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Fabrication of drug-loaded biodegradable microcapsules for controlled release by combination of solvent evaporation and layer-by-layer self-assembly

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Abstract

The initial burst release of drug from polymer microparticles remains an unsolved problem. Here, we deposited polysaccharides on drug-loaded microspheres using layer-by-layer self-assembly to produce core–shell microparticles for sustained drug release. The ibuprofen (IBU)-loaded poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) microparticles were fabricated by conventional solvent evaporation. The processing parameters, such as pH of water phase, drug/polymer ratio, polymer type, and emulsifier concentration, were optimized according to the encapsulation efficiency and drug loading as pH 4.0, drug/polymer ratio = 10/50 (wt), HV in PHBV = 6 wt.%, and PVA concentration = 1% (w/v). The multilayer shells of chitosan (CHI)/sodium alginate (ALG) and poly(diallyldimethylammonium chloride) (PD)/sodium poly(styrenesulfonate) (PSS) were formed on the IBU-loaded PHBV microparticles using layer-by-layer self-assembly. The in vitro release experiments revealed that, as for the microparticles with three CHI/ALG bilayer shells, the initial burst release of IBU from the microparticles was significantly suppressed and the half release time was prolonged to 62 h from 1 h for the microparticles without coverage. The compact CHI/ALG multilayer film was observed with an atomic force microscopy (AFM) due to the matched distance of charges along the CHI chain and those along the ALG chains. The present combination for encapsulating drug-loaded microparticles demonstrates an effective way to prolong the drug release with reduced initial burst.

Keywords: Solvent-evaporation method; Layer-by-layer self assembly; Microcapsule; Initial burst release; Drug controlled release

1. Introduction

Various drug delivery systems, such as liposomes, micelles, emulsions and polymer micro/nanoparticles, facilitate application in controlled and targeted delivery (Kataoka et al., 2001; Soppimath et al., 2001; Agnihotri et al., 2004; Constantinidesa et al., 2004; Simoes et al., 2004). Recently, some remarkable progress has been achieved for polymer micro/nanoparticles in drug delivery due to their biocompatibility and biodegradability. Several biodegradable polymers were popularly used for this purpose, such as poly(glycolide) (PGA), poly(lactic acid) (PLA), poly(lactide-*co*-glycolide) (PLGA), poly (ε-caprolactone) (PCL), poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), gelatin, chitosan (CHI), alginate (ALG) and so on (Soppimath et al., 2001; Edlund and Albertsson, 2002; Panyam and Labhasetwar, 2003; Jilek et al., 2005). The technique for preparing polymer micro/nanoparticles includes solvent evaporation/extraction, emulsion polymerization, interfacial polycondensation and spray drying. Usually, the drug release from polymeric micro/nanoparticles shows a biphasic pattern in terms of release rate: an initial burst release due to a rapid dissolution of drugs at or close to the particle surface and a subsequent exponential release due to drug diffusion from the interior of the particle (Govender et al., 1999; Khang et al., 2001; Hedberg et al., 2004). One promising method for reducing the release rate and suppressing the initial burst is encapsulating the drug-loaded particles with thin polymer films.

Layer-by-layer self-assembly has been confirmed as a powerful method for micro-encapsulation (Caruso, 2001; Decher and Schlenoff, 2003; Peyratout and Dahne, 2004), where polyelectrolyte multilayer films are elaborated on various micro/nanoparticles through alternate deposition of oppositely charged polyelectrolytes mainly caused by electrostatic attrac-

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tion (Sun et al., 2005). Micro-encapsulation via layer-by-layer self-assembly has potential applications in biochemistry, pharmacy, controlled release, cosmetic and catalyst (Decher and Schlenoff, 2003; Liang et al., 2004). The first approach directly uses proteins, for instance, as the depositing species to prepare bioactive core–shell particles (Caruso and Möhwald, 1999; He et al., 2005; Liang et al., 2005). The second approach involves direct coverage of drug microcrystals, such as ibuprofen (IBU) (Qiu et al., 2001a,b; An et al., 2004), furosemide (Ai et al., 2003), vitamin K_3, insulin (Dai et al., 2004), dexamethasone (Pargaonkar et al., 2005; Zahr et al., 2005), and indomethacin (Chen and Lin, 2005; Ye et al., 2005a) with polyelectrolyte multilayer films for prolonged release. The third approach involves fabricating hollow microcapsules with polyelectrolyte multilayer walls by removing the template cores and to load drugs, enzymes, and proteins into the capsules for delivery (Tiourina and Sukhorukov, 2002; Mao et al., 2005; Ye et al., 2005b, 2006). The fourth approach is to encapsulate the drug-loaded nano or microparticles with polyelectrolyte multilayer films to suppress the initial burst. Following this thought, we have proposed a new combination method for sustained drug release as adsorbing IBU with porous $CaCO_3$ microparticles and encapsulating the IBU-loaded porous $CaCO_3$ microparticles with multilayer films of protamine sulfate (PRO) and sodium poly(styrene sulfonate) (PSS) formed through layer-by-layer self-assembly (Wang et al., 2006).

In this work, IBU-loaded microparticles were made with biodegradable copolymer PHBV by the conventional solvent-evaporation method, which was well described in some recent reviews (O'Donnell and McGinity, 1997; Freitas et al., 2005). And the IBU-loaded PHBV microparticles were encapsulated with biocompatible polysaccharide multilayer films formed through layer-by-layer self-assembly. IBU was selected as a model drug for it was widely used as an anti-inflammatory drug and its property was well documented (Babazadeh, 2006; Chen et al., 2006). PHBV and polysaccharides CHI and ALG were chosen for their biocompatibility and/or biodegradability (Chandra and Rustgi, 1998; Soppimath et al., 2001; Edlund and Albertsson, 2002; Sinha et al., 2004). This combination approach is expected to improve the capsule size and morphology compared with that fabricated through direct deposition on drug microcrystals with polyelectrolyte multilayers, and to increase the loading capacity compared with that spontaneous drug loading into the microcapsules of polyelectrolyte multilayer after removing the template cores.

2. Materials and methods

2.1. Materials

Bacteria-synthesized poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV, MW 2.4×10^5, Aldrich) with hydroxyvalerate (HV) content of 6, 8 and 10 wt.%, ibuprofen (IBU, Juhua Group Co., China), chitosan (CHI, MW 1.5×10^4, Fluka), poly(diallyldimethylammonium chloride) (PD, MW 2–3.5×10^5, Aldrich) and sodium poly(styrenesulfonate) (PSS, MW 7×10^4, Aldrich), polyethyleneimine (PEI, MW 2.5×10^4, Aldrich) and poly(vinyl alcohol) (PVA, MW 1×10^5, Guangzhou Chemical Reagent Factory, China) were used as received. Sodium alginate (ALG, MW 1.2×10^5) obtained from Kimitsu Chemical Industries Co., Japan was dialyzed and freeze-dried before use. The deionized water used in experiments was purified with a Millipore purification apparatus as the resistivity higher than 18 MΩ cm.

2.2. Preparation of IBU-loaded PHBV microparticles

IBU-loaded PHBV microparticles were prepared with the conventional solvent-evaporation method of oil-in-water type as described previously (Sendil et al., 1999). Briefly, PHBV and IBU were dissolved in 2 mL chloroform, and injected dropwise into 60 mL water containing certain amount of PVA as an emulsifier to reach the desired PVA concentrations. The mixture was intensively dispersed with an Ultra-Turrax T25 (IKA, Germany) at 21,500 rpm for 5 min and kept at a low temperature by using an ice water bath. It immediately turned into an emulsion, and the organic solvent was gradually evaporated to fabricate microparticles of polymer and drug under slow stirring at room temperature for 18–24 h. The IBU-loaded PHBV microparticles were finally collected by centrifugation at 10,000 rpm for 10 min (Sigma 3K15, Germany) and rinsed twice with IBU-saturated aqueous solution (pH 4.0, adjusted with acetic acid) to remove any residual emulsifier and free drug which were not entrapped into the microparticles. Microparticles were freeze-dried at −5 °C for 2–3 days (Christ, Alpha 1–4, Germany) and then stored at 4 °C prior to use.

The microparticles were fractionated by centrifugation into three fractions using the following procedure: 5 mL of the prepared suspension was centrifuged at 5000 rpm for 2 min, the precipitated particles were separated as the first fraction (big microparticles). The supernatant was then centrifuged at 8000 rpm for 2 min and the precipitated particles were separated as the second fraction (medium sized microparticles). The supernatant was then centrifuged at 10,000 rpm for 10 min to precipitate the third fraction (small microparticles).

2.3. Encapsulation by polyelectrolyte multilayers

Polyelectrolyte multilayers of CHI/ALG or PD/PSS were deposited on the IBU-loaded PHBV microparticles through layer-by-layer self-assembly in aqueous solution. To prevent IBU from dissolving during the layer-by-layer process, all the polyelectrolyte solutions and the rinsing water used were adjusted to pH 4.0 with acetic acid. The first layer was deposited by adding 20 mg of IBU-loaded PHBV microparticles to 2 mL of CHI or PD solution (2 mg/mL, 0.5 M NaCl). The mixture was incubated for 20 min under gently shaking. The excess CHI or PD was removed by two repeat refining circles of centrifugation (10,000 rpm, 10 min, 10 °C)/washing/re-disperse in water at pH 4.0. The following ALG or PSS layer was deposited using the same procedure with 2 mL of ALG or PSS solution (2 mg/mL, 0.5 M NaCl). Alternating CHI and ALG or PD and PSS layers were deposited subsequently in the identical way until the desired number of layers was achieved.

2.4. Fabrication of polyelectrolyte multilayers on quartz slides

In order to observe the surface morphology of the films with an atomic force microscopy (AFM), multilayers of CHI/ALG and PD/PSS were prepared on flat quartz slides as the substrate. Prior to use, the quartz slide was cleaned with the "piranha" solution (98% H_2SO_4 and 30% H_2O_2 mixture of 7:3 in volume) at 80 °C for 1 h and then dipped in the H_2O/30%:H_2O_2/29%:NH_3 (5:1:1 in volume) mixture at 70 °C for 1 h accompanied with plentiful pure water rinse after each treatment. The concentration of aqueous CHI, ALG, PD and PSS deposition solutions was 2 mg/mL with 0.5 M NaCl and the CHI solution containing 0.3 vol.% of acetic acid for dissolution. The concentration of PEI aqueous solution, used for the first layer, was 1 g/L without NaCl. The polyelectrolytes were alternately deposited on the quartz slide to form ALG/CHI or PSS/PD multilayer films by immersing the slide into a deposition solution for 20 min and rinsed with water for three times per layer.

2.5. Encapsulation efficiency (EE) and drug loading (DL)

The fresh suspension was centrifuged and the amount of unincorporated drug in the supernatant was measured by UV absorbance at 221 nm (Hitachi U-3010, Japan). The encapsulation efficiency (EE) and drug loading (DL) were calculated as follows:

$$\mathrm{EE}(\%) = \frac{\text{weight of IBU fed} - \text{weight of IBU in the supernatant}}{\text{weight of IBU fed}} \times 100$$

$$\mathrm{DL}(\%) = \frac{\text{weight of IBU in microparticles}}{\text{weight of microparticles}} \times 100$$

2.6. In vitro IBU release

A certain weight of IBU-loaded microparticles with or without polyelectrolyte multilayer coverage was sealed in a dialysis bag (cut-off MW $8–10 \times 10^3$) and immersed in 200 mL of phosphate buffered saline solution (PBS, pH 7.4), which was continuously stirred with a magnetic stirrer at a fixed speed of about 100 rpm at 37 °C. At a specified time interval, 3 mL of solution was drawn from the release medium and IBU concentration was determined with UV absorbance at 221 nm. The solution was then returned to the release medium. Each data point was taken from the average of three measurements.

2.7. Characterization

Size and size distribution of the microparticles were measured in pure water with a Malvern Autosizer 4700. ζ-Potential of the microparticles dispersed in pure water was determined with a Brookhaven zeta-potential analyzer and the ζ-potential value was the average of three measurements.

Scanning electron microscopy (SEM) observation was performed with a Philips XL 30 at the acceleration voltage of 10 kV. Samples were prepared by dropping the microparticle suspension on a quartz wafer, air-dried overnight, then sputtered with gold.

Transmission electron microscopy (TEM) observation was performed with a Philips CM 300 at the acceleration voltage of 15 kV. Samples were prepared by dropping the microparticle suspension on a carbon-coated copper grid and then air-dried before measurement.

Infrared (IR) spectra were measured with a Bruker Vector 33 on the carefully dried samples embedded in KBr pellets of ca. 1 wt.%. Thirty scans were carried out for each spectrum at 4 cm^{-1} resolution and 0.5 cm^{-1} interval.

IBU concentration in solutions was determined from UV absorbance at 221 nm using a Hitachi U-3010 spectrometer with the same solution without IBU as the reference.

The morphology of the air-dried multilayer films was observed with a Seiko SII atomic force microscope (AFM) in tapping mode using a silicon tip with the cantilever spring constant of 10 N/m and the resonance frequency of ca. 120 kHz at room temperature. Several images were taken from different fields of the film to test the representation of the AFM images. The ambient humidity was controlled at about RH 50%.

3. Results and discussion

3.1. Fabrication optimization for IBU-loaded PHBV microparticle

The IBU-loaded PHBV microparticles were fabricated by o/w solvent evaporation, including two major steps, the formation of stable emulsion and evaporation of organic solvent from the emulsion. During such a process, a number of parameters, such as pH of water phase, drug/polymer ratio, polymer type and concentration of the emulsifier, will substantially determine the properties of the microparticle. Hence, we optimized the processing parameters to increase the encapsulation efficiency and drug loading of the microparticles.

The solubility of IBU is 6.14 mg/mL at pH 7.4 and 0.036 mg/mL at pH 1.4 in aqueous solutions (Qiu et al., 2001a,b), so that the pH value of the water phase will alter the encapsulation efficiency and drug loading. The observed results are shown in Fig. 1. At pH 6.5, the encapsulation efficiency and drug loading were only 21.1 and 3.5%, respectively. While at pH 4.0, they increased dramatically to 59.0 and 9.8%, respectively, because the high solubility of IBU in water of pH 6.5 caused a large loss into the water phase. Decreasing pH of the water phase reduces the IBU solubility and enhances the drug entrapment into the microparticles. Thereafter, the IBU-loaded PHBV microparticles in this work were all prepared at pH 4.0.

Seven batches of microparticles with different formulas were fabricated and the results are summarized in Table 1. The maximum encapsulation efficiency was 59.0% (B1) while the minimum encapsulation efficiency was 45.2% (B3). The entrapment of IBU into PHBV microparticles decreased significantly with increasing drug/polymer ratio (B1–B3) and PVA concentration (B1, B6, B7). Because the PHBV concentration in our experiments was fixed at 25 mg/mL, increasing the

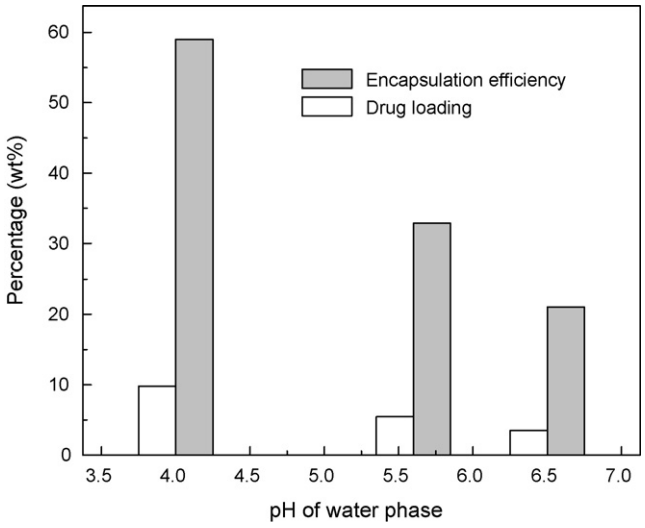

Fig. 1. The encapsulation efficiency and drug loading for IBU into the PHBV microparticles vary with the pH value of the water phase.

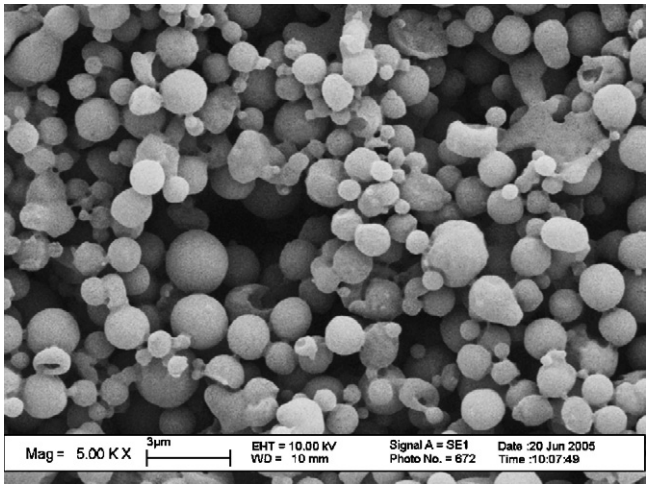

Fig. 2. SEM photo of the IBU-loaded PHBV microparticle B1 (see Table 1).

drug/polymer ratio meant increasing the drug concentration. A possible reason is that the polymer is insufficient to cover the drug completely, leading to a higher leakage of the drug into the water phase. This may be also due to the increase in drug concentration gradient from the emulsion droplet to the external aqueous medium, which is the driving force for drug diffusion. This increased drug concentration gradient reduces the encapsulation efficiency. At the same time, the drug loading was increased from 9.8% (B1) to 16.9% (B3), directly due to the increase in drug/polymer ratio. Dhanaraju et al. (2003) chose levonorgestrel and ethinylestradiol as model drugs, and poly(ε-caprolactone) (PCL) as the drug carrier. An increase in drug/polymer ratio from 1/10 to 1/5 also resulted in a decrease in the encapsulation efficiency from 57.7 to 46.3% and an increase in the drug loading from 5.25 to 7.7%.

As the HV content in the PHBV copolymer was increased from 6 to 10% (B1, B4, B5), only a very slight decrease in the encapsulation efficiency and drug loading was observed. This change in HV content is too small to induce obvious effects on the encapsulation efficiency and drug loading.

In the solvent-evaporation method, emulsifier is usually used to stabilize the suspended droplets over the whole process (Lemos-Senna et al., 1998). The effect of emulsifier on the preparation of drug-loaded microparticles has been reported (Nihant et al., 1994; Mu and Feng, 2002). In the present work, decreasing PVA concentration (B7, B6, B1) enhanced the drug loading from 7.6 to 9.8% and the encapsulation efficiency from 45.6 to 59.0%. High PVA concentration may favor the distribution of IBU in water phase and cause more drug leakage to the water phase.

The average diameter and its distribution as-prepared and after 3-month storage are listed in Table 1. The average diameters were between 500 and 800 nm. By increasing any one factor among the drug/polymer ratio, PVA concentration, and HV content in the PHBV copolymer, the average diameter of the IBU-loaded PHBV microparticles was decreased with narrower distribution due to the enhancement of dispersion capacity for the organic phase in aqueous medium. After 3-month storage, an increase in the particle size was observed. The SEM photo of the microparticle B1 in Fig. 2 shows that all particles are in fine spherical shape with smooth surface without any aggregation or adhesion. Fig. 3 is the FTIR spectra of IBU (a), PHBV (b) and the IBU-loaded microparticle B1 (c). The existence of IBU in the microparticle is confirmed by the appearance of adsorption bands at 1513 and 778 cm^{-1} for the phenyl group in IBU.

The in vitro release profiles of the above seven batches of IBU-loaded microparticles in pH 7.4 PBS at 37 °C are presented in Fig. 4. For all samples, the IBU release curves exhibit a biphasic pattern: the initial burst release to about 50–90% in the first 2 h and the subsequent steady release at almost a constant rate.

Table 1
Processing parameters and properties of IBU-loaded PHBV microparticles

Batch	Drug/polymer ratio (mg/mg)	HV content (wt.%)	PVA concentration (w/v%)	EE (wt.%) ± S.D.	DL (wt.%) ± S.D.	Size (nm) (Polydispersity index)	
						Original	After 3 months
B1	10/50	6	1	59.0 ± 0.6	9.8 ± 0.1	766 (0.769)	998 (0.938)
B2	20/50	6	1	55.5 ± 2.1	15.9 ± 0.6	654 (0.513)	746 (0.336)
B3	30/50	6	1	45.2 ± 1.5	16.9 ± 0.6	568 (0.096)	577 (0.555)
B4	10/50	8	1	57.8 ± 4.0	9.5 ± 0.7	632 (0.685)	633 (0.684)
B5	10/50	10	1	56.3 ± 1.2	9.4 ± 0.2	647 (0.066)	765 (0.760)
B6	10/50	6	2	55.1 ± 2.6	9.2 ± 0.4	706 (0.718)	854 (0.644)
B7	10/50	6	4	45.6 ± 6.4	7.6 ± 1.1	610 (0.315)	692 (0.315)

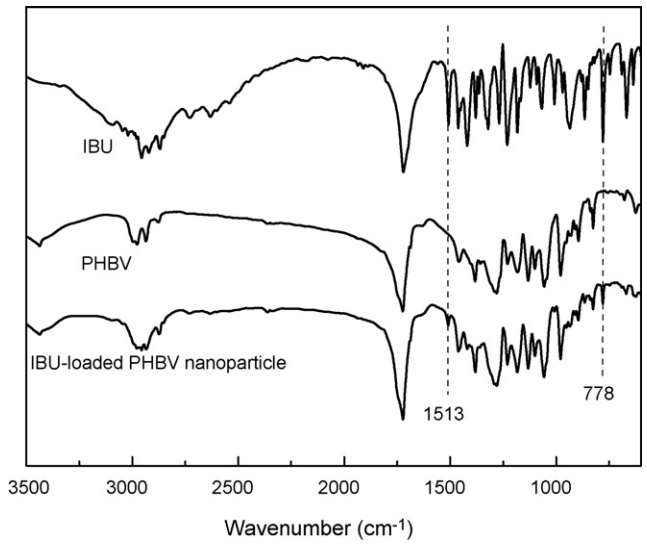

Fig. 3. FTIR spectra of IBU (a), PHBV (b) and the IBU-loaded PHBV microparticle B1 (c).

Complete release of the carried IBU from the microparticles took more than 200 h. Messaritaki et al. (2005) observed with NMR and confocal microscopy that 5-fluorouracil distributed heterogeneously across all regions within the poly(D,L-lactide-*co*-glycolide) (PLGA) microparticles. They found that the initial burst release was associated with the swelling of the polymer matrix and the cavity size growing during swelling. Their finding suggested that the rate-limiting step in drug release was the rate of polymer swelling and/or drug diffusion within the polymer matrix. With an increase of drug/polymer ratio from 10/50 (mg/mg) to 30/50 (mg/mg) (B1–B3), the IBU released in the first 2 h from the microparticles was 70, 84 and 88%. This is a direct result of increasing the drug loading in the microparticles, consequently increasing the drug concentration gradient. 70, 59 and 55% of IBU was released respectively in the first 2 h with increasing HV content in PHBV from 6 to 10 wt.% (B1, B4, B5). Increasing HV content decreases the crystallinity of PHBV copolymer and increases the compatibility with IBU, suppressing the release of IBU from the microparticles.

From the above results, sample B5 prepared under the conditions of drug/polymer ratio = 10/50 mg/mg, HV content = 10 wt.%, PVA concentration = 1% (w/v) had the slowest release rate for IBU with the size of 647 nm. However, sample B1 prepared under conditions of drug/polymer ratio = 10/50 mg/mg, HV content = 6 wt.%, PVA concentration = 1% (w/v) with the size of 766 nm was used in the following experiments due to its maximum IBU encapsulation efficiency (59.0%).

3.2. Encapsulation by polyelectrolyte multilayer films

We tried to suppress the initial burst from the IBU-loaded PHBV microparticles by encapsulation with polyelectrolyte multilayer films prepared through layer-by-layer self-assembly. By following this procedure, oppositely charged polyelectrolytes were alternately deposited onto the IBU-loaded PHBV microparticles till desired layer numbers were reached. The formation of polycation and polyanion films on the IBU-loaded microparticle B1 was monitored with ζ-potential as plotted against the layer number in Fig. 5, which reflected the change in the surface charge with deposition. ζ-Potential of the bare IBU-loaded PHBV microparticle was −3.61 mV. Therefore, either of positive species PD or CHI was used for the first layer and the alternation of positive and negative ζ-potentials due to the charge overcompensation indicated the successful deposition of polyelectrolytes on the microparticles. The absolute ζ-potential values of PD/PSS pair are higher than those of CHI/ALG pair, because PD and PSS are strong polyelectrolytes while CHI and ALG are weak polyelectrolytes.

Table 2 lists the drug loading of the microparticles after encapsulation with the polyelectrolyte multilayers. IBU-loaded PHBV microparticles coated with CHI/ALG, $(CHI/ALG)_3$ and $(PD/PSS)_3$ films were referred to as IBU-PHBV/CHI/ALG, IBU-PHBV/$(CHI/ALG)_3$, and IBU-PHBV/$(PD/PSS)_3$, respectively. The drug loading decreased from 9.8% (IBU-loaded

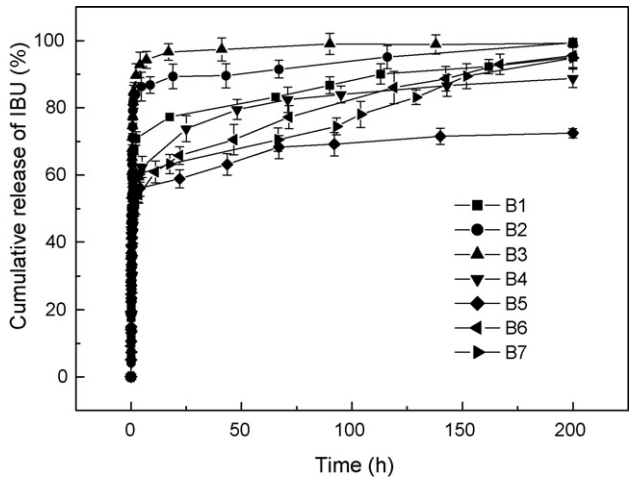

Fig. 4. Release profiles of the IBU-loaded PHBV microparticles in pH 7.4 PBS at 37 °C.

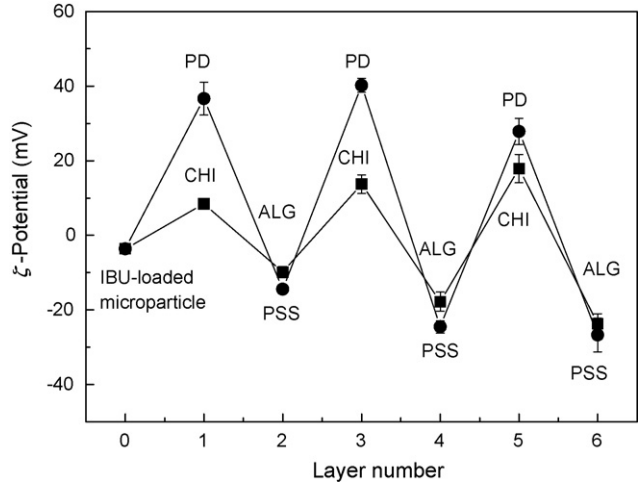

Fig. 5. ζ-Potential plotted against the number of layers deposited on microparticle B1.

Table 2
Drug loading of IBU-loaded PHBV microparticles coated with CHI/ALG multilayers

Microparticles	Drug loading (wt.%) ± S.D.
IBU-loaded PHBV microparticle	9.8 ± 0.1
IBU-PHBV/CHI/ALG	9.1 ± 0.1
IBU-PHBV/$(CHI/ALG)_3$	7.9 ± 0.1
IBU-PHBV/$(PD/PSS)_3$	6.5 ± 0.2

PHBV microparticle) to 7.9% (IBU-PHBV/$(CHI/ALG)_3$) or 6.5% (IBU-PHBV/$(PD/PSS)_3$) with increasing layer number of adsorbed polyelectrolytes up to 6, which was due to the IBU loss during deposition, rinse, and centrifuge processes.

The TEM image of the IBU-loaded PHBV microparticle coated with three CHI/ALG bilayers is shown in Fig. 6. It is clearly seen that the polyelectrolyte multilayer film of about 10–20 nm thickness appears at the surface of the IBU-loaded PHBV microparticle as a shell.

Fig. 7 displays the in vitro release profiles of IBU-loaded PHBV microparticles, IBU-PHBV/CHI/ALG, IBU-PHBV/$(CHI/ALG)_3$, and IBU-PHBV/$(PD/PSS)_3$ in pH 7.4 PBS at 37 °C. The half release time ($t_{1/2}$) of the IBU-loaded PHBV microparticles was 1 h and the multilayer film of $(PD/PSS)_3$ had almost no restriction on the burst release with $t_{1/2}$ of about 3 h. However, the IBU initial burst was successfully suppressed even with only one CHI/ALG bilayer and the half release time of IBU-PHBV/CHI/ALG and IBU-PHBV/$(CHI/ALG)_3$ microcapsules was prolonged up to 13 and 62 h, respectively. After a period of 200 h, 95% of IBU was released from the bare microparticles, but only 60% from the IBU-PHBV/$(CHI/ALG)_3$ microcapsules. These results indicate that depositing polysaccharide multilayer films is an effective barrier to the diffusion of encapsulated IBU and the release rate can be easily tuned by varying the layer number of the deposited polysaccharides.

Direct deposition of polyelectrolyte multilayer films on the drug microcrystals can also prolong the release of encapsulated drug. However, the reported time scale for the drug microcrystal

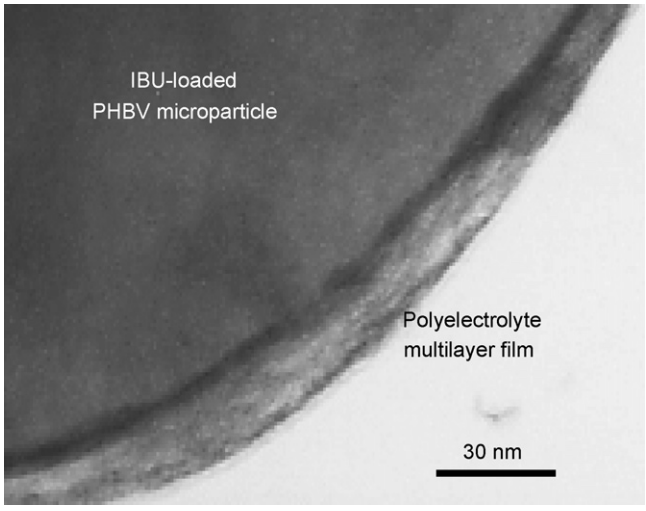

Fig. 6. TEM image of IBU-PHBV/$(CHI/ALG)_3$.

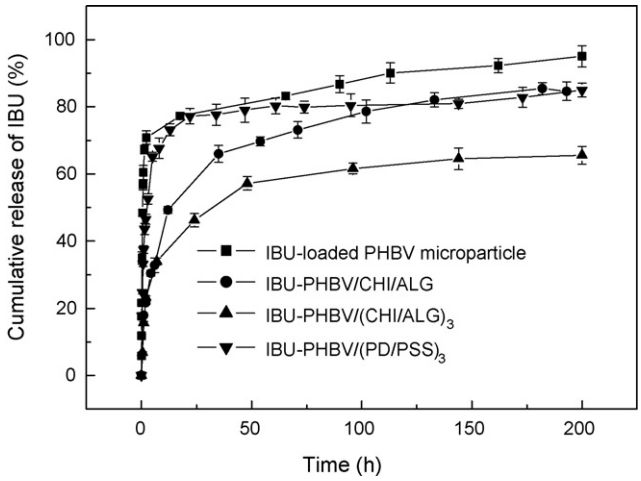

Fig. 7. Release profiles of the IBU-loaded PHBV microparticle B1 coated with multilayer films in pH 7.4 PBS at 37 °C.

release without and with the coverage of polyelectrolyte multilayer films was only about tens of second to minutes (Qiu et al., 2001a,b; An et al., 2004; Dai et al., 2004; Ye et al., 2005a). In the present case, the layer-by-layer self-assembled CHI/ALG multilayer shells prolonged to 10–100 h the release from the IBU-loaded PHBV microcapsules made through solvent evaporation.

AFM images of the surface morphology of $(PSS/PD)_3$ and $(ALG/CHI)_3$ multilayer films on the quartz slice are depicted in Figs. 8 and 9, respectively. The surface of $(PSS/PD)_3$ film was smooth and the average roughness was 1.88 nm. There were many cracks in the $(PSS/PD)_3$ film and the longest crack reached about 1 μm with the width of about 50 nm. In contrast to the $(PSS/PD)_3$ film, there were no evident cracks in the $(ALG/CHI)_3$ film. Its surface was somewhat rough and the average roughness was 4.38 nm, being 2.5 times that of the $(PSS/PD)_3$ film. The cracks in $(PSS/PD)_3$ film appeared to be the main origin for the fast release of IBU from the IBU-PHBV/$(PD/PSS)_3$ microparticles. Another explanation is that the polyelectrolyte multilayer film is a type of physically cross-linked network through electrostatic attraction. The permeability would be controlled by the mobility of sub-chains between the cross-linkers, which depends on the cross-linking density and film cavity size (Qiu et al., 2001a). Because the distance between the charged groups along the CHI chain closely matches that of the ALG chain, a higher cross-linking density in the multilayer film is achieved, which in turn attenuates drug permeability. In contrast, the charge distance along the PD chain does not match that of the PSS chain, leading to a multilayer film with large cavities and higher permeability.

The drug release rate also depends on the size of microparticles. The IBU-loaded microparticles were fractionated by centrifugation into three fractions, referred to as big, medium and small microparticles. The size distribution curves of these IBU-loaded PHBV microparticles are shown in Fig. 10. The mean diameter of big, medium, and small MP is 2278, 669, and 239 nm, respectively, with narrow distribution. The release profiles of the small, medium, and big microparticles encapsulated

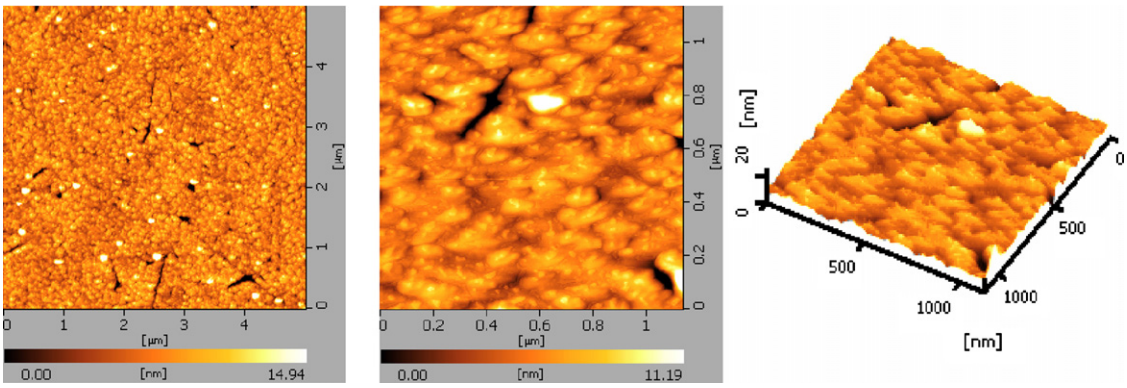

Fig. 8. AFM images of the $(PSS/PD)_3$ film on quartz slide.

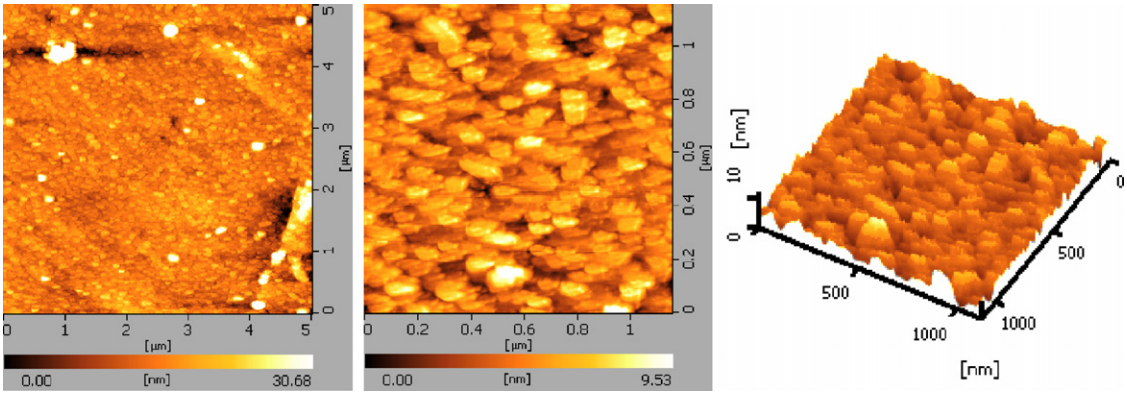

Fig. 9. AFM images of the $(ALG/CHI)_3$ film on quartz slide.

with the $(CHI/ALG)_3$ multilayer are illustrated in Fig. 11. As expected, the small microparticle released faster than the big microparticle. The half release time of small, medium and big IBU-PHBV/$(CHI/ALG)_3$ was 15, 32 and 62 h, respectively. The fast release of the small microparticle is due to the large surface-to-volume ratio, and the coverage with $(CHI/ALG)_3$ multilayer still maintains the similar release property.

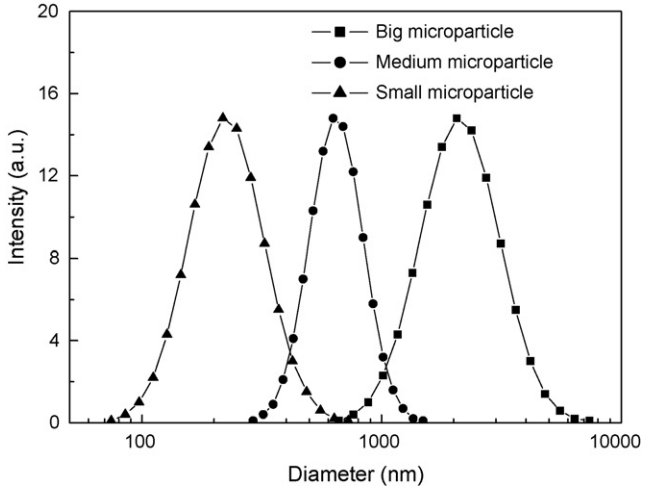

Fig. 10. Size and size distribution of fractionated IBU-loaded PHBV microparticles.

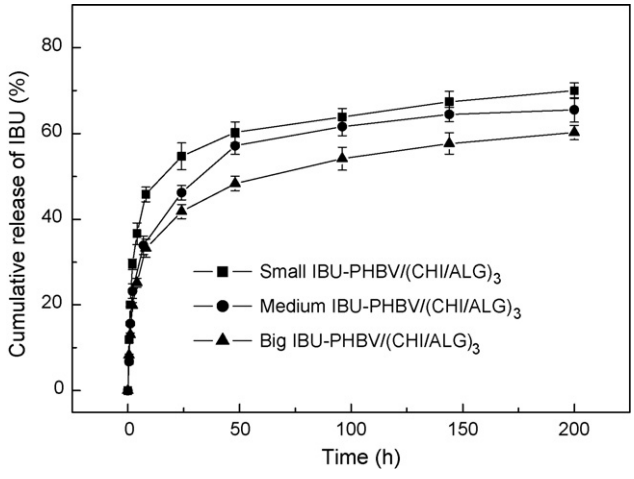

Fig. 11. Release profiles of microparticles with different size coated with $(CHI/ALG)_3$ bilayers in pH 7.4 PBS at 37 °C.

4. Conclusions

Combining the solvent-evaporation method for preparing drug-loaded microparticles through layer-by-layer self-assembly for depositing polyelectrolyte multilayer films, we successfully fabricated the IBU-loaded PHBV microcapsules. Only several CHI/ALG bilayer films were required to sig-

nificantly suppress the initial burst release of IBU from the microparticles. The proposed combination method for the drug-loaded microcapsules illustrates an effective way to prolong the drug release with reduced initial burst.

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