Preparation of Poly(l-lactic acid) Microencapsulated Vitamin E

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Abstract

Poly(l-lactic acid) (PLLA) microencapsulated vitamin E was prepared in oil in water (O/W) emulsion system using solvent evaporation technique. The influence of PLLA:vitamin E weight ratio on the encapsulation was studied. In the case of low molecular weight PLLA, the optimum ratio is 25:1. It was found that using smaller amount of PLLA, microcapsule could not formed. In contrast, vitamin E was well enveloped with lower amount of high molecular weight PLLA, PLLA:vitamin E at 3:1. The obtained capsule was observed with an optical and scanning electron microscopes. The amount of the encapsulated vitamin E was measured with gel permeation chromatograph.

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Keywords: Poly (l-lactic acid); Encapsulation; Polymer capsule; Vitamin E; Solvent evaporation

1. Introduction

Vitamin E or tocopherol is an important nutrient as antioxidant in the cell. It is popularity added to beverages, cosmetics, nutritional and other products. There are four structures those are α-, β-, γ and δ-tocopherols. Among them, α-tocopherol is the most widely used form in many applications [1]. It is degraded when exposed light, heat and peroxidation in cell membrane which effect on health [2]. Then, the direct utilization is quite limit. To prevent degradation, the encapsulation is interesting means. It can perform by various techniques such as coating [3], coacervation [4], monomer polymerization [5]
nanoprecipitation [6] and solvent evaporation [7, 8]. It is not only protecting the core substance from the outside environment but also controlling the release of the encapsulated substance.

Recently, the biodegradable polymers have been attractive for a wide range of applications such as biomedical, pharmaceutical, food packaging and agricultural industries [9, 10]. It was used as the polymer shell for various encapsulations. It was reported the preparation of α-tocopherol loaded poly ε-caprolactone by solvent evaporation in oil in water (O/W) system [11]. The encapsulation of β-carotene in poly(hydroxybutyrate-co-hydroxyvalerate) using the solution enhanced dispersion by supercritical fluids technique was also reported [12].

One of the most popular biodegradable polymers is poly(l-lactic acid) (PLLA) which is non-toxic and environmental friendly. It is aliphatic polyester produced from renewable resources such as corn starch or sugarcane available in Thailand. PLLA is produced from the direct polycondensation of l-lactic acid or the ring-opening polymerization of l-lactide [13]. There are many researches reported the utilization of PLLA as polymer shell encapsulating various materials. It was reported that PLLA was used to encapsulate urea by solvent evaporation in water in oil in water system [14]. The smooth spherical capsules were formed. The influence of PLLA molecular weight on the formation of the polymer shell was also studied. Cholecystokinin composed of seven amino acids was encapsulated in poly(lactide-co-glycolide) microspheres prepared by a multiple emulsion solvent evaporation method [15]. The study of PLLA encapsulation and release properties of encapsulated ribozymes in water-in-oil-in-water emulsion system was also carried out [16].

In this work, PLLA is used as polymer shell to encapsulate vitamin E for food application. The encapsulation of vitamin E with PLLA shell in O/W emulsion using solvent evaporation technique is studied. The influence of weight ratio of PLLA: vitamin E and molecular weight of PLLA on the encapsulation are investigated.

2. Experimental

2.1. Materials

L-lactic acid (Sigma-Aldrich; purity, 80%) was used as received for the synthesis of low molecular weight (LMW) PLLA. Analytical grade p-toluene sulfonic acid (p-TSA) (Carlo Erba) was used by recrystallization. Poly (vinyl alcohol) (PVA) (Aldrich; degree of saponification, 87-90%) was used as received. Chloroform (CHCl₃) (RCI Labscan; purity, 99.8%) was used as received. Commercial grade PLLA from B.C. Polymers Marketing, Co., Ltd was used as high molecular weight (HMW) PLLA.

2.2. Preparation of low molecular weight PLLA

LMW PLLA was prepared by direct poly-condensation using p-TSA (2% wt of monomer) as catalyst. Lactic acid (100 g) was charged to the reactor at 140°C for water removal about 2 hours. After that, p-TSA was added to start the polymerization with gentle stirring rate for 6.5 hour using vacuum pump. Number average molecular weight ($M_n$) of PLLA was measured by gel permeation chromatograph (GPC; Water 2414, Water, USA) with two poly(styrene-divinylbenzene) gel columns (Phenogel 5 x 10² and 5 x 10⁴ Å, 7.8 mm i.d x 30 cm, Phenomenex, USA) connected in series. The flow rate of chloroform as eluent was maintained at 1.0 mL/min with column temperature of 40 °C and the elution was monitored with refractive index detector. The columns were calibrated with six standard polystyrene samples (2.5x10³-6.0x10⁵, $M_w/M_n = 1.05-1.15$).
2.3. Capsules preparation

PLLA capsule encapsulated vitamin E was prepared in O/W system under the condition listed in Table 1. Firstly, PLLA was dissolved in 10 ml of chloroform containing certain amount of vitamin E as oil phase. It was added to 50 ml PVA aqueous solution (1%wt) and then homogenized at 5,000 rpm for 5 min to prepare polymer droplets in O/W emulsion as shown in Fig 1. After that, the obtained polymer droplets were stirred to evaporate chloroform for about 3 days resulting in polymer capsules. Finally, they were centrifuged at 5,000 rpm for 10 min and dried overnight in vacuum oven.

The effect of PLLA molecular weight on the preparation of PLLA microencapsulated vitamin E was also investigated using LMW ($M_n = 1,500$ g/mol) and HMW ($M_n = 90,000$ g/mol) PLLA as the recipe shown in Table 2.

![Fig. 1. Schematic of PLLA microencapsulated vitamin E preparation by solvent evaporation in O/W emulsion system](image)

Table 1. Reagent amounts for the preparation of PLLA microencapsulated vitamin E at various weight ratios of PLLA:vitamin E using LMW PLLA by solvent evaporation in O/W system

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>PLLA:vitamin E</th>
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<tbody>
<tr>
<td></td>
<td>25:1</td>
</tr>
<tr>
<td>PLLA (g)</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin E (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Chloroform (g)</td>
<td>10.0</td>
</tr>
<tr>
<td>PVA solution (g)</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Table 2. Reagent amounts for the preparation of PLLA microencapsulated vitamin E at various molecular weights of PLLA using PLLA:vitamin E at 3:1 by solvent evaporation in O/W system

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>$M_n$ of PLLA (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,500</td>
</tr>
<tr>
<td>PLLA (g)</td>
<td>3.9</td>
</tr>
<tr>
<td>Vitamin E (g)</td>
<td>1.3</td>
</tr>
<tr>
<td>Chloroform (g)</td>
<td>10.0</td>
</tr>
<tr>
<td>PVA solution (g)</td>
<td>50.0</td>
</tr>
</tbody>
</table>
2.4. Characterization of PLLA capsules

The PLLA droplets and capsules encapsulated vitamin E after solvent removal were observed with an optical microscope (OM; SK-100EB & SK-100 ET, Seek Inter Corporation Ltd., Thailand) and scanning electron microscope (SEM; JSM-6510, Jeol, Jeol Ltd., Japan) to investigate the morphology of the surface and the structure of the microcapsules. For SEM observation, few of capsules was placed on a nickel SEM stub and dried before Au-coated.

The amount of the encapsulated vitamin E was determined by GPC. Approximately 0.05 g of dried PLLA capsule was dissolved in 5 ml of chloroform, filtered and then measured with GPC. To determined vitamin E concentration, the area of vitamin E analyzed by liquid chromatography mode was compared with the standard curve of vitamin E solutions at 0.05, 0.1, 0.2, and 0.4 %wt and then calculated.

Fig. 2. Optical micrographs of PLLA/vitamin E droplets before (a, b, c) and PLLA microencapsulated vitamin E (a’, b’, c’) after solvent evaporation at various weight ratios of PLLA:vitamin E. PLLA:vitamin E: a, a’) 25:1; b, b’) 12:1 and c, c’) 3:1

3. Results and discussion

3.1. Weight ratio of PLLA:vitamin E

LMW PLLA was used as the polymer shell to encapsulate vitamin E. The polymer droplets were prepared using PVA as nonionic stabilizer. Long polymer chain of PVA stabilize polymer droplet via steric stabilization. Then, oil phase of PLLA solution was dispersed in aqueous medium as spherical droplets with high colloidal stability as shown in Fig. 2(a-c). After solvent evaporation, the polymer capsules were formed. At the initial stage, PLLA was completely dissolved with vitamin E and chloroform as the homogeneous solution. During solvent evaporation, the miscibility of all components gradually reduces, PLLA gradually separate from solution and diffuse to the droplet interface. The separated PLLA chains adsorb at the interface to form PLLA shell encapsulating vitamin E as the capsule core. This mechanism is well known and named as an “internal phase separation” [17, 18]. By OM observation, after solvent evaporation, the polymer droplet became dark due to phase separation increases.
the heterogeneity in the droplet as shown in Fig. 2(a'-c'). Various weight ratios of PLLA:vitamin E were studied. It was found that, at high PLLA content (25:1), vitamin E was well enveloped with PLLA shell (Fig. 3) resulting in the formation of the spherical capsules. However, the decreasing of PLLA content as 12:1 and 3:1 weight ratios, polymer capsule could not form. It indicated that low amount of LMW PLLA was not enough to encapsulate large amount of vitamin E. Moreover, it may be due to small amount of the short PLLA chain can easily dissolved in chloroform. In this case, phase separation lately occurred when the internal viscosity was high. It reduces the movement of PLLA chain diffusing to interface of the droplet [19]. Then, polymer capsule could not form.

Fig. 3. SEM micrograph of PLLA microencapsulated vitamin E at weight ratios of PLLA:vitamin E of 25:1

Fig. 4. Optical micrographs of PLLA microencapsulated vitamin E after solvent evaporation at 3:1 of PLLA:vitamin E using different $M_n$ of PLLA. $M_n$ of PLLA (g/mol): a) 1,500 and b) 90,000

3.2. Molecular weight of PLLA

The influence of molecular weight of PLLA on the encapsulation of vitamin E was also investigated. HMW PLLA was used to prepare microcapsule at 3:1 weight ratio of PLLA:vitamin E compare with the LMW one. It showed that after solvent evaporation for 4 days, phase separation of HMW PLLA was clearly observed as shown in Fig. 4b. In contrast, the heterogeneity of LMW PLLA droplets due to phase separation of PLLA chains could not observed (Fig. 4a). This indicate that phase separation earlier occurred for HMW chains than the lower molecular weight polymer chain leading to the formation of
PLLA capsule encapsulating vitamin E. SEM micrograph (Fig. 5) confirms the formation of the HMW PLLA capsule at 3:1 weight ratio of PLLA:vitamin E. The spherical HMW PLLA microcapsules having smooth outer surface were obtained. It can be explained that HMW polymer chains is less miscible with vitamin E and solvent than the lower molecular weight chains. Then, during solvent evaporation, the miscibility of PLLA with the other components in the HMW polymer droplet more quickly reduced resulting in phase separation at the earlier stage than the LMW polymer droplet. At that point, the internal viscosity is not so high then polymer chains can easily diffuse to the droplet interface forming the polymer shell.

Fig. 5. SEM micrograph of PLLA microencapsulated vitamin E at 3:1 of PLLA:vitamin E using HMW PLLA

Fig. 6. GPC chromatograms of (a) HMW PLLA, (b) vitamin E and (c) PLLA microencapsulated vitamin E
The GPC chromatograms of PLLA, standard of vitamin E and PLLA microencapsulated vitamin E were shown in Fig. 6. The retention times of PLLA and vitamin E in individual chromatogram were 12.5 and 19.5 min, respectively. In the case of PLLA microencapsulated vitamin E chromatogram, PLLA and vitamin E peaks were observed and the retention times accorded to those of the original chromatograms. This result indicated that there is some vitamin E incorporated in the PLLA capsule consistent with the above data. Moreover, the concentration of encapsulated vitamin E was determined with GPC compared with the standard curve. The vitamin E concentration of 0.38 % wt with 3.49 %RSD was obtained in the case of 3:1 PLLA:vitamin E. This result indicated that the encapsulation of vitamin E with PLLA shell using solvent evaporation method is successful.

4. Conclusions

PLLA microencapsulated vitamin E is successfully prepared in O/W system using simple solvent evaporation technique. In the case of LMW PLLA, only 25:1 weight ratio of PLLA:vitamin E, the capsule was formed. Using lower amount of PLLA, polymer is not enough to completely envelop vitamin E. In contrast, using HMW PLLA, polymer capsules were completely formed. It was found that smaller amount of HMW PLLA can well encapsulate large amount of vitamin E than LMW as in the case of 3:1 weight ratio of PLLA:vitamin E. The spherical capsules with smooth outer surface were obtained. The measurement of the encapsulated vitamin E concentration confirms the successful encapsulation.

References


