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Synthesis of Collagen-Modified Polylactide and Its Application in Drug Delivery

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ABSTRACT: In this article, collagen modified polylactide (CPLA) was synthesized by means of graft modification, and its structure was confirmed by FTIR and FITC-labeled fluorescence spectra. Subsequently, the performance of CPLA was characterized with hydrophilicity test and degradability test. After that, the aspirin sustained release microspheres of the synthetic copolymers were prepared via the emulsion-solvent evaporation technique, followed with its measurements of morphology, size, and encapsulation efficiency. Finally, the controlled release properties of the obtained microspheres were investigated. The results showed that the aspirin sustained release microspheres exhibited well-defined morphology with smooth spherical surface, with average size of 3.990 μm and encapsulation efficiency of 51.83%. Furthermore, compared with aspirin-loaded PLA microspheres, at the initial 32 h, the drug release was faster for aspirin-loaded CPLA microspheres favored by its increased hydrophilicity, and then the drug release was slower than that of PLA microspheres because the $-NH_2$ group on the introduced collagen inhibited acidic autocatalytic degradation. The results suggested that CPLA showed a great potential as particles for drug delivery. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 3290–3296, 2013

KEYWORDS: synthesis and processing; morphology; drug delivery systems

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INTRODUCTION

Drug delivery systems are promising approaches to prevent the potentially harmful and serious side effects of various therapeutic agents on other organs,[1] in which a number of polymers have been used as delivery vehicles. Polylactide (PLA) is one of most common polymers due to its nontoxicity and biodegradability. However, the hydrophilicity of PLA needs to be improved since its high hydrophobicity retards cell attachment[2] and reduces the drug intake of intestinal epithelia.[3] Therefore, hydrophilic and hydrophobic balance of PLA should be controlled to facilitate oral particle absorption.[4] Moreover, the drug release from PLA particles is difficult to be controlled because of long-term acid autocatalytic degradation of polymer matrix.

To improve polymer hydrophilicity and degradability, many different methods have been developed to prepare copolymers, which contain block copolymerization and graft copolymerization. The block copolymer has been synthesized by two or more monomers. Wu et al.[5] reported on the synthesis of amphiphilic pluronic (F68)-PLGA copolymer by ring-opening polymerization of pluronic (F68), DL-lactide and glycolide. Compared with block copolymerization, graft modification improved the hydrophilic properties with less influence on the properties of the main chain. Moreover, the mechanism of graft modification was much simpler than that of block polymerization, and it was a more affordable way to synthesize our required materials. There were some methods in the literature of graft modification, which contained UV-photo grafting,[6,7] gamma irradiation grafting,[8] and aminolysis grafting.[9,10] Gutierrez-Villarreal et al.[6] introduced *N*-Vinylpyrrolidone (NVP) onto PLA film by photo-initiated grafting to modify the nature hydrophobic PLA behavior. Another alternative approach was to introduce collagen into PLA. Yang et al.[8] grafted collagen to PLLA by gamma irradiation grafting with poly(acrylic acid) as a coupling agent. Hong et al.[10] introduced collagen to the surface of PLA microspheres by covalent linking via aminolysis grafting with glutaraldehyde as a crosslinking agent. These two techniques were effective ways to produce collagen-modified PLA, but the synthesis required a two-step procedure. The first step was to introduce functional groups (carboxyl groups or amino group) on the surface of the materials, and the second step was to covalently couple collagen with these groups by coupling agents. So, we choose a method of grafting to modify PLA by introducing collagen with dicyclohexylcarbodiimide (DCC) as a condensing agent, and this method just needed one-step procedure.

Aspirin is a common drug used for its analgesic and antipyretic effects. Previous studies show that it has been used frequently

in prevention cardiovascular events.[11] However, long-term use of low-dose aspirin increases the risk of serious gastrointestinal complications.[12] So a controllable delivery of aspirin is of importance to lighten those side effects.

In this study, collagen-modified PLA was synthesized by grafting collagen on both ends of PLA with DCC as a condensing agent, and its structure was characterized by FTIR and FITC-labeled fluorescence spectra. Then, the microsphere of that polymer with aspirin was prepared by O/W emulsion/solvent evaporation technique and some of its properties were tested, such as size, hydrophilicity, and morphology. Finally, the encapsulation efficiency and release profile was analyzed.

EXPERIMENTAL

Materials

Lactic acid (AR) and DCC were purchased from Sinopharm Chemical Reagent. Collagen was purchased from Shandong Qingzhou Longbei biotechnology, (relative molecular weight of 600–3000). Fluorescein isothiocyanate (FITC, HPLC grade) was purchased from Sigma Company. All reagents were used as received without further purification.

Synthesis of CPLA

PLA was directly synthesized by melt polycondensation with stannous chloride ($SnCl_2$, AR) as catalyst.[13,14] The relative molecular mass of PLA was measured with gel permeation chromatograph (AP2000, OI, USA) (Weight Average molecular weight, $M_W = 10{,}053$, Polydispersity index, PDI = 2.309).

The PLA and collagen were dissolved in dimethyl sulfoxide (DMSO) in certain proportion, to this was added the solution of DCC in DMSO. After that, the solution was stirred at 20°C for 12 h. Then the reaction solution was added to lots of distilled water to precipitate the white polymer (CPLA). It was filtered and washed repeatedly until the filtrate could not be colored with ninhydrin. Finally, the CPLA was dried in vacuum at 37°C for 48 h.

Structure Characterization of CPLA

FTIR spectra were obtained using fourier transform infrared spectrometer (IRPrestige-21, Shimadzu, Japan). Fluorescence spectra were obtained by fluorescence labeling methods[15] using fluorescence spectrophotometer (F-4500, Hitachi, Japan). Briefly, 100 mg CPLA was dissolved in DMSO and *N*-methyl morpholine was added to adjust the pH value between 9 and 9.5. Then, 10 mg FITC was added under low-speed stirring and the reaction was continued for 2 h away from light at room temperature. After reaction finished, the reaction mixture was added to lots of distilled water. The precipitate was collected and washed thoroughly with distilled water until the filtrate was no significant fluorescence peaks. The solution of FITC-labeled CPLA (0.1 mg/mL) in tetrahydrofuran (THF) was prepared to measure fluorescence emission spectroscopy and excitation spectroscopy.

Measurement of Collagen Content

The graft ratio of collagen on PLA was determined by ninhydrin reaction.[16] Briefly, 1 mL of ninhydrin (5 mg/mL) in DMSO solution and 5 mL of CPLA (0.1 mg/mL) in DMSO solution were mixed and heated for 20 min at 100°C, and then cooled for 15 min at room temperature. After that, the absorbance at 580 nm of this mixed solution was measured on UV–Vis spectrophotometer (TU-1901, CN). The collagen content was quantified by means of a collagen calibration curve at same condition.

Performance Characterization of CPLA

Hydrophilicity test was carried out through the determination of water absorption. Water absorption was calculated as the percentage ratio of the amount of water absorbed to the initial dry weight. Briefly, the specimen was submerged in phosphate buffer salines (PBS) (pH = 7.4, 0.1*M*) at 37.0 ± 0.5°C for up to 1 week, the samples were removed at 2-day intervals, the surface water dried off and the samples weighed. Determinations were run in triplicate.

Degradability test was preformed by measuring pH value of distilled water and weight loss of CPLA. The former was measured by submerging samples in distilled water (pH = 6.45) at 37.0 ± 0.5°C and surveying pH value of medium at 2 days intervals. The latter was measured by submerging samples in PBS (pH = 7.4, 0.1*M*) at 37.0 ± 0.5°C. Samples were removed, dried at 40°C for 24 h and weighted at 1 week intervals.

The Preparation of Aspirin-Loaded Microspheres

The aspirin-loaded microspheres were prepared using O/W emulsion solvent-evaporation method.[17,18] Briefly, the CPLA (0.5 g) was dissolved in 9 mL of dichloromethane to this was suspended aspirin (0.1 g). The solution was processed by ultrasonic dispersion for 15 min. It was then slowly added to aqueous phase, which contained 0.75% (w/v) PVA, 0.5% (v/v) Tween-80. The mixture was stirred and emulsified for 3 h. Then the solvent was evaporated by stirring at 300 rpm for 3 h at 35°C. Finally, the harden microspheres were centrifuged at 4500 rpm, washed with distilled water and dried. The blank microspheres were also prepared at the same condition.

The Characterization of Aspirin-Loaded Microspheres

The size distribution of microspheres was measured by laser particle size analyzer (Mastersizer2000, Malvern, UK).The morphology of microspheres was observed by the environmental scanning electron microscope (Quanta-200E, Neth). Thermal analysis of blank microspheres, the mechanical mixture of aspirin and blank microspheres and aspirin-loaded microspheres were performed with differential scanning calorimeter (Q20, TA, USA) under an atmosphere of N_2 (at a rate 20 mL/min) at a heating rate of 15°C/min, scanning from 0 to 200°C.

Aspirin Content Determination

Microspheres (20.0 mg) were dissolved in dichloromethane (2 mL) and 20 mL of PBS (pH = 7.4, 0.1*M*) was added. The suspension was processed through bath sonication for 5 min to precipitate the polymer at 37°C and then centrifuged at 3000 rpm. The polymer was removed and the supernatant was collected. Aspirin content of microspheres in filtrate was determined with an UV spectrophotometer at 268 nm by means of a calibration curve. The encapsulation efficiency of aspirin in the microspheres was calculated by eq. (1).

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Table I. Mathematical Models Used to Describe the Kinetics of the Drug Dissolution Curves

Mathematical model	Kinetic equation
Zero order	$Q_t = K_0 t + C_0$
First order	$\ln(1 - Q_t) = -K_1 t + C_1$
Higuchi	$Q_t = K_H t^{1/2} + C_H$

$$\text{Encapsulation efficiency} = \frac{m_1}{m_2}\% \quad (1)$$

m_1 : total amount of drug measured in microspheres

m_2 : total amount of drug theoretically associated with microspheres

In Vitro Drug Release and Kinetic Evaluation

In vitro release of drug from microspheres was studied by rotating flask method at 37°C. Aspirin microspheres (150 mg) were put in 250 mL single flask containing 150 mL of PBS (pH = 7.4, 0.1*M*).The flask was sealed and rotated using thermostat magnetic stirring apparatus (DF-1, CN). The samples of the release mediums (10 mL) were collected by centrifugation for 4 min at 3000 rpm and replaced with the same volumes of fresh mediums at predesigned time intervals. The samples were assayed using UV–Vis spectrophotometer (TU-1901, CN) at 268 nm.

There are many mathematical models to describe drug release from pharmaceutical systems, such as Zero order, First order, and Higuchi. In this study, the dissolution profiles of aspirin from microspheres were studied by applying these three mathematical models (Table I).

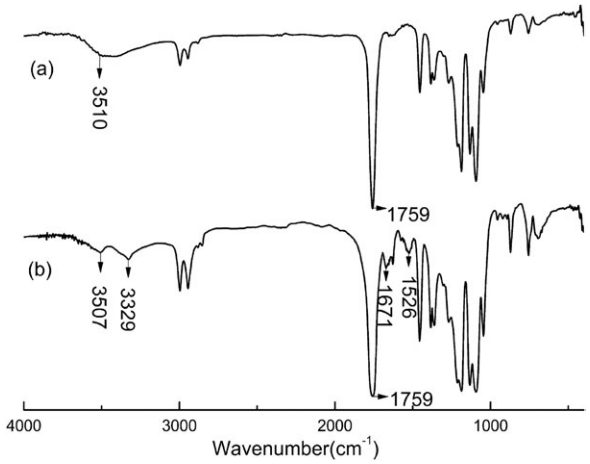

Figure 1. FTIR spectra of (a) PLA and (b) CPLA.

RESULT AND DISCUSSION

FTIR Analysis

The FTIR spectra of PLA and CPLA were shown in Figure 1. From the FTIR spectra of PLA [Figure 1(a)], it could be seen that the wide peak of stretching vibration at 3510 cm^{-1} was attributed to O—H in hydroxyl group and carboxyl groups. FTIR spectrum of CPLA [Figure 1(b)] showed the following conclusions. The peaks of stretching vibration at 3507 and 3329 cm^{-1} were attributed to O—H, NH_2, N—H in amide group. The C=O peak at 1759 cm^{-1} was wider than that of PLA. The peaks at 1671 and 1526 cm^{-1} were attributed to the stretching vibration of C=O and bending vibration of N—H in amide group. These results indicated that collagen had been grafted into the PLA.

Fluorescence Analysis

The isothiocyanate groups of FITC can react with the amino groups of collagen under alkaline conditions. This resulted in

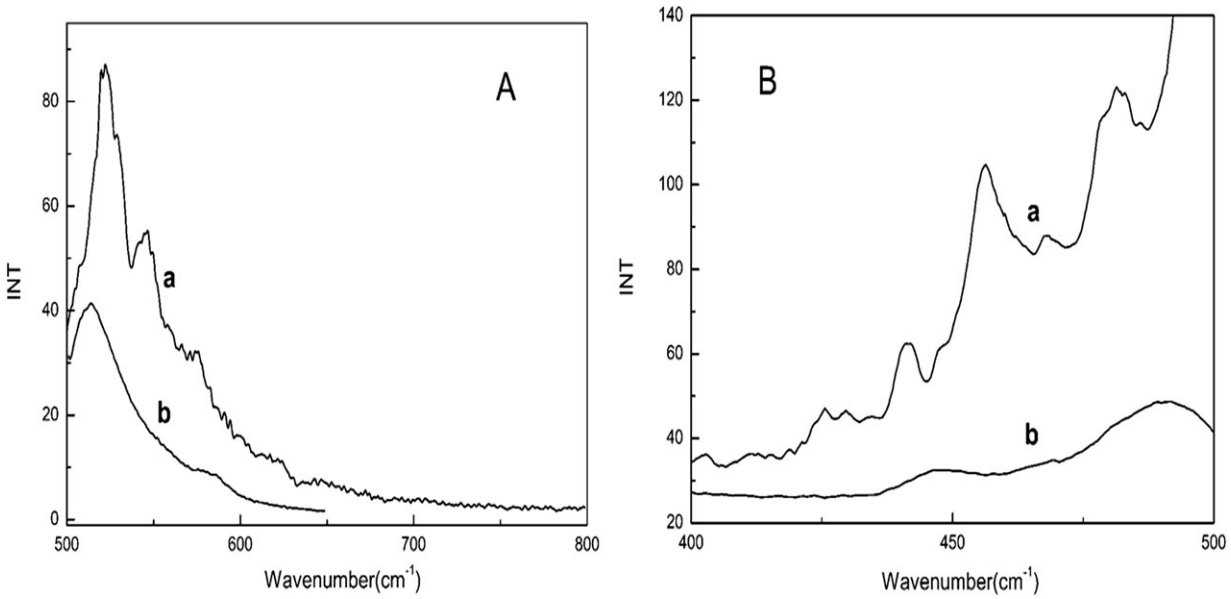

Figure 2. (A) Fluorescence emission spectra and (B) excitation spectra of FITC-labeled (a) CPLA and (b) collagen.

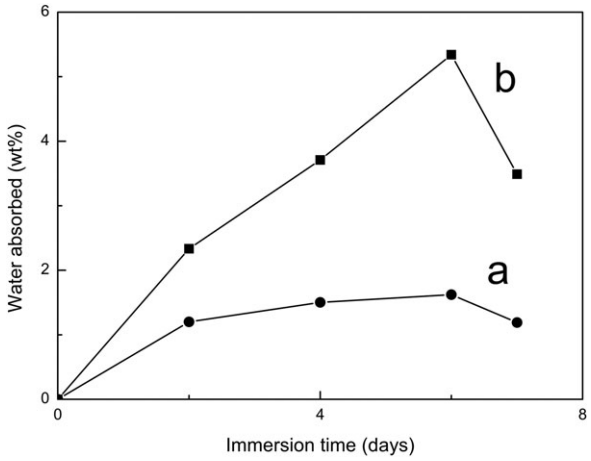

Figure 3. Water absorption behavior of (a) PLA and (b) CPLA.

maximum excitation spectra in the region of 450–500 nm and the maximum emission spectra in the region of 500–530 nm. As was shown in Figure 2, both hydrolyzed collagen and CPLA had obvious emission and excitation spectra in relative region. The above results indicated that the collagen had been introduced to PLA.

The Graft Ratio of Collagen on the CPLA

Ninhydrin can react with collagen to generate blue-violet condensation compound, which has maximum absorption peak at 580 nm. Therefore, we can make use of the ninhydrin reaction for the quantitative determination of collagen content on CPLA. The graft ratio was measured about 5%.

Hydrophilicity Test

The water absorption illustrated the balance between the dissolution of oligomers and water uptake of the residual material.[19] Water absorption behavior in Figure 3 indicated that the water absorption of CPLA was significantly higher than PLA and exhibited downward trend after 6 days. It could be explained as

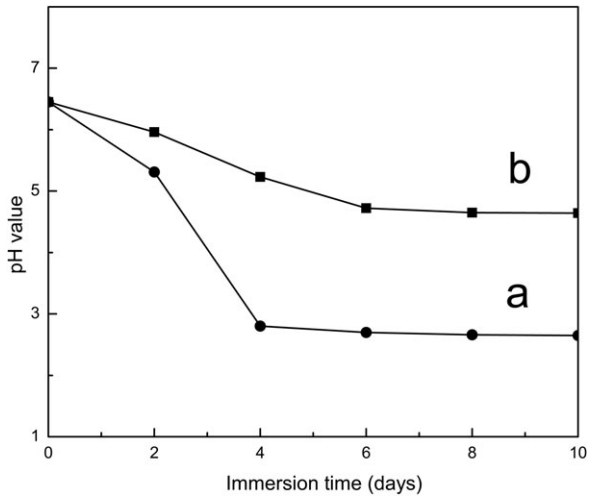

Figure 4. The pH change of medium as a function of time during degradation of (a) PLA and (b) CPLA.

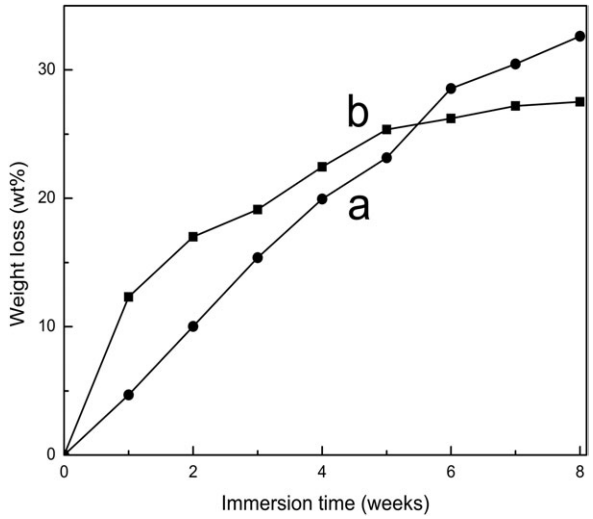

Figure 5. The weight loss change of (a) PLA and (b) CPLA with degradation time.

that there were more hydrophilic groups in CPLA than that in PLA, such as —NH_2, —COOH, and —OH, which could increase water absorption and degradation of copolymers. After 6 days, the dissolution of oligomers predominated in the system, which contributed to the decline of water absorption.

Degradability Test

The relationship between pH value and degradation time of PLA and CPLA was shown in Figure 4. It was mainly influenced by the release of —COOH, which was produced in the hydrolysis process of the ester bond in the main chain of polymer.[20] At first two days, the pH value of PLA changed from 6.45 to 5.31, and then its change was from 5.31 to 2.80. After that, the curve of pH value versus time remained essentially flat. But for CPLA, its change was from 6.45 to 4.64 in the 10 days. It could be concluded that the pH value of CPLA was higher than PLA. The reason was that —NH_2 group on the introduced collagen can neutralize H^+ generated by ionization of —COOH.

The weight loss of PLA and CPLA versus degradation time was shown in Figure 5. Weight loss in samples came from the dissolution of both water-soluble compounds and oligomeric degradation products into the PBS.[19,20] In the first week, the weight loss of CPLA was obviously higher than PLA. This was mainly due to the increased water absorption generated by the degradation of water-soluble group of collagen on CPLA. After that, the weight loss of CPLA increased at a slow rate while the weight loss of PLA increased at a relatively constant rate, which was attributed to less cumulation of oligomeric degradation

Table II. The Average Size and Span of CPLA Microspheres and Aspirin-Loaded CPLA Microspheres

Microspheres	Average size (μm)	Span
CPLA-MS	1.730 ± 0.120	1.156 ± 0.366
ASP-CPLA-MS	3.738 ± 0.225	1.579 ± 0.262

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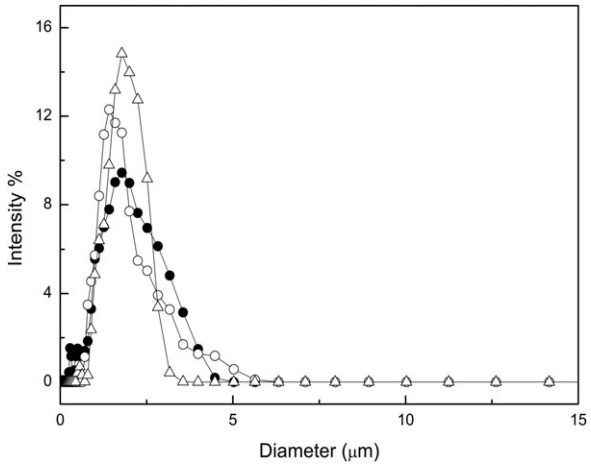

Figure 6. The size distribution curves of CPLA microspheres (3 measurements).

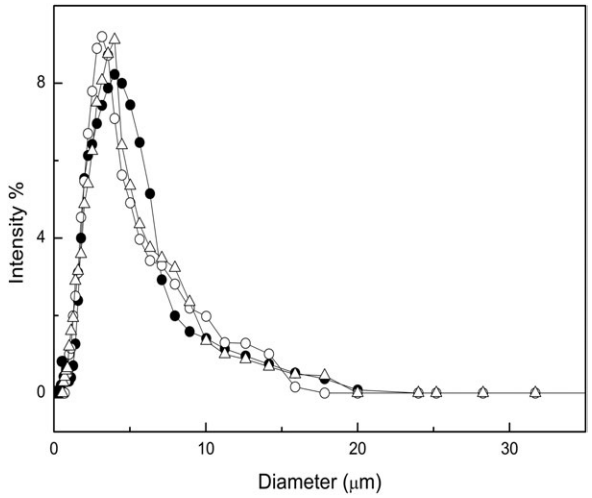

Figure 7. The size distribution curves of aspirin-loaded CPLA microspheres (3 measurements).

products of CPLA. That is, there was no obvious acid-catalyzed self-accelerating degradation of CPLA and the pH value no longer cut down. When compared with CPLA, the considerable acid-catalyzed self-accelerating degradation of PLA resulted in a high weight loss rate at the sixth week.

Particle Size Distribution

The average size and span of CPLA-MS and ASP-CPLA-MS were shown in Table II. The sizes of CPLA and aspirin-loaded CPLA microspheres were from 0.25 to 5.64 μm (Figure 6) and from 0.44 to 20.00 μm (Figure 7), respectively. It could be seen that the average size of ASP-CPLA-MS was larger than CPLA-MS. These results were consistent with the observations obtained by ESEM.

Morphological Characterization

The ESEM images [Figure 8(a,b)] revealed that all microspheres are spherical shape with smooth surface. However, the size of ASP-CPLA-MS was obviously larger than CPLA-MS, which could be attributed to aspirin dispersed in and absorbed on the CPLA matrix.[21]

Thermal Analysis

The DSC curves of CPLA-MS, mixture of aspirin and CPLA-MS and ASP-CPLA-MS were reported in Figure 9. Glass-transition temperature (T_g) of microspheres were 43.89, 46.84, and 56.61°C, respectively. The later was 12.72°C higher than the first one, which indicated that aspirin was encapsulated in CPLA microspheres instead of attaching to its surface.

Determination of Aspirin Content

To compare the property of CPLA microspheres with PLA microspheres for controlled delivery system, aspirin was employed as a model drug. It was showed in Table III that the encapsulation efficiency and drug content of aspirin in CPLA-MS were all slightly higher than PLA-MS.

In Vitro Drug Release Studies and Kinetic Evaluation

The *in vitro* release profiles of aspirin from PLA and CPLA microspheres were reported in Figure 10. The drug release rates were obviously affected by the type and degradation way of polymer.[22,23] In this study, ASP-CPLA-MS exhibited an initial burst of 6.03%, which was higher than that of ASP-PLA-MS (4.97%). Then, it was followed by a diffusion-controlled release,

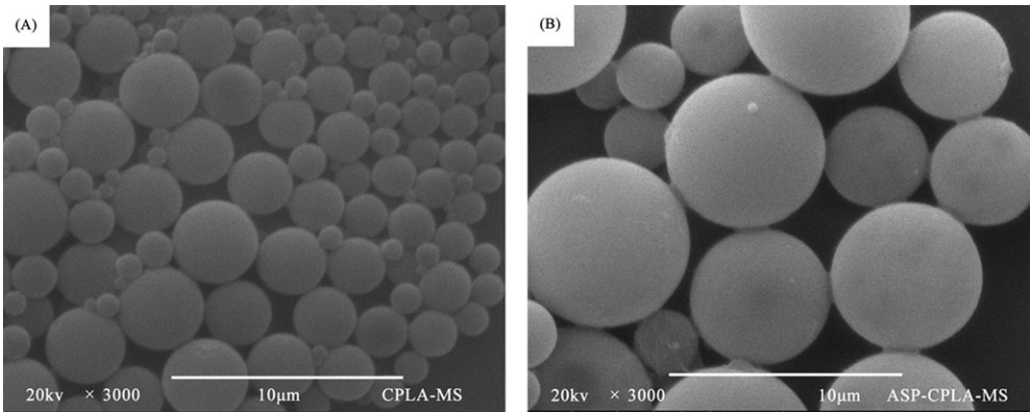

Figure 8. ESEM images of (A) CPLA microspheres and (B) aspirin-loaded CPLA microspheres.

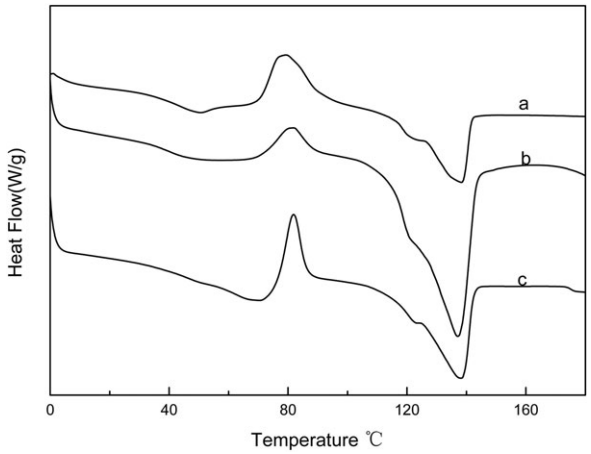

Figure 9. DSC curves of (a) CPLA microspheres, (b) mixture of aspirin and CPLA microspheres, and (c) aspirin-loaded CPLA microspheres.

which was faster than that of ASP-PLA-MS. After 32 h, the drug release of ASP-PLA-MS was faster than ASP-CPLA-MS. After 144 h, 97.74% aspirin was released from PLA microspheres while only 61.13% aspirin was released from CPLA microspheres. After 480 h, 96.80% aspirin was released from CPLA microspheres. These results showed that the release time of aspirin from CPLA-MS was much longer than that of PLA-MS. The different rates of drug release could be explained to different degradation rates of polymer matrix. The higher initial burst and faster short-term release of ASP-CPLA-MS might be derived from the increased hydrophilicity of polymer, which accelerated the hydrolysis of CPLA matrix.

Table III. Drug Content and Encapsulation Efficiency of Different Microspheres

Microspheres	Drug content (%)	Encapsulation efficiency (%)
ASP-CPLA-MS	8.638 ± 0.122	51.830 ± 0.733
ASP-PLA-MS	8.128 ± 0.227	48.770 ± 1.361

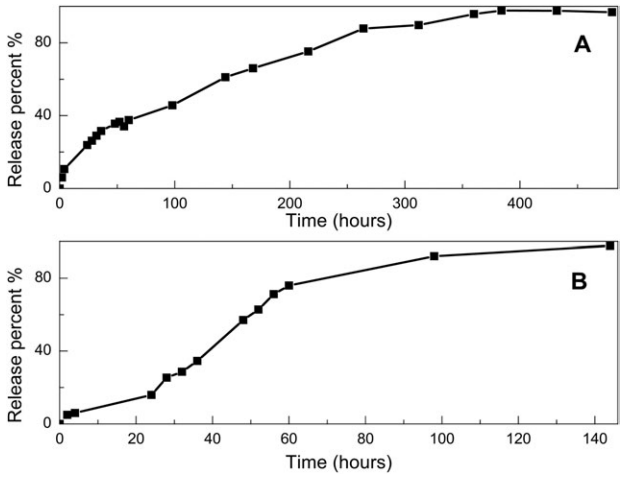

Figure 10. *In vitro* release profiles of aspirin from (A) CPLA microspheres and (B) PLA microspheres.

Table IV. Kinetic Equations of Different Microspheres

Microspheres	Kinetic model	Fitting equation
ASP-CPLA-MS	Zero order	$Q_t = 0.1917t + 23.5604$, $R = 0.9181$
	First order	$\ln(1 - Q_t) = -0.0081t + 0.0078$, $R = 0.9642$
	Higuchi	$Q_t = 4.8483t^{1/2} + 1.0096$, $R = 0.9880$
ASP-PLA-MS	Zero order	$Q_t = 0.7789t + 9.0424$, $R = 0.8561$
	First order	$\ln(1 - Q_t) = -0.0281t + 0.3535$, $R = 0.9698$
	Higuchi	$Q_t = 10.4733t^{1/2} - 19.0891$, $R = 0.8952$

Q_t, percentage of drug released; t, time of drug released; R, linear correlation coefficient.

The results obtained by Kinetic evaluation (Table IV) showed that the release of aspirin from CPLA microspheres and PLA microspheres followed a Higuchi model and a first order model,[24] which indicated that CPLA had a potential application in drug delivery.

CONCLUSIONS

The collagen-modified PLA was successfully synthesized by the method of graft modification. FTIR and fluorescence spectra confirmed that collagen had grafted on the PLA. The graft ratio of collagen on CPLA was about 5%. The hydrophilicity of this copolymer was better than PLA. Moreover, there was no obvious acid-catalyzed self-accelerating degradation behavior in the degradation process of CPLA.

The aspirin-loaded CPLA microspheres were produced by O/W emulsion-solvent evaporation method, which had smooth spherical surfaces and larger size than PLA microspheres. When compared with aspirin-loaded PLA microspheres, aspirin-loaded CPLA microspheres showed much longer release time of aspirin and exhibited better controlled release performance. The results suggested that CPLA was a promising candidate for drug delivery.

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