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Preparation and Physicochemical Characteristics of Polylactide Microspheres of Emamectin Benzoate by Modified Solvent Evaporation/Extraction Method

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Supporting Information

ABSTRACT: Emamectin benzoate is highly effective against insect pests and widely used in the world. However, its biological activity is limited because of high resistance of target insects and rapid degradation speed in fields. Preparation and physicochemical characterization of degradable microcapsules of emamectin benzoate were studied by modified solvent evaporation/extraction method using polylactide (PLA) as wall material. The influence of different compositions of the solvent in internal organic phase and external aqueous phase on diameter, span, pesticide loading, and entrapment rate of the microspheres was investigated. The results indicated that the process of solvent extraction and the formation of the microcapsules would be accelerated by adding water-miscible organic solvents such as ethyl ether, acetone, ethyl acetate, or *n*-butanol into internal organic phase and external aqueous phase. Accelerated formation of the microcapsules would result in entrapment rates of emamectin benzoate increased to as high as 97%. In addition, by adding ethanol into the external aqueous phase, diameters would reduce to 6.28 μm, whereas the loading efficiency of emamectin benzoate did not increase. The PLA microspheres prepared under optimum conditions were smoother and more spherical. The degradation rate in PLA microspheres of emamectin benzoate on the 10th day was 4.29 ± 0.74%, whereas the degradation rates of emamectin benzoate in methanol solution and solid technical material were 46.3 ± 2.11 and 22.7 ± 1.51%, respectively. The PLA skeleton had combined with emamectin benzoate in an amorphous or molecular state by using differential scanning calorimetry (DSC) determination. The results indicated that PLA microspheres of emamectin benzoate with high entrapment rate, loading efficiency, and physicochemical characteristics could be obtained by adding water-miscible organic solvents into the internal organic phase and external aqueous phase.

KEYWORDS: *solvent evaporation/extraction method, emamectin benzoate, polylactide microspheres, physicochemical characteristics, differential scanning calorimetry*

INTRODUCTION

Insect pests are the main limiting factor for vegetable production in tropical Asia. Farmers often use synthetic insecticides indiscriminately, and heavy insect resistance to insecticides is very common in the fields. To control pests efficiently, some new pesticides, with novel modes of action, have been developed recently. Among them, emamectin benzoate is a novel insecticide deriving from the natural product avermectin family with improved thermal stability, greater water solubility, and a broader spectrum of insecticidal activity than those of avermectin.[1] Emamectin benzoate, with novel modes of action similar to that of avermectin (GABA- and glutamate-gated chloride channel agonist), high selectivity, and greater safety to nontarget organisms, is highly effective against lepidopteran pests and widely used in the world. It is 18−80400-fold more potent against diamondback moth, *Plutella xylostella*, cabbage looper, *Trichoplusia ni* (Hübner), and beet armyworm, *Spodoptera exigua* (Hübner), than other traditional insecticides, such as fipronil, chlorfenapyr, and tebufenozide.[2] However, significant resistance to emamectin benzoate in target pests was found because of long-term heavy usage of this insecticide.[3] On the other hand, emamectin benzoate was sensitive to light and had a rapid degradation rate in natural conditions so that its biological activity was limited greatly in fields.[4] The problems could be solved by application of a microencapsulation formulation of emamectin benzoate.

Microencapsulation of pesticides is a method used to obtain products with controlled release properties, and it is a process that allows an active product to be isolated from the external medium by forming microspheres or microcapsules.[5] Biodegradable microsphere-based controlled release systems have been widely studied for pesticides and drug-delivery devices.[6−9] A wide range of pesticides have been processed into microspheres to prolong their effectiveness using a variety of materials such as ethyl cellulose, lignin, and chitosan.[10−14] Biodegradable poly(D,L-lactic acid) (PLA) and poly(D,L-lactic-*co*-glycolic acid) (PLGA) were the most useful among the microparticles and implants, which promised platforms to provide long-acting systemic.[9,15]

There were many methods to prepare microspheres of pesticides including emulsion solvent evaporation, spray-drying, and phase separation. Emulsion solvent evaporation is the most widely investigated with the advantages that it required neither

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elevated temperatures nor phase separation inducing agent.[16−18] In the application process of the traditional emulsion solvent evaporation method, the pesticide and the polymer were dissolved into an organic solvent to form the organic phase. Then the organic phase was dispersed into a large volume of aqueous phase in the presence of an emulsifier. Then organic solvent was removed by evaporation, and solid microspheres were formed with the pesticide encapsulated.[19,20]

In our previous study, PLA microencapsulation of spinosad and chlorpyrifos was prepared by using an emulsion solvent evaporation method, respectively,[21,22] and chlorpyrifos microencapsulated by PLA had a slower degradation rate than that in traditional pesticide formulations in natural condition.[23] The microencapsulation of emamectin benzoate should be studied because of its high toxicity and wide use and its high degradation rate in the field. However, on the basis of our previous study, the entrapment rate of emamectin benzoate was lower when the traditional emulsion solvent evaporation method was used because emamectin benzoate was slightly soluble in water and easily diffused into the external aqueous phase. In addition, microspheres prepared from the traditional solvent evaporation method usually had a rough surface as emamectin benzoate diffused into the external aqueous phase was adsorbed in the surface of the microspheres.[17] Therefore, how to prevent emamectin benzoate from diffusing into the external aqueous solution became an urgent problem. To solve the problems, modified emulsion solvent evaporation/extraction method was used to prepare PLA microspheres containing emamectin benzoate.

Different from the traditional emulsion solvent evaporation method, in a modified emulsion solvent evaporation/extraction method, the water-miscible organic solvent such as acetone, methanol, or ethanol was added into the organic phase as extracting solvent, and the mixed organic solvent could be extracted more quickly into the external aqueous phase after the formation of organic liquid droplets. Therefore, the mixed organic solvent containing water-miscible organic solvent would accelerate the formation of microspheres.[24,25] Quick formation of microspheres would result in an increase of the entrapment rate of pesticide because the loss of pesticide mainly occurred in a short time before the formation of microspheres.[26] Furthermore, the adsorption of pesticide on the surface of microspheres could be decreased by quick formation of microspheres at the same time. It was suggested that microspheres with a smooth and rounded surface could be obtained by using the solvent evaporation/extraction method.[27]

To study the optimized conditions for preparation of emamectin benzoate microspheres, some factors influencing the characteristics of microspheres were optimized in our previous work (see the Supporting Information). However, the entrapment rate of emamectin benzoate microspheres was <80%. Because emamectin benzoate was slightly soluble in water, emamectin benzoate was easily diffused into the external aqueous solution, which resulted in the decline of the entrapment rate. In this study, we changed the composition of internal organic phase and external aqueous phase to accelerate the formation process of microspheres. The diameter of microspheres would change not only with the composition of the internal organic phase or external aqueous phase but also with the emulsifying speed. The influence of the composition of the internal organic phase or external aqueous phase emulsifying speed and that of the emulsifying speed on the physicochemical characteristics of microspheres were focused on to obtain PLA microspheres of emamectin benzoate with high entrapment rate and smooth and rounded surface by using the modified solvent evaporation/extraction method in this study.

MATERIALS AND METHODS

Materials. The chemicals were provided by the manufacturers: technical grade emamectin benzoate (95.8% pure) from Jiamusi Xingyu Biotechnique Development Co., Ltd., Heilongjiang, China; polylactide (PLA) (technical grade, M_w = 80000) from Esun Industrial Co., Ltd., Shenzhen, China; gelatin (chemically pure) from Sinopharm Chemical Reagent Limited Corp., Co., Ltd., Beijing, China. Methanol and acetonitrile used for high-performance liquid chromatography (HPLC) were of chromatograpy grade. Others were of analytical reagent grade.

Preparation of Microspheres. Briefly, 0.3132 g of emamectin benzoate and 0.6 g of PLA were dissolved in 10 mL of dichloromethane or the mixed organic solvents of dichloromethane and one other organic solvent (ethyl ether, acetone, ethyl acetate, or *n*-butanol) in an ice−water bath. In this study, dichloromethane (or the mixed organic solvents) containing emamectin benzoate and PLA was hereafter defined as the internal organic phase. The blends of 0.3132 g of emamectin benzoate, 0.6 g of PLA, and 10 mL of organic solvent(s) were emulsified in a previously prepared aqueous solution of gelatin (250 mL) at 7000 rpm for 30 s to form an oil/water (O/W) emulsion using a high-speed disperser. The aqueous solution of gelatin 1.5% (w/v) was hereafter defined as the external aqueous phase. The 250 mL solution of O/W emulsion was immediately added to 1000 mL of distilled water solution after 0.3 mL of organic silicon defoamer was added into the O/W emulsion. The organic solvent components in the water solution of the O/W emulsion were extracted and evaporated by stirring the solution overnight at 700 rpm and 30 °C, and the solid microspheres were formed gradually. After extraction and evaporation of organic solvent components for 4 h, the solid microspheres were collected by centrifugation and drying. In addition, blank PLA microspheres were prepared using the same method without the addition of emamectin benzoate.

Effects of Different Factors on the Characteristics of Microspheres. A single-factor exploration method was used to study the effects of different factors (including composition of internal organic solvents and external aqueous phase) on the diameter, span, pesticide loading, and entrapment rate of microspheres. The effects of different kinds and contents of extracting solvent in the internal organic phase or in the external aqueous phase on the characteristics of microspheres were studied, respectively. For instance, different kinds of water-miscible organic solvent such as ethyl ether, acetone, ethyl acetate, and *n*-butanol in the internal organic phase, different contents of extracting solvent such as 25, 37.5, 50, and 62.5% acetone in the internal organic phase, and different contents of extracting solvent such as 0, 0.4,0.8, 1.2, and 1.6% ethanol in the external aqueous phase were used. In addition, the effects of different emulsifying speeds (6000, 7000, 8000, and 9000 rpm) were studied.

Microsphere Characteristics. *Morphology of Microspheres.* In the preparation process of microspheres, the morphology of microspheres was observed by light microscope. Dry microsphere samples were sputter-coated with gold by using a JFC-1200 Sputter Coater 9 (Japan Electron), and the morphology of microspheres was analyzed by scanning electron microscopy (SEM) (JSM-5310LV, Japan Electron).

Entrapment Rate and Pesticide Loading. The entrapment rate was defined as the ratio of actual-to-theoretical pesticide content. The pesticide loading was defined as the actual content of emamectin benzoate in microspheres. The 0.02 g microsphere solid was dissolved with 2 mL of dichloromethane. After dichloromethane had evaporated completely, the sample was washed by using 1.5 mL of methanol under ultrasonic condition five times, and the methanol solution was collected and diluted to 10 mL. Emamectin benzoate concentration in the methanol solution was determined by HPLC using an Amemyst c18-H column at 25 °C. The mobile phase was composed of

Table 1. Characteristic of Microspheres of Different Conditions

emulsifying speed (rpm)	external aqueous phase	internal organic phase	diameter (μm)	span	pesticide loading (%)	entrapment rate (%)
6000	1.5% gelatin	DCM	14.4 ± 1.09 fg	1.50 ± 0.04 cd	26.0 ± 0.34 fh	79.2 ± 1.02 fh
7000	1.5% gelatin	DCM	11.6 ± 0.51 e	1.45 ± 0.03 cd	24.4 ± 0.78 ef	74.3 ± 2.37 ef
8000	1.5% gelatin	DCM	9.02 ± 0.59 cd	1.44 ± 0.03 cd	22.4 ± 0.58 de	68.2 ± 1.76 de
9000	1.5% gelatin	DCM	6.02 ± 0.57 a	1.40 ± 0.04 bc	19.7 ± 0.36 c	60.1 ± 1.11 c
7000	1.5% gelatin	DCM/ether (1:1)	17.2 ± 0.64 i	1.47 ± 0.05 cd	24.2 ± 0.82 ef	73.8 ± 2.50 ef
7000	1.5% gelatin	DCM/*n*-butanol (1:1)	9.77 ± 0.98 d	1.55 ± 0.03 d	23.5 ± 0.90 ed	71.6 ± 2.75 ed
7000	1.5% gelatin	DCM/acetic ether(1:1)	13.4 ± 1.10 f	1.53 ± 0.08 d	28.0 ± 1.00 ig	85.3 ± 3.05 ig
7000	1.5% gelatin	DCM/acetone(1:1)	16.5 ± 1.41 hi	1.49 ± 0.04 cd	31.8 ± 0.73 k	97.0 ± 2.22 k
7000	1.5% gelatin	DCM/acetone (1.5:0.5)	12.2 ± 0.74 e	1.24 ± 0.09 a	25.9 ± 0.75 fh	79.0 ± 2.30 fh
7000	1.5% gelatin	DCM/acetone (1.25:0.75)	14.8 ± 0.44 fg	1.44 ± 0.05 bcd	29.4 ± 1.16 g	89.4 ± 3.54 g
7000	1.5% gelatin	DCM/acetone (0.75:1.25)	15.4 ± 0.47 gh	1.72 ± 0.04 e	27.7 ± 0.73 hi	84.3 ± 2.22 hi
7000	1.5% gelatin + 0.4% EtOH	DCM	10.2 ± 0.38 d	1.40 ± 0.02 bc	23.2 ± 0.44 def	70.5 ± 1.34 def
7000	1.5% gelatin + 0.8% EtOH	DCM	9.04 ± 0.36 cd	1.33 ± 0.02 b	21.4 ± 0.44 d	65.1 ± 1.34 d
7000	1.5% gelatin + 1.2% EtOH	DCM	7.99 ± 0.36 bc	1.42 ± 0.03 bcd	17.1 ± 1.25 b	52.1 ± 3.82 b
7000	1.5% gelatin + 1.6% EtOH	DCM	6.82 ± 0.36 ab	1.43 ± 0.03 bcd	13.2 ± 0.70 a	40.3 ± 2.12 a

[a]DCM, dichloromethane; EtOH, ethanol. Different lower case letters indicate significant difference ($P < 0.05$).

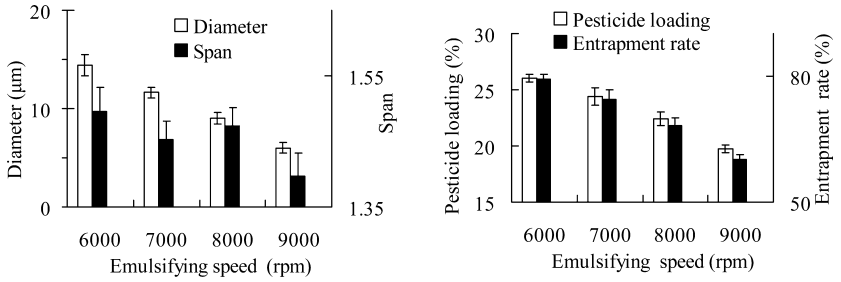

Figure 1. Effects of emulsifying speed on the quality of microcapsules.

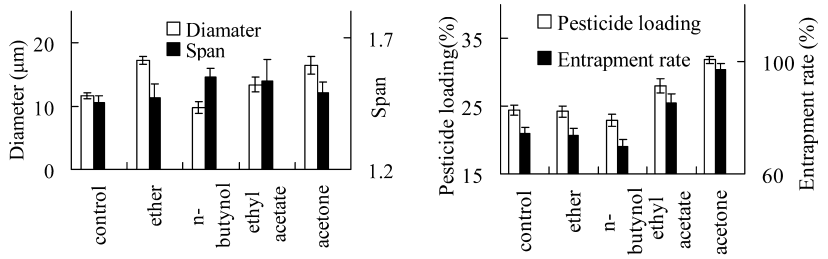

Figure 2. Effects of internal organic phase on the quality of microcapsules. In control, dichloromethane alone was used. In other treatments, the dichloromethane was mixed with ether, *n*-butynol, ethyl acetate, or acetone at the ratio of 1:1, respectively.

methanol/acetonitrile/triethylamine solution (0.1%, 50:40:10), with a flow rate of 1.0 mL/min. The detection wavelength was 245 nm, and the injection amount was 20 μL. From the concentration, the entrapment rate and content of emamectin benzoate in the prepared microspheres were calculated.

Mean Diameter and Span. Amounts of 0.25 g of microspheres and 0.2 g of sodium phosphate were dissolved with 30 mL of distilled water under ultrasonic condition. The mean diameter was measured using an LS-POP(7) laser particle size analyzer (OMEC Instrument Co., Ltd., Zhuhai, China) after high-speed sample introduction. Span = $(D_{90} - D_{10})/D_{50}$. D_{50} represents the mean diameter of microspheres, D_{90} represents the diameter of 90% of the microspheres, and D_{10} represents the diameter of 10% of the microspheres.

Differential Scanning Calorimetry (DSC). Ten milligrams of four samples, including emamectin benzoate, PLA microspheres of emamectin benzoate, a physical mixture of emamectin benzoate and blank PLA microspheres, and blank PLA microspheres without emamectin benzoate, was used for analysis by DSC. Thermal analyses were performed using a DSC instrument (Netzsce, Co., Ltd., Germany) with a heating rate of 5 °C/min and the atmosphere was air.

Photolysis Stability. The samples, that is, emamectin benzoate microsphere solid (0.03g) and the mixture of blank PLA microspheres and emamectin benzoate (0.03g), were weighed and put in 10 mL small beakers, respectively. In addition, emamectin benzoate technical (0.03g) was mixed with 10 mL of methanol in a 10 mL colorless volumetric flask. The three samples were sealed and kept under natural sunlight condition. The degradation rate of the three samples, that is, emamectin benzoate microspheres, mixture of blank PLA microspheres and emamectin benzoate, and emamectin benzoate technical, was determined, respectively, by HPLC after the samples were kept under natural sunlight condition for 0, 1, 4, 7, or 10 days. At least three replicates were performed for each assay.

RESULTS

Effects of Emulsifying Speed on Microsphere Characteristics. The mean diameter and the entrapment rate

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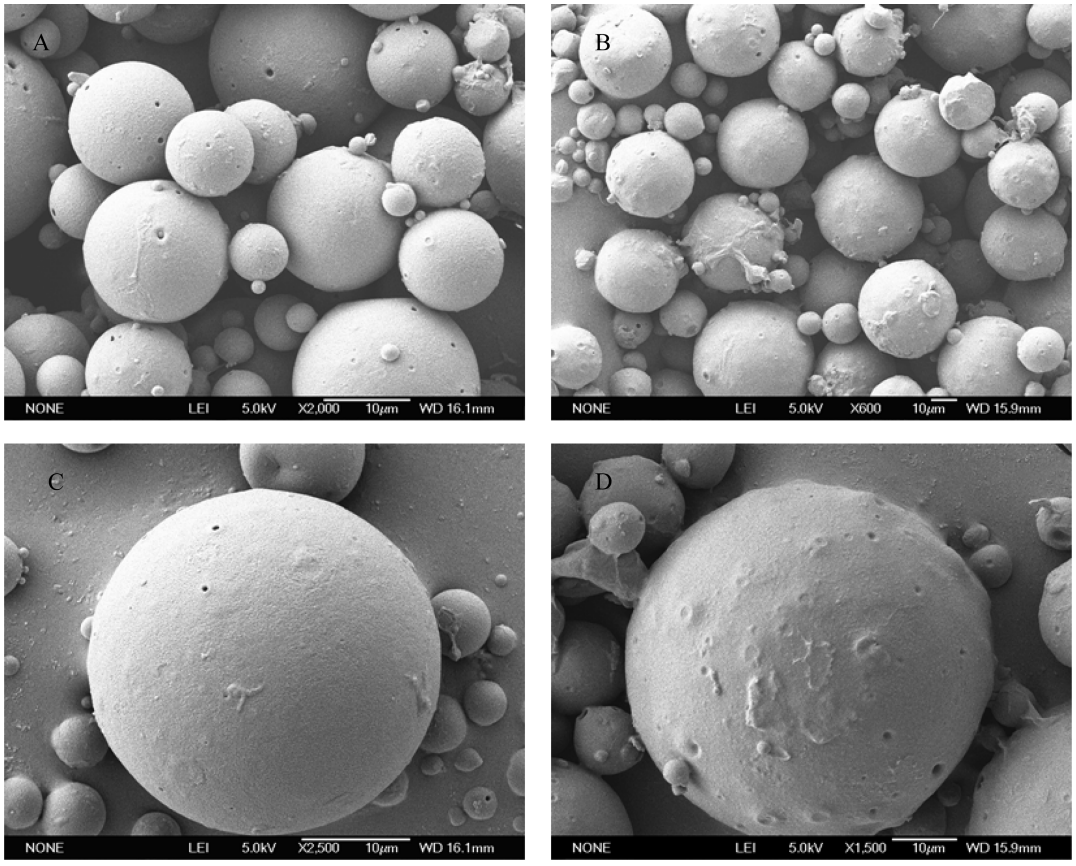

Figure 3. Scanning electron micrographs of microspheres prepared using different internal organic phases: (A, C) internal organic phase was the mixture of dichloromethane with acetone (1:1); (B, D) internal organic phase was dichloromethane alone.

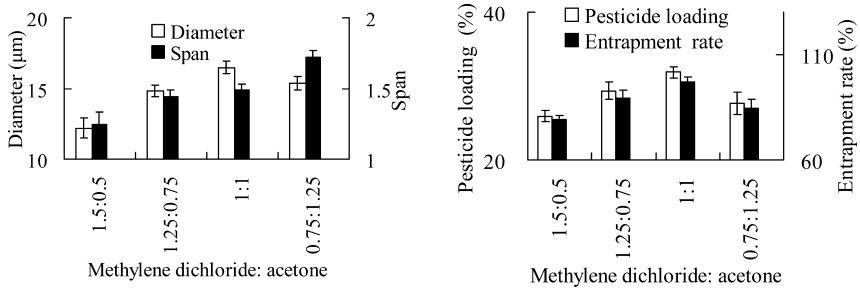

Figure 4. Effects of acetone content in internal organic phase on the quality of microcapsules.

reduced from 14.4 to 6.02 μm and from 79.2 to 60.1%, respectively, when the emulsifying speed was increased from 6000 to 9000 rpm. In addition, the span of microspheres showed a decreased tendency with the increase of emulsifying speed (Table 1; Figure 1).

Influences of Internal Organic Phase on Microsphere Characteristics. *Internal Organic Phase Species.* Several kinds of organic solvents with greater polarity than dichloromethane were selected as extraction solvent to be mixed with dichloromethane at a certain proportion. To improve the diffusion speed of organic solvents to the external water phase, accelerate the formation process of microspheres, and improve the entrapment rate finally, solvents of *n*-butyl alcohol, acetone, ethyl acetate, and ether with different polarities were used. Among the extraction solvents, *n*-butyl had the strongest polarity and ether had the weakest.

Microspheres with a smooth and round surface could be obtained after mixing dichloromethane with the extraction solvents. The mean diameter of microspheres prepared from different solvents decreased from diethyl ether, acetone, ethyl acetate, and *n*-butyl alcohol in turn, whereas the span of microspheres displayed an opposite tendency (Table 1; Figure 2). The particle size of microspheres increased when the microspheres were prepared from the mixtures of dichloromethane with ether, acetone, or ethyl acetate, respectively, as compared to the microspheres prepared from dichloromethane alone. However, the entrapment rate of microspheres increased significantly, and the highest entrapment rate could be found when acetone was used as organic solvent composition (Table 1; Figure 2). The diameter of microspheres increased but entrapment rate reduced when ether was used as organic solvent composition (Table 1; Figure 2). However, when *n*-butyl alcohol was used as organic solvent composition, the

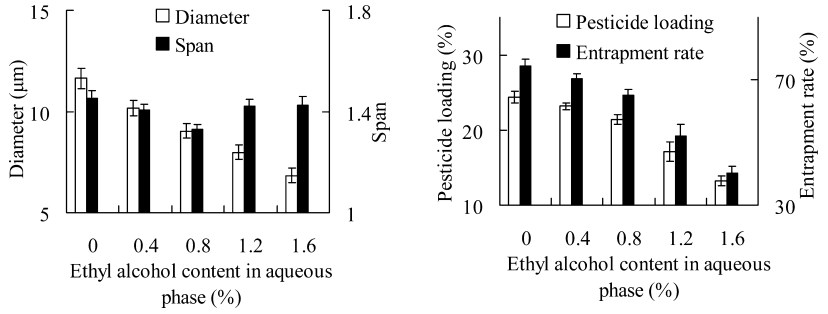

Figure 5. Effects of ethyl alcohol content in external aqueous phase on the quality of microcapsules.

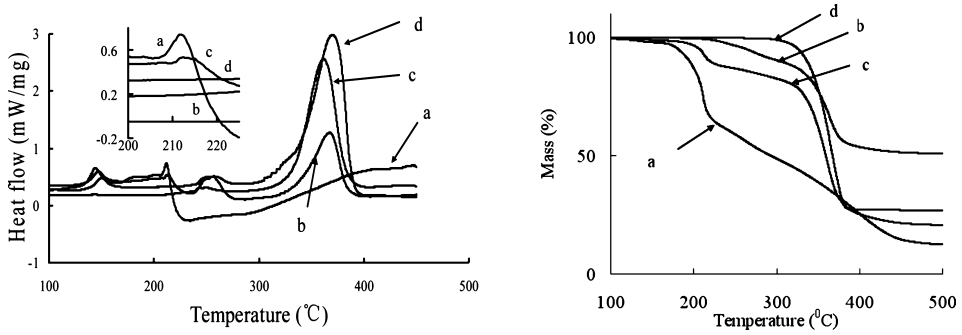

Figure 6. DSC spectrogram diagram (left) and thermal weight loss (right): a, emamectin benzoate; b, PLA microspheres of emamectin benzoate; c, physical mixture of emamectin benzoate and blank PLA microspheres; d, blank PLA microspheres without emamectin benzoate.

diameter of microspheres decreased significantly because alcohol could reduce the surface tension of the oil−water interface. At the same time, the entrapment rate of microspheres decreased significantly as the specific surface area of the emulsion droplets increased (Table 1; Figure 2).

Content of Acetone in Internal Organic Phase. Dichloromethane and acetone were mixed (at the ratio of dichloromethane/acetone = 1.5:0.5, 1.25:0.75, 1:1, or 0.75:1.25, respectively) to form the internal organic phase to investigate the influence of different contents of acetone in the internal organic phase on the characteristics of microspheres. Microspheres prepared from different contents of acetone could be formed quickly, and the solid shell of microspheres could be observed within a few minutes under a light microscope. Compared with the surface of microspheres prepared using the single solvent dichloromethane, the surface of microspheres prepared using a mixture of dichloromethane and acetone was smoother and more rounded, without adsorption of emamectin benzoate on the surface (Figure 3).

With the increasing content of acetone, the entrapment rate of microspheres gradually increased from 79.02 to 97.02% and the diameter and span of microcapsules increased slightly. However, when the amount of acetone in the internal organic phase was >50%, the entrapment rate of microspheres decreased substantially (Table 1; Figure 4). Therefore, the entrapment rate of microspheres could be improved by adding a suitable content of acetone in the internal organic phase.

Effect of Extracting Solvent in the External Aqueous Phase on Microsphere Characteristics. The diameter and entrapment rate of emamectin benzoate microspheres decreased with increasing ethanol content in the external aqueous phase, and the trend of span variation was not pronounced (Table 1; Figure 5).

Analysis on Differential Scanning Calorimetry. DSC is often used to analyze the physical state of drugs encapsulated in polymeric microspheres.[28,29] As shown in Figure 6, the melting peaks of emamectin benzoate and blank PLA microspheres appeared at 210 °C (thermogram a) and 370 °C (thermogram d), respectively. The result of thermal weight loss indicated that emamectin benzoate and blank PLA microspheres lost the most weight at 180−220 °C (thermogram a) and 340−360 °C (thermogram d), respectively, corresponding to the DSC results. When emamectin benzoate and blank PLA microspheres were mixed as a physical mixture (thermogram c), the melting peak appeared at both 210 and 370 °C approximately. However, after emamectin benzoate was encapsulated in PLA microspheres (thermogram b), its melting peak appeared at 360 °C and the melting peak of emamectin benzoate at 210 °C disappeared.

Photolysis Stability. Among the three samples, that is, emamectin benzoate in PLA microspheres solid, the mixture of blank PLA microspheres and emamectin benzoate, and the methanol solution of emamectin benzoate, the methanol solution of emamectin benzoate had the highest degradation rate, whereas emamectin benzoate in PLA microspheres had the lowest degradation rate. The degradation rates of emamectin benzoate in methanol solution and emamectin benzoate solid technical material were 46.3 ± 2.11 and 22.7 ± 1.51%, respectively, under natural sunlight condition on the 10th day. However, the degradation rate of emamectin benzoate in PLA microspheres on the 10th day was 4.29 ± 0.74%. Significant differences in the degradation rates of emamectin benzoate could be found on the first day between emamectin benzoate in methanol solution and in PLA microspheres or on the third day between solid emamectin benzoate and PLA microspheres of emamectin benzoate after the three samples had been kept under natural light condition (Figure 7). The results indicated that microencapsulation of emamectin benzoate displayed a significantly lower degradation rate of emamectin benzoate under natural light condition, as

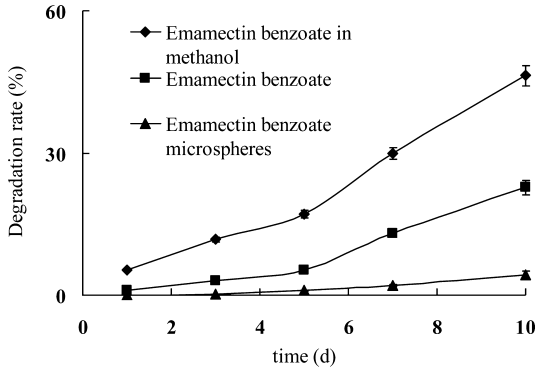

Figure 7. Light degradation curve of emamectin benzoate in PLA microspheres, solid emamectin benzoate, and methanol solution of emamectin benzoate.

compared to emamectin benzoate in methanol solution or solid emamectin benzoate.

DISCUSSION

It was known that emulsifying speed was the main parameter for controlling the dispersion's droplet size and directly affected the diameter of microspheres under the existence of sufficient emulsifier condition.[16] Our results indicated different diameters of microspheres could be obtained by changing the emulsifying speed according to actual needs in experiments. With the increase of emulsifying speed, the dispersion and the specific surface area of organic emulsion droplets in continuous phase increased, but the organic emulsion droplet sizes decreased. The facts resulted in the diffusion probability of the emamectin benzoate to external aqueous phase increased and the entrapment rate decreased. Generally speaking, higher emulsifying speed and stronger shear force would result in better dispersion effect of dispersed organic phase in external aqueous phase and smaller particle size of microspheres.[30] Therefore, in the preparation process of emamectin benzoate microspheres by using the emulsion solvent evaporation/extraction method, the emulsifying speed was the critical factor that affected the emulsion droplet size and finally influenced the size and entrapment rate of microspheres.

Bodmeier et al. reported that the entrapment rate of drug increased when dichloromethane was mixed with organic solvents such as acetone, methanol, ethanol, dimethyl sulfoxide, and ethyl acetate in the preparation of poly(D,L-lactide) microspheres containing quinidine or quinidine sulfate prepared by the solvent evaporation method.[26] Niwa et al. reported that nanospheres with higher entrapment rate and lower size could be obtained when a mixture of acetone and dichloromethane (or acetone and chloroform) was used as organic solvent in the preparation of PLGA nanospheres containing indomethacin and 5-fluorouracil, and the diameter of nanospheres decreased when the amount of acetone in internal organic phase increased.[25] In this paper, the entrapment rate of emamectin benzoate increased when a suitable amount of acetone or ethyl acetate was added into the internal organic phase. However, different from the results above,[25,26] the diameters of microspheres increased when the amount of acetone in internal organic phase increased. The increase of microphere diameter might be related to the fact that the formation speed of microspheres was so fast that microspheres gathered together easily. On the other hand, microspheres with high entrapment rate and suitable diameter could be also obtained by changing the emusifying speed. In conclusion, microspheres of emamectin benzoate with high entrapment rate and nice diameter could be obtained by adding a suitable amount of polar solvent and using appropriate emusifying speed.

The influence of different contents of ethanol in the external aqueous phase was investigated in this paper. It was reported that the entrapment rate of microspheres increased when methanol was added into external aqueous phase as extracting solvent in the preparation of egg albumin microspheres.[31] On the contrary, Yao et al. reported that diameter and entrapment rate of microspheres decreased when ethanol was used as extracting solvent in the preparation of Tranilast poly(D,L-lactide) microspheres.[32] In the present study, the entrapment rate of emamectin benzoate did not increase after the addition of organic solvent in the external aqueous phase, but the diameters of microspheres decreased. Our results were similar to the results of Yao et al.[32] Our results provided evidence that the entrapment rate of microspheres would decline if ethanol was added into the external aqueous phase, because ethanol would result in reducing the surface tension of the emulsion and in smaller diameter, larger specific surface area, and lower entrapment rate. In addition, emamectin benzoate might diffuse into the external aqueous phase with ethanol as it was soluble in methanol, even though emamectin benzoate microspheres could be formed quickly.

The DSC results show that the combination of PLA and emamectin benzoate presented a synergistic effect in reducing the T_m of PLA. The facts indicated some affinity between the polymers and emamectin benzoate. The DSC results revealed that PLA had combined with the active ingredient of the pesticide in the amorphous or molecular state, but not in a simple physical mixing or adsorption on the surface of the microspheres. Our result indicated that DSC was an available method for the analysis of the combination of microspheres and pesticide. Similar analysis for characteristics of microspheres by using DSC determination was also used in the analysis of chlorpyrifos microspheres.

Photolysis stability was an important indicator to measure characteristics of microspheres. The results from the study of photolysis stability also indicated that emamectin benzoate in microspheres could be protected from light degradation by wall material PLA. In addition, the results obtained by both DSC and photolysis stability indicated that emamectin benzoate and PLA had combined with each other well.

Release characteristic of pesticide was also an important indicator of microspheres characteristics. It was proved that microsphere formulations of medicines or pesticides prepared using the solvent evaporation method or the solvent evaporation/extraction method had an obviously sustained effect and could meet the needs of slow release.[10−14,24,25]

Emamectin benzoate, a slightly soluble chemical, was prepared as a microsphere formulation of emamectin benzoate with high entrapment rate and great photolysis stability by using the solvent evaporation/extraction method. It was suggested that the solvent evaporation/extraction method, which has been widely used in the medical field, would be a good method in the microencapsulation of pesticides, in particular, for pesticides with slight water solubility. Our study broadened the scope of application of pesticide microcapsules and provided a reference for the microencapsulation of pesticides.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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