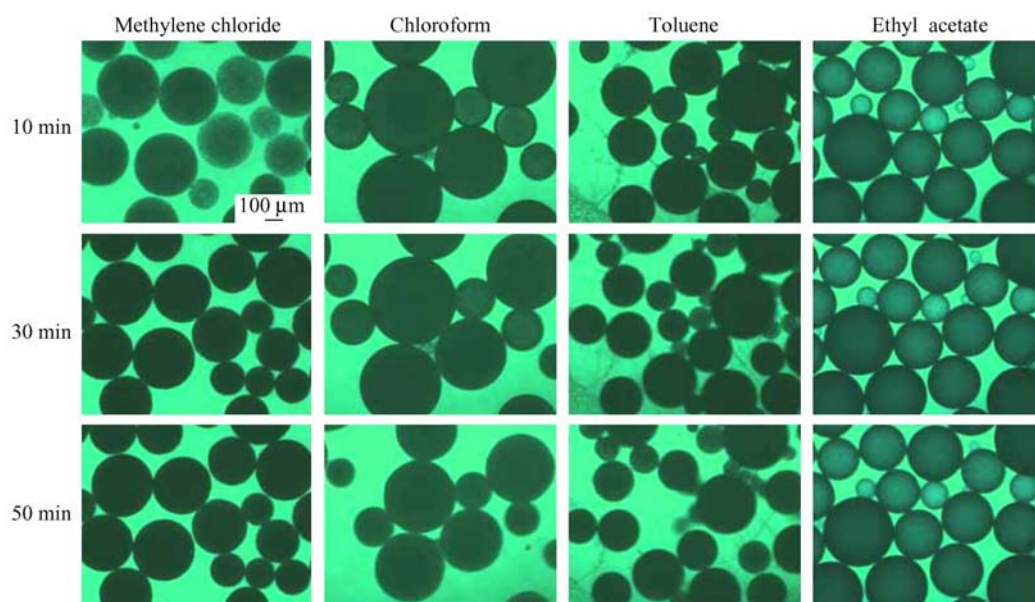


Fig. 5 Optical photomicrographs of PLA microspheres prepared with different solvents during the solvent removal process

Figure 6 shows the relative shrinkage of microdroplets calculated from the microscopy photographs of microdroplets prepared with different organic solvents after pouring the primary emulsion into the outer aqueous phase. The size of PLA/ethyl acetate microdroplets did not change. While, the PLA/methyl chloride microdroplets showed a slight shrinkage in the initial 20 min and then kept unchanged. However the size of PLA/chloroform and PLA/toluene microdroplets gradually decreased during the observation process. In our experiments, the PLA/ethyl acetate microdroplets were found to become quickly hard even within few minutes, while PLA/toluene microdroplets took a longer stirring

time to become hard due to the slow evaporation of toluene. Therefore, based on our observation and the results in Table 1, the solvent removal rate decreased in the order: ethyl acetate > methylene chloride > chloroform > toluene.

In the emulsion solvent evaporation method, the complete removal of organic solvents results in the formation of polymer microspheres^[22, 27]. During the formation process of microspheres, organic solvents diffuse from the microdroplets into the outer aqueous phase, and then get through the air/liquid interface and finally evaporate into the atmosphere. Therefore, the solvent removal rate usually includes diffusion rate and evaporation rate, which could be

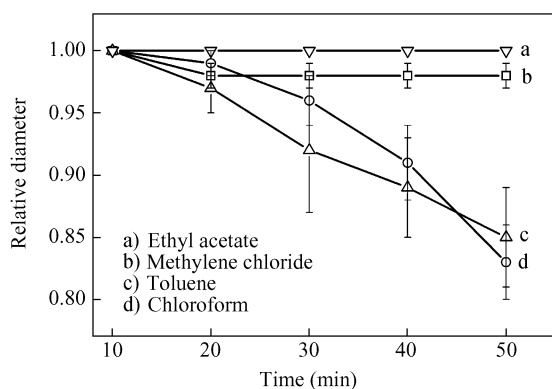


Fig. 6 Plot of relative microsphere shrinkage throughout the solvent removal process of PLA microsphere prepared with different solvents

respectively represented by the solubility in water and boiling point of solvent. For solvents with high solubility, the diffusion rate decides the solvent removal rate at most. Although ethyl acetate has a higher boiling point of 76.7°C, the high solubility makes it diffuse rapidly from microdroplets into outer aqueous phase. The quick diffusion of ethyl acetate led to the rapid solidification of PLA/ethylene acetate microdroplets and resulted in no change of microspheres size. While for organic solvents with

low solubility, the evaporation rate of solvent plays a significant role in determining its removal rate. Therefore, microdroplets prepared with methylene chloride, chloroform and toluene shrank according to their evaporation rate.

Based on the above discussion, a formation mechanism of porous PLA microspheres prepared with different solvents was proposed, as shown in Fig. 7. When the solvents with relatively lower removal rate such as methylene chloride, chloroform and toluene are used, the slow removal process of solvents produces a concentrated and viscous microenvironment in microdroplets. The sites of inner aqueous droplets develop into pores within microspheres, which finally leads to microspheres with homogeneous porous structure. When the solvents have a rapid removal rate such as ethyl acetate, the fast leaching of solvents results in the high osmotic pressure between the microdroplet and the outer aqueous phase. This high osmotic pressure drives the still unstable inner contents of microdroplets out and leads to the coalescence of some inner aqueous droplets, which finally results in

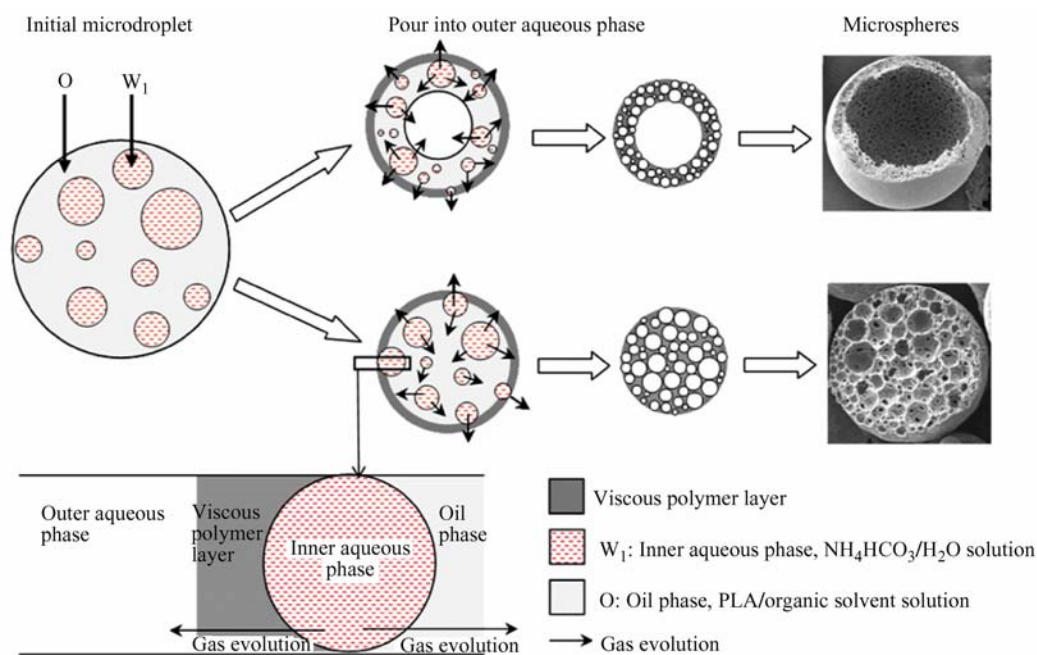


Fig. 7 A schematic diagram of the mechanism resulting in porous microspheres by the modified emulsion solvent evaporation method

The enlarged diagram indicates that initial polymer solidification happens at the microspheres surface during the early stage of the solvent removal process.

the formation of microspheres with hollow structure and porous shell. Besides the influence on the porous structure of PLA microspheres, organic solvent also affects the size and size distribution of microspheres. The long stirring time resulted from the slow removal rate of organic solvents is good to the decrease of the size and size distribution of polymer microspheres. Therefore, microspheres prepared with ethyl acetate have the largest size and size distribution; while those prepared with toluene have the smallest size and size distribution.

4 CONCLUSIONS

Porous PLA microspheres were prepared by the

modified emulsion solvent evaporation method. When the solvents with different boiling points and aqueous solubility were used, microspheres with different porous structure, size, size distribution and surface morphology were obtained. These differences were mainly resulted from the various solvent removal rates which usually included diffusion rate and evaporation rate. A formation mechanism of porous PLA microspheres was proposed, which may give a simple method to control the porous structure of microspheres fabricated by the modified emulsion solvent evaporation method.

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