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Preparation and Characterization of Cyhalothrin-Loaded Poly(2-hydroxyethyl methacrylate)-*co*-polylactide (PHEMA-*co*-PLA) Ultrafine Particles

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ABSTRACT: Poly(2-hydroxyethyl methacrylate)-*co*-polylactide (PHEMA-*co*-PLA) and its corresponding cyhalothrin-loaded ultrafine particles were successfully synthesized and prepared, respectively. The chemical structures of the copolymers have been confirmed by Fourier transform infrared spectroscopy (FTIR), ^{1}H-nuclear magnetic resonance (^{1}H-NMR), ^{13}C-nuclear magnetic resonance (^{13}C-NMR), and thermogravimetric analysis (TGA). Furthermore, the particle size, the cyhalothrin loading content (LC), and the cyhalothrin release behavior were investigated. PHEMA-*co*-PLA proved to be a good material for the preparation of ultrafine particles for lipophilic pesticide delivery. The developed cyhalothrin-loaded PHEMA-*co*-PLA ultrafine particles showed good dispersity in water and sustained release behavior. In addition, it is easy to be prepared by both nanoprecipitation method and emulsion/solvent evaporation method. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 1861–1867, 2013

KEYWORDS: poly(2-hydroxyethyl methacrylate); polylactide; ultrafine particles; cyhalothrin delivery system

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INTRODUCTION

Pesticides have been widely used in bulk form in crops, which leads to contamination of soil, groundwater, animals, and non-target organisms. To decrease the heavy burden on the environment, it is necessary to use pesticide more reasonably and more effectively. The manufacture controlled-release formulation is a good strategy to solve the environment problem, considering that it reduces the frequency of agrochemicals application.[1,2]

So far, nanotechnology applications have already been explored in crop protection, especially in the aspect of agriculture chemical application. For example, the nano-encapsulation method is used[3] in which chemicals are delivered in a controlled and targeted manner like nanomedicine implications for drug delivery in humans. Some companies employ suspensions of nanoscale particles (nanoemulsions), which can be either water- or oil-based and contain uniform suspensions of pesticidal or herbicidal nanoparticles (or ultrafine particles) in the range of 200–400 nm. They can be easily incorporated in various media such as gels; creams, liquids, etc. and have multiple applications in preventative measures, treatment or preservation of the harvested product.

By developing pesticide-loaded ultrafine particles that protects the pesticide from degradation it is possible to reduce the total amount of pesticide needed. And then, pesticide can be released quickly or slowly depending on the property of carrier. Furthermore, compared with microcapsules, ultrafine particles are more efficiently absorbed into plants due to their small size, which can increase the solubility of lipophilic pesticide so that they can penetrate into the plants more easily.[4,5]

There are several methods to fabricate ultrafine particles using amphiphilic polymers as carriers especially for hydrophobic pesticide, among which, emulsion/solvent evaporation method and nanoprecipitation method[6–8] are very classical. The key factor in the two methods is a suitable amphiphilic polymer. Therefore, it is very important to choose an excellent material as carrier, which cannot only form micelle but also be friendly to environment. Furthermore the mole ratio of the hydrophilic segment to the hydrophobic segment is very important for the loading content and release behavior of ultrafine particles.[9]

Polylactide (PLA) is a kind of biodegradable materials that has been widely used in biomedical applications such as sutures, screws for bone fractures, and drug delivery. However, there are

some drawbacks such as the low hydrophilicity and high crystallinity, confining its use as drug carrier, for example, the low hydrophilicity and high crystallinity.[10,11] So PLA are often grafted to other macromoleculer as hydrophobic segments of the copolymer.

Poly(2-hydroxyethyl methacrylate) (PHEMA) is widely used in medicine especially in the fabrication of intraocular and soft contact lenses that prove no harm to human body. Furthermore, the successful synthesis of PHEMA-*co*-PLA has been reported.[12,13] As we expected, PHEMA-*co*-PLA is a grafted-amphiphilic copolymer which can be utilized as carrier of ultrafine particles through regulating the mole ratio of hydrophilic segments and hydrophobic segments.

In this work, a grafted-amphiphilic copolymer was synthesized by reacting DL-lactide (DLLA) with poly(2-hydroxyethyl methacrylate) (PHEMA). Then cyhalothrin was used as a model drug to fabricate cyhalothrin-loaded PHEMA-*co*-PLA ultrafine particles. Cyhalothrin, targeting a wide range of insects including aphids, Colorado beetles, and butterfly larvae and so on, belongs to the pyrethroid chemical class of pesticides. When loaded into the ultrafine particles, cyhalothrin showed a high loading content and sustained release behavior. Therefore, it is expected that the cyhalothrin-loaded PHEMA-*co*-PLA ultrafine particles show a promising strategy for crop protection. In the previous work, the chitosan-PLA copolymer was synthesized and imidacloprid-loaded ultrafine particles were fabricated successfully.[14]

EXPERIMENTAL

Materials

Poly(2-hydroxyethyl methacrylate) was purchased from Sigma and used as received. D,L-Lactide (DLLA) was obtained from Alfa Aesar (Ward Hill, MA, USA). Cyhalothrin was purchased from Nanjing Hairui Group Co., Ltd (China). Dimethyl sulfoxide (DMSO) was distilled under reduced pressure and stored over molecular sieves (4 Å). All other reagents and solvents used in the study were analytical grade and obtained from commercial sources.

Preparation of PHEMA-*co*-PLA Graft Copolymer

PHEMA-*co*-PLA graft copolymer was prepared according to the method reported by Wu Y et al.[15] with slight change. 0.5 g of PHEMA and 5 g of DLLA were put in a three-neck flask, and dissolved in 15 mL of dimethyl sulfoxide. The system was degassed under vacuum for 0.5 h, and then purged by nitrogen for 0.5 h. After addition of triethylamine, the temperature was adjusted to 86°C under moderate stirring. The reaction was performed for 10.5 h under nitrogen atmosphere. The resulting product was dropped into 500 mL ice water and the precipitate was collected, washed three times with distilled water. After dried at 30°C under vacuum, the mixture was extracted with hot-toluene (80°C). Finally, PHEMA-*co*-PLA graft copolymer can be obtained after dried under vacuum.

Characterization of PHEMA-*co*-PLA Graft Copolymer

IR spectra was performed on a Fourier-transform infrared (FTIR) spectrometer (Perkin-Elmer, Fremont, CA, USA) over potassium bromide pellets and the wavelength was set from 4000 to 500 cm^{-1}.

^1H NMR was recorded on a Bruker AVANCE 400 NMR spectrometer (Billerica, MA, USA). PHEMA was dissolved in $CDCl_3$. PHEMA-*co*-PLA copolymer was dissolved in $(CD_3)_2SO$.

The thermal properties of samples were determined on a Perkin Elmer (Fremont, CA, USA) thermo-gravimetric analysis apparatus (TGA). The temperature range was 30–900°C under nitrogen flow with a heating rate of 20°C/min.

Fabrication of Cyhalothrin-Loaded PHEMA-PLA Ultrafine Particles

Nanoprecipitation Method. Cyhalothrin-loaded PHEMA-PLA ultrafine particles were prepared by the nanoprecipitation method as previously described by Fessi et al.[16,17] Briefly, 1.5 ml of the PHEMA-PLA copolymer solution in acetone (2 mg/mL) with cyhalothrin (1 mg/mL) (at weight ratios of 100/1, 50/1, and 10/1 copolymer/cyhalothrin) was then dropped into 10 mL of water using a syringe positioned with the needle under magnetic stirring. After 30 min, the acetone was evaporated by reduced pressure. The cyhalothrin-loaded nanoparticles were collected by centrifugation at 15,000 rpm for 10 min, and washed with ethanol three times.

Emulsion/Solvent Evaporation Method. Briefly, 10 mg of PHEMA-PLA copolymer was dissolved in 1 mL of methylene chloride followed by the addition of cyhalothrin at weight ratios of 50/1, 10/1, and 5/1 copolymer/cyhalothrin. Then the mixture was dropped to 10 mL of 0.6% (w/v) of polyvinyl alcohol (PVA, PVA-124) solution under magnetic stirring for 10 min then sonicationed by a microtip probe sonicator. Methylene chloride was evaporated by reduced pressure. Finally, the ultrafine particles were formed and were collected by centrifugation at 13,000 rpm for 10 min, and washed three times with ethanol.

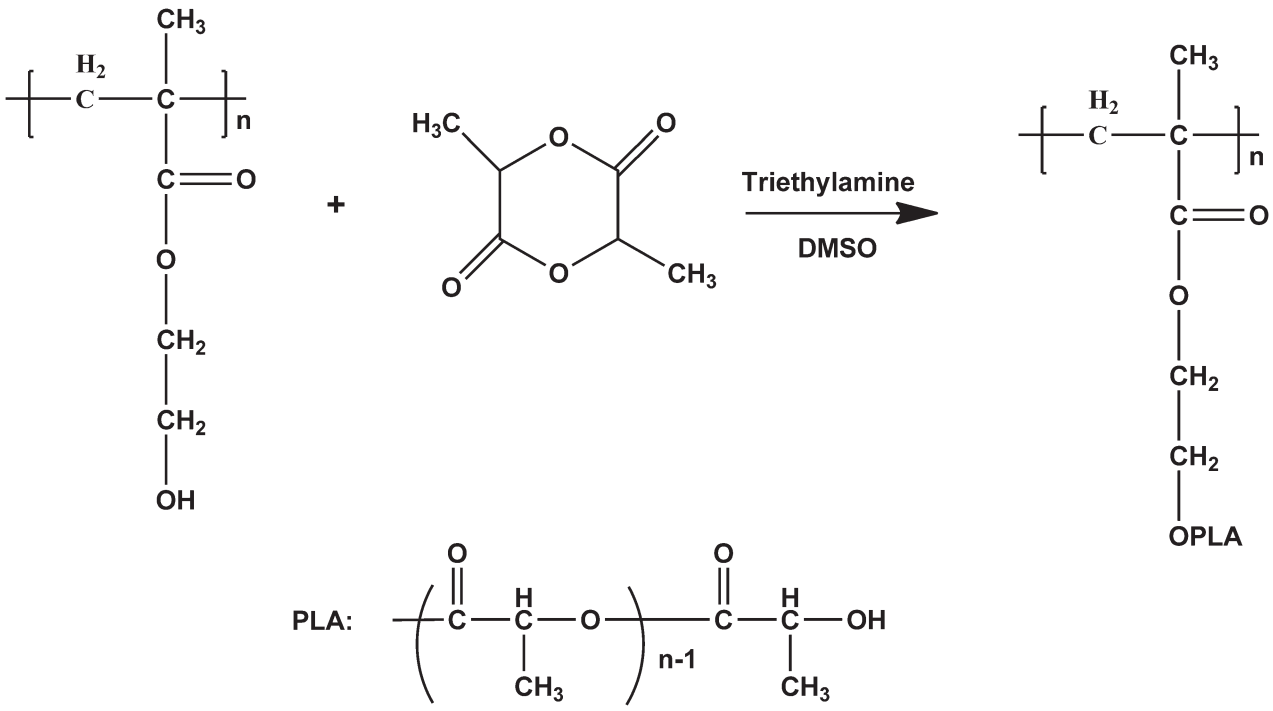
Characterization of the Ultrafine Particles

The morphology of particles was observed using a transmission electron microscopy (TEM, FEI, Tecnai G^2 20). A drop of samples was deposited onto carbon coated copper grids, dried at room temperature, and were stained with uranyl acetate.

The average size of particles was determined by dynamic light scattering (DLS) using a Zetasizer NanoZS Analyzer.

Cyhalothrin-Loading Content. After centrifugation, the particles were collected and lyophilized. The resulting product was dispersed in 1 mL acetonitrile, so that cyhalothrin can be extracted from the particles. The content of cyhalothrin can be determined by high-performance liquid chromatography (HPLC) (Waters 600, 2487 UV detection; Hypersil C18 column, 4.5 × 250 mm).

***In Vitro* Release.** In vitro release experiment, 5 mg of cyhalothrin-loaded PHEMA-*co*-PLA ultrafine particles was reconstituted into 3 mL of distilled water and then introduced into dialysis bags (MWCO 12,000 g/mol). The dialysis bags were placed into 30 mL of distilled water, and the media was stirred at 37°C and 100 rpm using a magnetic stirrer. 1 mL media was withdrawn for cyhalothrin concentration analysis at specific time intervals. After that, 1 mL fresh distilled water was added



Scheme 1. Synthesis of PHEMA-*co*-PLA copolymer.

into the media. The concentration of released cyhalothrin was determined by HPLC. The total amount of released cyhalothrin was calculated by the following mathematical formulas as described:[18]

$$m_{\mathrm{t-act}} = \left(C_t + \frac{v}{V} \sum_{0}^{t-1} C_t \right) V$$

where $m_{\mathrm{t\text{-}act}}$ is the actual quality of released cyhalothrin at time t, C_t is the pesticide concentration in release fluid at time t measured by HPLC, v is the sampled volume taken at a predetermined time interval, and V is the total volume of release fluid.

RESULTS AND DISCUSSION

Synthesis and Characterization of PHEMA-*co*-PLA Copolymer

The synthesis route of PHEMA-*co*-PLA copolymer is shown in Scheme 1. The FTIR spectra of PHEMA and PHEMA-*co*-PLA are shown in Figure 1. In Figure 1(a) the peak at 1726 cm^{-1} was attributed to carbonyl group of the PHEMA and the spectrum of PHEMA-*co*-PLA copolymer [Figure 1(b)] has a new absorption peak appearing at 1756 cm^{-1} corresponding to the carbonyl group of PHEMA and the branched PLA. The peak at 3421 cm^{-1} is attributed to the hydroxyl group. The methyl and methene asymmetric deformation can be seen at 1456 cm^{-1} and the methyl symmetric vibration appearing at 1381 cm^{-1}. In Figure 1(c), the new peak at 1589 cm^{-1} was attributed to the aromatic group of cyhalothrin.

The chemical identities of PHEMA and PHEMA-*co*-PLA were confirmed by ^{1}H NMR [Figure 2(a, b)] and ^{13}C NMR [Figure 3(a, b)]. In Figure 2(b), compared to Figure 2(a), 1.28 ppm corresponds to the methyl protons of the $HOCH(CH_3)$ at the end of chains and 1.46 ppm corresponds to the methyl protons of the $HOCH(CH_3)$ in the chain. The absence of 4.8 ppm indicates that hydrogen atom of —OH at the end of HEMA has been replaced. Also the signals at 4.18 and 5.18 ppm in Figure 2(b) were attributed to the methenyl protons of the PLA moiety located in the terminal groups and repeat units, respectively. Compared to PHEMA [Figure 3(a)], the ^{13}C NMR spectra of PHEMA-*co*-PLA copolymer [Figure 3(b)] showed some change at 170 ppm, which was attributed to the C=O group carbon peak of PLA. The signals at 68 and 70 ppm were attributed to the CH group carbon peak of the PLA moiety located in the

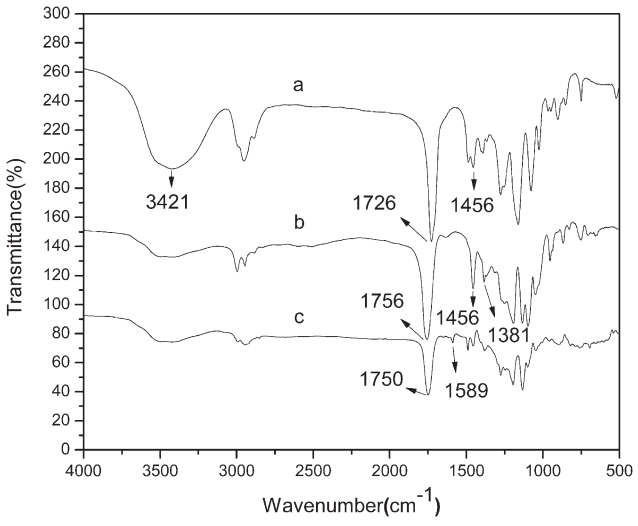


Figure 1. FTIR spectra of PHEMA (a), PHEMA-PLA copolymer (b) and complex of PHEMA-PLA and cyhalothrin(c).

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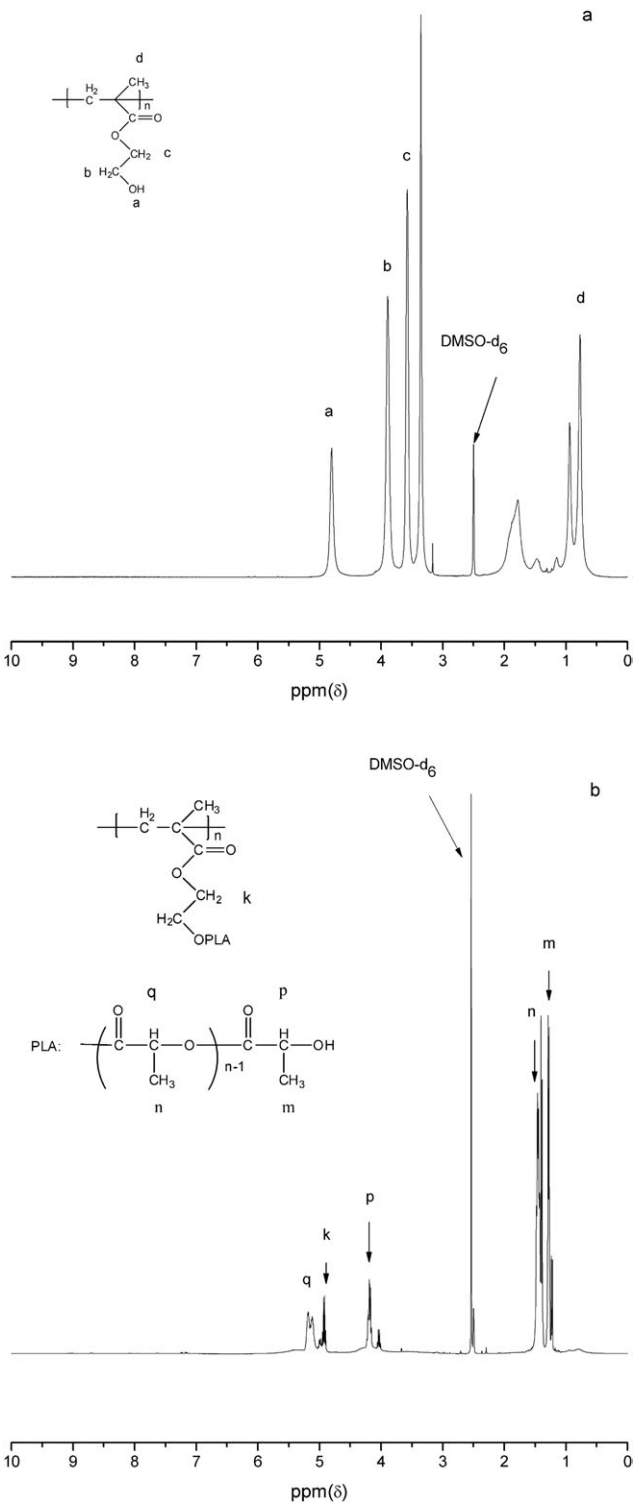

Figure 2. The 1H-NMR spectrum of (a) PHEMA and (b) PHEMA-PLA copolymer.

repeat units and terminal groups. The signals at 17 and 20 ppm were attributed to the CH_3 group carbon peak of the PLA moiety located in the repeat units and terminal groups [Figure 3(b)]. All above in Figures 1–3 evidenced that the polylactide side chain has been successfully grafted to PHEMA.

TGA graphs observed for PHEMA and PHEMA-*co*-PLA grafted copolymer (1 : 10) are shown in Figure 4. Compared to PHEMA, grafted polymer shows lower thermal degradation temperature, which indicates the decrease of the thermal stability for PHEMA-*co*-PLA graft copolymer. In the TGA graphs of PHEMA in the thermal degradation range is above 125°C while PHEMA-*co*-PLA grafted copolymer range is above 250°C. The results showed the decrease of the thermal stability for PHEMA-*co*-PLA copolymer relative to the original PHEMA.

Fabrication and Characterization of Cyhalothrin-Loaded Ultrafine Particles

In this case, two methods were adopted to fabricate cyhalothrin-loaded ultrafine particles which were shown in Figure 5. Dynamic light scattering (DLS) was used to measure the size and size distribution of both empty nanoparticles and

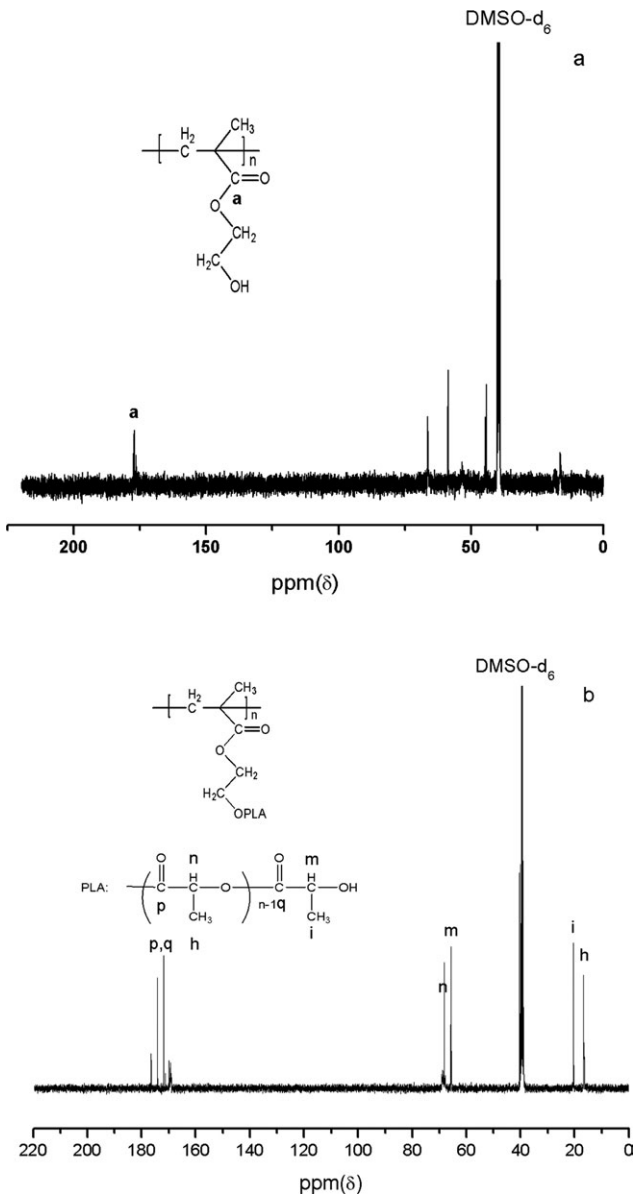

Figure 3. The 13C-NMR spectrum of (a) PHEMA and (b) PHEMA-PLA copolymer.

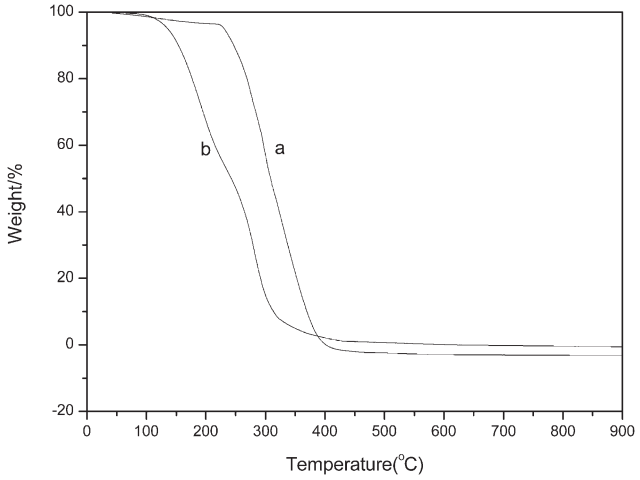

Figure 4. TG thermograms of (a) PHEMA and (b) PHEMA-*co*-PLA copolymer.

cyhalothrin-loaded ultrafine particles. And the results were showed in Tables I and II. Table I shows the results of emulsion/solvent evaporation method, from which we can see that higher percentage of drug content can form larger particles and higher loading content (LC). After cyhalothrin-loading, the size

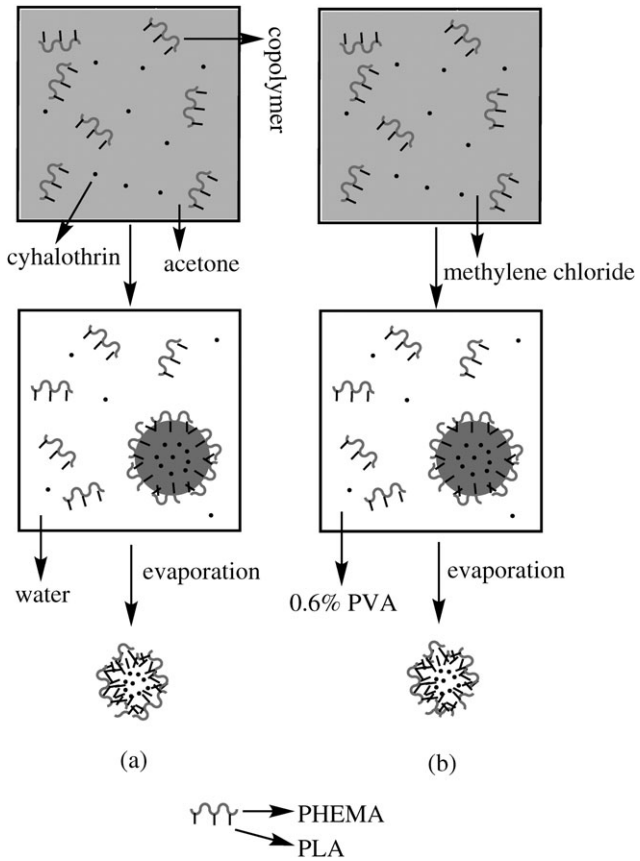

Figure 5. Mechanisms of encapsulation into the ultrafine particles from the PHEMA-PLA copolymer using (a) nanoprecipitation methods and (b) emulsion solvent evaporation methods.

Table I. The Size, Size Distribution, Loading Content (LC) of Ultrafine Particles by the Emulsion Solvent Evaporation Method

Copolymer/cyhalothrin weight ratio	Size (nm)	PDI	LC (%)
5 : 1	340.4	0.029	13.08
10 : 1	316.1	0.237	7.2
50 : 1	293.6	0.184	0.84
100 : 0	273.0	0.232	-

of ultrafine particles increased significantly. As the weight ratio of copolymer/cyhalothrin increased from 5 : 1 to 50 : 1, the size of cyhalothrin-loaded ultrafine particles decreased from 340.4 to 293.6 nm. And all the cyhalothrin-loaded ultrafine particles were larger than empty ultrafine particles (273.0 nm). Meanwhile, cyhalothrin loading content decreased, too. As shown in Table II, the similar results was gotten by nanoprecipitation method(copolymer/cyhalothrin increased from 5 : 1 to 50 : 1, the size of cyhalothrin-loaded ultrafine particles decreased from 215.9 to 205.4 nm and the size of empty particles is 188.3 nm). The results can be explained that a greater amount of pesticide can originate a larger hydrophobic core, which resulted in a larger volume of particles. Though, the change of size was in correspondence with our previous work, the loading content is much higher than before.[14] Compared with two methods, the ultrafine particles made by nanoprecipitation method were much smaller than those made by emulsion/solvent evaporation method because the particles formed in different mechanisms that were shown in Figure 4.[19–21] In nanoprecipitation method, when the solution of a PHEMA-*co*-PLA copolymer and cyhalothrin in acetone was injected into an aqueous solution, the precipitation took place after the solvent evaporation and the single-layer nanoparticles fabricated. In emulsion/solvent evaporation method, moderate PHEMA-*co*-PLA copolymer and cyhalothrin were dissolved in methylene chloride. The mixture was emulsified by sonication in aqueous solution with 0.6% polyvinyl alcohol (PVA) and the nanoparticles were formed after the solvent evaporation.

Figure 6(a,b) shows the morphology of cyhalothrin-loaded ultrafine particles by two methods. It was confirmed that the nanoparticles were formed without aggregation. It should be pointed out that the size of particles was smaller than that measured by DLS because the sample was dried and placed in vacuum in TEM, so that particles were expected to get smaller.

Table II. The Size, Size Distribution, LC of Ultrafine Particles by Nanoprecipitation Method

Copolymer/cyhalothrin weight ratio	Size (nm)	PDI	LC (%)
5 : 1	215.9	0.072	14.6
10 : 1	211.6	0.027	5.6
50 : 1	205.4	0.044	1.2
100 : 0	188.3	0.205	-

Release Behavior

The dialysis method was used to study the release behavior of cyhalothrin-loaded ultrafine particles. Figure 7 shows the accumulative release curve of cyhalothrin from the ultrafine particles in vitro, which were prepared using emulsion/solvent evaporation method. Ultrafine particles containing 13% of cyhalothrin were studied. An initial burst release occurred at the first 2 days, 26% of cyhalothrin was released. Then the release rate became a constant that is slower than the first 2 days. Compared with our previous work,[14] the initial burst effect becomes inconspicuous. And the release speed became slower than before. The reason is that the loading content was much higher than before, and the property of polymer and active ingredient were different. The release behavior of cyhalothrin-loaded nanoparticles can be explained by the mechanism of bulk erosion. It is assumed that cyhalothrin is released out by bulk erosion in which the drug diffuses out through channels that formed before or during nanoparticle core bulk degradation.[22] Also the interaction between cyhalothrin and copolymer plays an important part in the release process.[23]

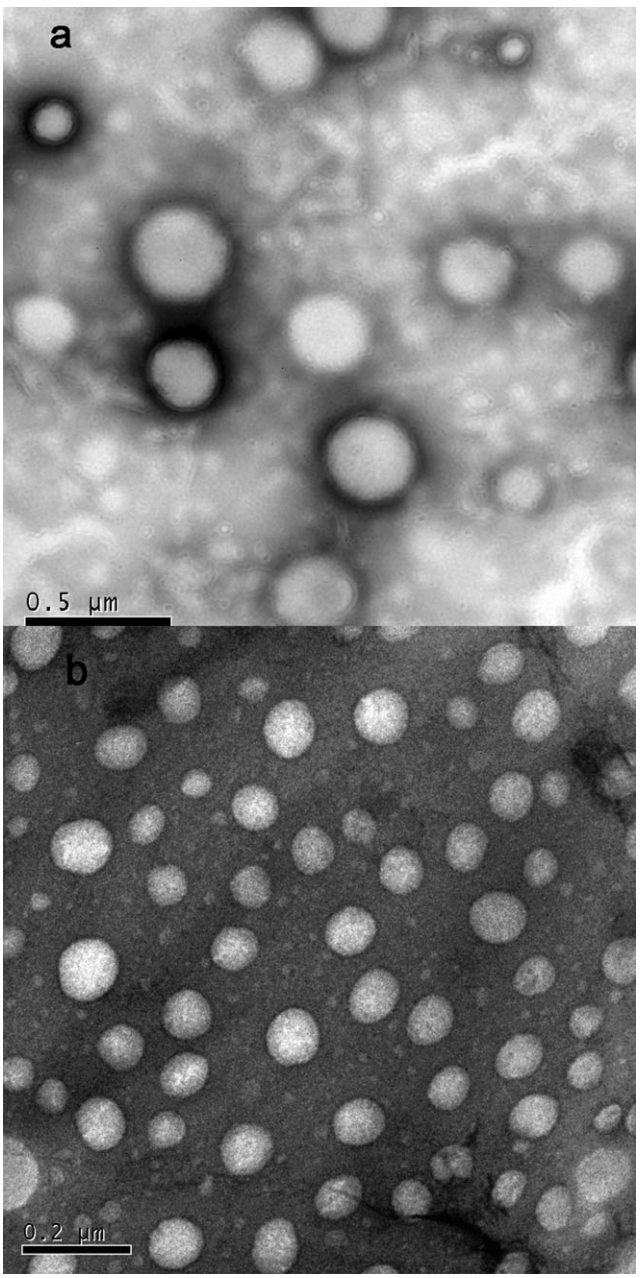

Figure 6. TEM of cyhalothrin- loaded ultrafine particles by (a) emulsion solvent evaporation method and (b) nanoprecipitation method.

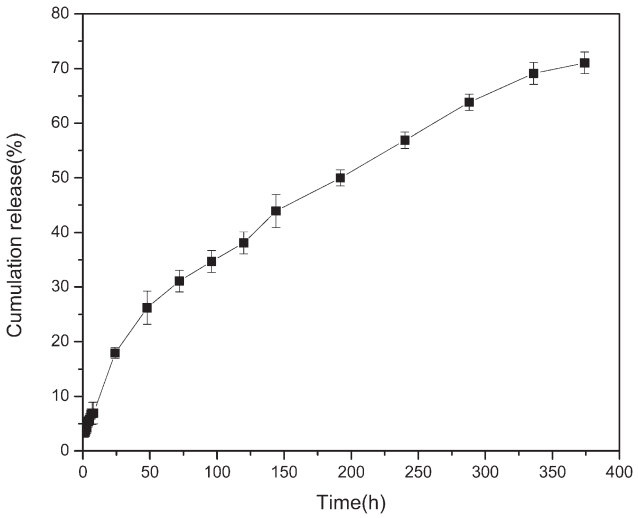

Figure 7. Release profiles of cyhalothrin from cyhalothrin loaded PHEMA-*co*-PLA ultrafine particles (emulsion solvent evaporation).

CONCLUSIONS

An amphiphilic copolymer with a hydrophilic PHEMA segment and a PLA moiety was synthesized. The cyhalothrin-loaded copolymer ultrafine particles were prepared by nanoprecipitation and emulsion/solvent evaporation method. The size of ultrafine particles and cyhalothrin loading content can be controlled by using different fabrication methods and by changing the mass ratio of polymer and technical chemical. Compared with our previous work,[14] cyhalothrin-loaded PHEMA-*co*-PLA nanoparticles shows higher loading content and lower release speed. The results suggest that PHEMA-*co*-PLA ultrafine is a good material to fabricate pesticide-loaded ultrafine particles.

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