# Preparation and *In Vitro* Drug-Release Behavior of 5-Fluorouracil-Loaded Poly(hydroxybutyrate-*co*-hydroxyhexanoate) Nanoparticles and Microparticles

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**ABSTRACT:** Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) copolymeric microparticles (MPs) and nanoparticles (NPs) were prepared by the double-emulsion solvent-evaporation technique. 5-Fluorouracil (5-Fu), an anticancer drug, was entrapped in PHBHHx NPs and MPs. A variety of parameters, including the species and concentration of different surfactants, power and time of ultrasonication for particle dispersion, and organic/aqueous solution ratio, that affected the production of the 5-Fu-loaded PHBHHx NP and MP particles and the release of 5-Fu were studied. The results show that the prepared NPs and MPs were spherical in shape and about 160 nm and 3 μm in size, respectively, when cetyltrimethyl ammonium bromide was used as the emulsifier. The drug-loading content (DLC) varied from 3.53 to 8.03% for 5-Fu-loaded NPs and from 4.83 to 18.87% for 5-Fu-loaded MPs and depended on the different initial feeding amounts of 5-Fu. The encapsulation efficiency decreased with increasing DLC. The *in vitro* drug-release characteristics appeared to have two phases with an initial burst effect occurring within the first 8 h; this was more obvious for the particles with low DLCs. The NPs with high DLC (8.03%) had the slowest release rate, 49.6% of 5-Fu within 24 h. Therefore, PHBHHx copolymeric NPs and MPs can possibly be applied as drug-delivery carrier materials in the future. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2944–2950, 2010

**Key words:** biomaterials; drug delivery systems; polyesters

## INTRODUCTION

In recent years, drug-releasing and drug-targeting systems have been developed to reduce drug degradation and side effects, improve bioavailability, and enhance the effectual concentration of drugs in a given area. Over the past several decades, a lot of work has been done and many delivery tools and methods have been developed, including liposome-based delivery systems, polymer-based delivery systems, and intelligent delivery systems.[1] Among these systems, biodegradable polymeric carriers have been shown to be very promising drug carriers and have recently received growing scientific attention.[2,3] A number of different polymers, both synthetic and natural, such as polylactides, poly(D,L-lactide-*co*-glycolide) copolymers, albumin, gelatin, alginate, collagen, and chitosan, have been used to formulate biodegradable nanoparticles (NPs) and microparticles (MPs).[4] NPs and MPs occupy a unique position in drug-delivery technology because of their attractive properties. In particular, NPs have several advantages in pharmaceutical applications, in that they offer possibilities for drug targeting and sustained release. NPs can easily penetrate different tissues and be absorbed by cells. They can even break through the blood–brain barrier and have been shown to be helpful in the treatment of some difficult-to-treat diseases such as brain tumors.[5–7]

Polyhydroxyalkanoates (PHA) are aliphatic polyesters that are synthesized by a wide range of bacteria. There are many kinds of structurally distinct PHAs, which have different physicomechanical properties and kinetics of biodegradation.[8,9] PHAs are generally biodegradable and have good biocompatibility, which makes them more and more attractive as biomedical materials.[10] Therefore, they have drawn the attention of scientists working in several fields, including medicine, pharmacy, and agriculture. In addition to their mechanical properties, these polymers are ideal for use as biomedical materials because of their unique and interesting physicochemical features, which are similar to those of polypropylene and poly(D,L-lactide-*co*-glycolide).[11] Poly(hydroxybutyrate-*co*-hydroxyvalerate) has been demonstrated as a drug carrier in the controlled release of tetracycline and gentamicin.[12,13] Also,

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certain PHAs, such as poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx), which contain both short-chain-length and middle-chain-length monomers, have better elastic properties, and many studies have illustrated the good biocompatibility and bioactivity of PHBHHx, which is more supportive of cell growth than polyhydroxybutyrate and poly(lactic acid).[10,14,15] PHBHHx was found to be totally biodegradable, and its degraded products are nontoxic and nonimmunogenic.[14] The main degraded product, 3-hydroxybutyrate, is a kind of ketone body, which can even been metabolized by extrahepatic tissues to supply energy. Thus, PHBHHx is a promising kind of biomaterial and is expected to have special applications, such as scaffolding for tissue engineering and drug-delivery carriers. Because of its ester backbone and alkane side chain, PHBHHx is a promising carrier candidate, specifically the delivery of hydrophobic therapeutic agents. In this study, the method for preparation of drug-loaded PHBHHx NPs and MPs and various parameters' effects were investigated with 5-fluorouracil (5-Fu), a chemotherapy drug for some types of cancer, as a model drug. The *in vitro* drug-release behaviors of PHBHHx NPs and MPs and the *in vitro* stability and degradation of PHBHHx NPs and MPs were also assessed.

## EXPERIMENTAL

### Materials

PHBHHx [weight-average molecular weight ($M_w$) = 466,500] containing 15.72 mol % of 3-hydroxyhexanoate (3-HHx) units was produced by *Aeromonas hydrophila*.[16] After fermentation, intracellular PHBHHx was extracted from the lyophilized cell mass with an excess of chloroform at 90°C for 4 h. The polymer was purified by repeat methanol precipitation.[17] All other chemicals were analytical grade and were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China)

### PHBHHx characterization

The polymer composition and monomer fraction were determined by gas chromatography (GC) spectrography (PerkinElmer Clarus 500, Waltham, MA) and proton nuclear magnetic resonance spectroscopy ($^1$H-NMR) spectra at 500 MHz on a Varian Unity Inova 500NB spectrometer.[18] For GC analysis, 8 mg of PHBHHx were subjected to methanolysis (4 h at 100°C) in an equal volume (2 mL) of chloroform containing methyl benzoate as an internal standard. After phase separation, the methyl esters were analyzed on a GC system equipped with a PE-1 column. For peak identification, a PHA standard (kindly donated by Tsinghua University, Beijing, China) was used. For NMR analysis, polymer samples were dissolved in deuterated chloroform (4 g/L), and tetramethylsilane was used as an internal reference. Polymer samples were operated at 500 MHz, and $^1$H spectra were recorded with a spectral width of 8003 Hz and an acquisition time of 1.892 s and were referenced internally to chloroform (7.26 ppm with respect to tetramethylsilane). The number-average molecular weight ($M_n$), $M_w$, and polydispersity ($M_n/M_w$) of the PHBHHx samples were determined with a high-performance liquid chromatograph–gel permeation chromatograph,[19] which consisted of a Spectra-System P2000 pump; an AS3000 autosampler with a 50-mL, fixed-loop injector; and a Shimadzu HSG-GO column (Shimadzu Corp., Kyoto, Japan). Chloroform was used as eluent at a flow rate of 1 mL/min. The sample concentration was 2 mg/mL, and the injection volume was 50 mL. The calibration curve was generated with polystyrene standards (Shodex, Japan).

### Preparation of the 5-Fu-loaded PHBHHx NPs and MPs

The double-emulsion solvent-evaporation technique (water-in-oil-in-water) was used to prepare the 5-Fu-loaded PHBHHx NPs and MPs.[20–22] Briefly, 5 mL of a certain concentration of 5-Fu solution was emulsified in 10 mL of PHBHHx–chloroform solution under high-speed stirring (BO-1 magnetic stirrer, Shanghai Shiyuan Science and Equipment Co., Ltd., Shanghai, China). The resulting primary emulsion was added to a certain amount of aqueous solution containing a given surfactant [anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethyl ammonium bromide (CTAB), or nonionic Triton X-100] under stirring to form the double emulsion. Chloroform was eliminated by evaporation under reduced pressure with a rotary evaporator (Chemat Technology, Inc., Northridge, CA). The resulting MPs were recovered by centrifugation, washed with distilled water, and lyophilized.

To obtain the 5-Fu-loaded NPs, the double emulsion prepared as described previously was dealt with sonication with a probe sonicator (Sonics & Materials, Newtown, CT) with a constant frequency of 20 kHz and power outputs of 40, 100, and 200 W, respectively, for 10–40 min (1-s performance with an interval of 1 s) at 25°C. Chloroform was evaporated as described previously, and NPs were collected with an ultracentrifuge and washed three times with distilled water. A fine NP powder was obtained by lyophilization.[23,24]

### Characterization of the 5-Fu-loaded PHBHHx NPs and MPs

The morphology of the prepared 5-Fu-loaded PHBHHx NPs and MPs was examined by a transmission electron

microscope (Hitachi H-600, Hitachi High-Technologies Corp., Tokyo, Japan). The mean particle size and size distribution of the NPs and MPs were determined with a Zetasizer 1000 particle size analyzer (Malvern Instruments, Ltd., Worcestershire, United Kingdom) and a Multisizer 3 submicrometer particle size analyzer (Beckman Coulter, Fullerton, CA), respectively. Measurements were carried out at 25°C in phosphate buffered saline. Each suspension sample was diluted to an appropriate concentration before the measurements and allowed to stand for 3 min to obtain a steady state. Each sample was analyzed in triplicate.[24]

### Determination of the drug-loading content (DLC) and entrapment efficiency of the 5-Fu-loaded PHBHHx NPs and MPs

The entrapment efficiency and drug loading of the 5-Fu-loaded PHBHHx NPs and MPs were assayed by UV spectrophotometry (Ultrospec 2100, Amersham Biosciences, Ltd., Piscataway, NJ) at a wavelength of 265 nm. The NP and MP yield, DLC, and drug-entrapment efficiency (EE) were calculated according to eqs. (1)–(3), respectively:

$$\text{NP and MP yield}(\%) = (M_p/M_i + M_h) \times 100\% \quad (1)$$

$$\text{DLC}(\%) = (M_t/M_p) \times 100\% \quad (2)$$

$$\text{Entrapment efficiency}\,(\%) = (M_t/M_t) \times 100\% \quad (3)$$

where $M_p$ is the mass of the NPs and MPs, $M_i$ is the mass of 5-Fu fed initially, $M_h$ is the mass of PHBHHx used, and $M_t$ is the total amount of 5-Fu in the NPs and MPs.[24,25] To measure $M_t$, 10 mg of NPs and MPs were dissolved in 1 mL of chloroform. The organic phase was then extracted with 95% ethanol. The content of 5-Fu in the extract was determined by a UV spectrophotometer at 265 nm[26] and calculated with a calibration curve.

### *In vitro* release study

*In vitro* release studies of the 5-Fu-loaded PHBHHx NPs and MPs were performed by the dialysis bag method.[24,25] Briefly, 5 mg of NP/MP powder were suspended in 10 mL of phosphate buffer and placed in a dialysis membrane bag with a molecular weight cutoff of 6000 g/mol. The bag was tied and immersed into 200 mL of a phosphate buffer solution (pH 7.4). The entire system was kept at 37°C with continuous stirring. We took 0.5 mL of the aqueous solution out of the release medium at predetermined time intervals and replaced it with fresh phosphate buffered saline buffer, and the absorbance of the solution was assayed at 265 nm with a UV spectrophotometer (Amersham Biosciences). Each sample was measured three times, and the release of 5-Fu was determined by a calibration curve. The reported values are the mean values for three replicate samples. $M_t$ in 5 mg of NP/MP powder was determined by the method mentioned in the previous section.[26] The release percentage was calculated according to eq. (4):

$$\text{Released percentage}\,(\%) = (M_r \times 200/0.5 \times M_t) \times 100\% \quad (4)$$

where $M_r$ is the amount of 5-Fu in the 0.5 mL of the aqueous sample solution.

### Statistical analysis

The results are expressed as the means plus or minus the standard deviations calculated from three independent experiments. The two-way analysis of variance was applied, and differences were termed statistically significant at $p < 0.05$.

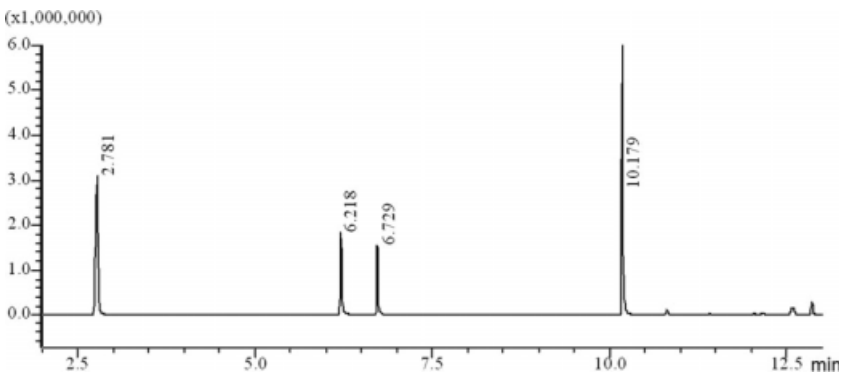


**Figure 1** GC spectrum of PHBHHx. The peak at 2.781 is methyl-3-hydroxybutyrate, that at 6.218 is methyl-3-hydroxyhexanoate, that at 6.729 is methyl benzoate, and that at 10.179 is methyl dodecanoic acid.

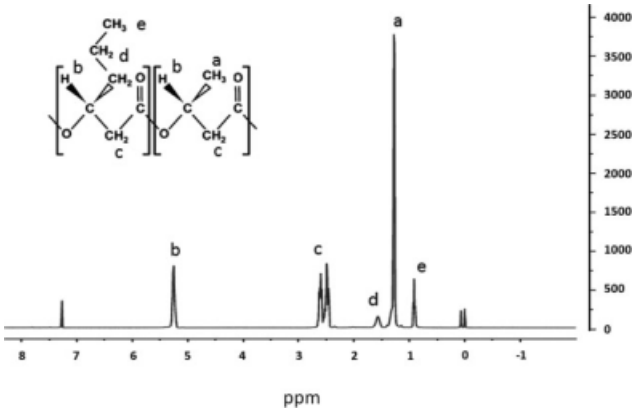


**Figure 2** $^1$H-NMR spectrum of PHBHHx.

## RESULTS AND DISCUSSION

### Characterization of PHBHHx

The polymer composition and monomer fraction were determined by GC and $^1$H-NMR spectra. Figure 1 shows the GC spectrum of the PHBHHx copolymer, which indicates that the copolymer contained both 3-hydroxybutyrate (84.28 mol %) and 3-HHx (15.72 mol %) monomers. The monomer fractions were calculated according to a PHBHHx standard containing 12 mol % 3-HHx. Figure 2 shows the $^1$H-NMR spectra of PHBHHx, which confirmed the chemical structure and monomer composition of the PHBHHx copolymer.

### Formation of the PHBHHx NPs and MPs

The 5-Fu-loaded PHBHHx NPs and MPs were both achieved by the double-emulsion solvent-evaporation technique. The morphology of the 5-Fu-loaded PHBHHx NPs and MPs was examined by transmission electron microscopy. Both NPs and MPs appeared spherical in shape and separated from each other. The sizes of the obtained NPs and MPs varied from 164 to 768 nm and from 2.63 to 8.03 μm, respectively, depending on different preparation conditions.

Three common surfactants, SDS, CTAB, and Triton X-100, were tested as emulsifiers during the preparation of the PHBHHx NPs and MPs. However, only CTAB helped to disperse the organic phase sufficiently and achieve a symmetrical system during the preparation of the PHBHHx NPs and MPs. A nonemulsified organic layer was formed even when 10 mmol/L SDS or Triton X-100 was used. This might be explained by the idea that, compared with anionic SDS and nonionic Triton X-100, the cationic CTAB might have dispersed the PHBHHx molecules more easily by interacting with the hydroxyl groups of PHBHHx at the surface of the NPs and MPs.

The size of NPs and MPs was affected by both the concentration of CTAB and the organic/aqueous (O/A) ratio (Tables I and II). Generally, higher concentrations of CTAB and a lower O/A ratio helped to achieve smaller NPs and MPs. When the MPs were prepared, the concentration of PHBHHx in chloroform also affected their size (Table I). Low concentrations of PHBHHx resulted in a decrease in the MP diameter. MPs with diameters of 3–4 μm could be achieved when 2 m*M* CTAB and a low O/A ratio of 1/10 was used. It is well accepted that the size of NPs and MPs is directly dependent on the rate of diffusion of the organic solvent to the outer aqueous environment. Reductions in the PHBHHx concentration and O/A ratio helped to both decrease the organic phase viscosity and facilitate solvent diffusion; thus, NPs and MPs of a smaller size were produced.[27] However, when 8 m*M* CTAB was used, the PHBHHx concentration and O/A ratio had little effect on the size of the MPs. The diameter of the MPs decreased slightly from 3.7 to 2.6 μm with decreasing O/A ratio. This indicated that 8 m*M* CTAB was more efficient for achieving small MPs.

**TABLE I**
**Effect of the Surfactant Concentration, PHBHHx Concentration, and O/A Solution Ratio on the size of the MPs**

| No. | O/A (v/v) | PHBHHx concentration (g/L) | CTAB concentration (m*M*) | | |
|---|---|---|---|---|---|
| | | | 2 | 5 | 8 |
| 1 | 1/4 | 20 | 5.27 ± 3.63 | 3.72 ± 1.85 | 3.65 ± 2.77 |
| 2 | 1/4 | 25 | 7.62 ± 3.48 | 4.87 ± 3.86 | 3.65 ± 2.76 |
| 3 | 1/4 | 30 | 8.03 ± 4.21 | 5.30 ± 3.69 | 3.71 ± 1.81 |
| 4 | 1/7 | 20 | 3.65 ± 2.30 | 3.02 ± 1.76 | 3.66 ± 2.31 |
| 5 | 1/7 | 25 | 3.92 ± 2.35 | 3.74 ± 2.77 | 3.65 ± 2.30 |
| 6 | 1/7 | 30 | 5.16 ± 3.84 | 4.04 ± 2.25 | 3.56 ± 2.51 |
| 7 | 1/10 | 20 | 3.75 ± 2.77 | 2.85 ± 1.85 | 2.63 ± 0.79 |
| 8 | 1/10 | 25 | 3.85 ± 2.69 | 3.01 ± 1.18 | 2.84 ± 1.73 |
| 9 | 1/10 | 30 | 4.88 ± 3.87 | 3.45 ± 2.29 | 2.98 ± 1.77 |

PHBHHx ($M_w$ = 466,500 produced by *Aeromonas hydrophila*) containing 15.72 mol % of 3-HHx units was dissolved in chloroform as the organic phase.

**TABLE II**
**Effect of the Concentration of CTAB and the O/A Solution Ratio on the Size of the NPs**

| No. | O/A (v/v) | CTAB concentration (m*M*) | Size (nm) | Polydispersity index |
|---|---|---|---|---|
| 1 | 1/4 | 2 | 767.9 ± 49.4 | 0.187 ± 0.094 |
| 2 | | 5 | 526.6 ± 80.2 | 0.230 ± 0.032 |
| 3 | | 8 | 409.4 ± 41.7 | 0.231 ± 0.044 |
| 4 | 1/7 | 2 | 434.6 ± 60.5 | 0.178 ± 0.016 |
| 5 | | 5 | 301.2 ± 18.8 | 0.156 ± 0.049 |
| 6 | | 8 | 267.8 ± 4.4 | 0.164 ± 0.020 |
| 7 | 1/10 | 2 | 166.9 ± 4.7 | 0.148 ± 0.008 |
| 8 | | 5 | 171.3 ± 7.2 | 0.150 ± 0.034 |
| 9 | | 8 | 163.7 ± 5.5 | 0.151 ± 0.020 |

Ultrasound treatment (20 kHz) with a power of 40 W was performed for 40 min at 25°C.

When the PHBHHx NPs were prepared, the double emulsion was dispersed by sonication under outputs of 40, 100, and 200 W, respectively, for 10–40 min (Table III). From the results, we observed that a high power of sonication for 10–20 min was more efficient for achieving small and uniformed PHBHHx NPs, but the diameters of NPs prepared with 40, 100, or 200 W of sonication had no significant difference when the processing time was 40 min. At the same time, the sonication process also caused the degradation of the copolymer, especially under high ultrasound power. Figure 3 shows the degradation curve of PHBHHx under the 40-, 100-, and 200-W sonication processes. The variation of these three independent experiments was quite small, which indicated that the degradation was uniform from batch to batch. From the figure, we observed that, when 40-W ultrasound was applied for 40 min, the molecular weight almost reached the limiting value, and it was just about a 30% decrease. A treatment of 40 min under 200 and 100 W of ultrasound caused 60 and 48%, respectively, decreases in the PHBHHx $M_w$. Therefore, sonication with an output of 40 W was adapted in later experiments because PHBHHx NPs could be achieved in this condition, and it only caused a slight degradation of PHBHHx, whose molecular weight decreased just about 30%. The NPs size obtained with 40 W of sonication for 40 min was smaller and more uniform compared with that obtained by 10–20 min of treatment. Consequently, sonication with an output of 40 W for 40 min was chosen for the subsequent preparation of drug-loaded PHBHHx NPs.

In addition to the power and time of the sonication treatment, the O/A ratio and concentration of CTAB also affected the size of the obtained NPs. The O/A solution ratio had the most notable effect on the NP diameter. The diameter of NPs prepared with a high O/A ratio (1 : 4 and 1 : 7) decreased significantly with increasing concentration of CTAB from 2 to 8 m*M* (Table II); this resulted from the cationic emulsification effect of CTAB, although, when the O/A ratio was 1 : 10, the concentration of CTAB had little effect on the size of the resulting NPs. This might have been because, first, the whole amount of CTAB was excessive compared with the amount of copolymers along with the increase of the aqueous phase volume, and second, the relatively low concentration of PHBHHx in the system repressed the aggregation of droplets and resulted in a decrease in the NP diameter. Because CTAB has some cytotoxicity and high concentrations of CTAB require a more intensive washing process, we used 2 m*M* CTAB as the emulsifier and an O/A ratio of 1 : 10 for the subsequent NP preparation.

### Effects of the 5-Fu drug loading on the DLC and EE (Table IV)

5-Fu-loaded PHBHHx NPs and MPs with mean particle sizes of 167 nm and 3.85 μm, respectively, were

**TABLE III**
**Effect of Sonication on the NP Size and Polymer Degradation**

| Power (W) | Time (min) | Mean size (nm) | Polydispersity index | $M_w$ (kDa) | $M_n$ (kDa) | $M_w/M_n$ |
|---|---|---|---|---|---|---|
| Control | | | | 467 ± 14.7 | 184 ± 10.3 | 2.53 ± 0.08 |
| 40 | 10 | 301 ± 19 | 0.177 ± 0.029 | 390 ± 7.7 | 260 ± 5.0 | 2.99 ± 0.12 |
| | 20 | 259 ± 30 | 0.196 ± 0.050 | 351 ± 10.1 | 237 ± 7.3 | 2.85 ± 0.16 |
| | 40 | 167 ± 5 | 0.148 ± 0.008 | 334 ± 8.4 | 237 ± 11.0 | 2.50 ± 0.15 |
| 100 | 10 | 237 ± 5 | 0.110 ± 0.016 | 357 ± 6.2 | 254 ± 7.3 | 2.90 ± 0.04 |
| | 20 | 199 ± 9 | 0.123 ± 0.048 | 290 ± 6.2 | 226 ± 12.9 | 2.82 ± 0.16 |
| | 40 | 176 ± 5 | 0.150 ± 0.034 | 243 ± 11.8 | 215 ± 28.3 | 2.54 ± 0.15 |
| 200 | 10 | 210 ± 13 | 0.105 ± 0.049 | 372 ± 9.1 | 338 ± 14.3 | 2.20 ± 0.07 |
| | 20 | 184 ± 8 | 0.109 ± 0.012 | 257 ± 8.2 | 346 ± 11.4 | 1.49 ± 0.02 |
| | 40 | 165 ± 6 | 0.159 ± 0.020 | 184 ± 10.6 | 242 ± 8.6 | 1.52 ± 0.03 |

The samples were prepared with a PHBHHx concentration of 25 g/L, an O/A solution ratio of 1/10, and a CTAB concentration of 2 m*M*. Ultrasound treatments were performed under 20 kHz at room temperature.

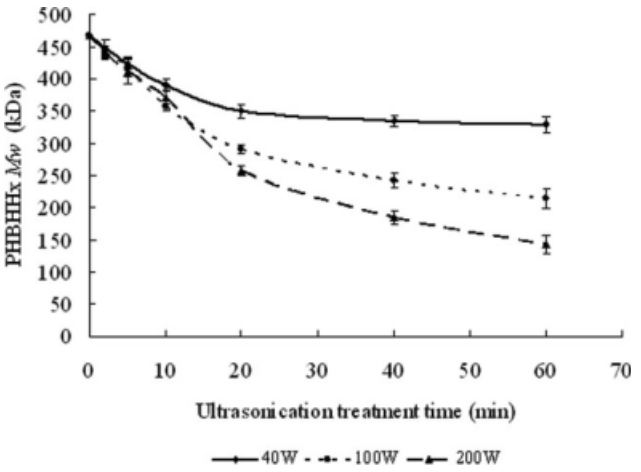


**Figure 3** Degradation curve of PHBHHx under ultrasonication treatment.

prepared with 2 m*M* CTAB as the emulsifier and an O/A ratio of 1 : 10. The DLC and EE were greatly affected by the initial feeding amount of 5-Fu. With an increase in the drug loading from 5 to 75 mg, the DLC of PHBHHx NPs and MPs increased drastically from 3.53 to 8.03% and from 4.83% to 18.87%, respectively, although the EE of the PHBHHx NPs and MPs decreased from 44.62 to 13.55% and from 71.69 to 38.54%, respectively. A possible explanation is that higher drug loadings resulted in increased drug amounts in the NPs and MPs, which could be illustrated by the increased DLC. At the same time, they also enlarged the concentration gradient between the polymer matrix and the outer aqueous phase, which, in turn, led to more drug loss in the fabrication process and a corresponding decrease in EE. Both the DLC and EE of the 5-Fu-loaded MPs were higher than those of the 5-Fu-loaded NPs. This might have been because of the sonication treatment, which caused the breakdown of the PHBHHx molecule and attenuated the interaction between the drug and the materials.

Because PHBHHx is a kind of hydrophobic polyester, the polymer itself may have a limited capacity to encapsulate a specific drug. 5-Fu is a water-soluble drug, and therefore, it is difficult to encapsulate it in hydrophobic polymers. The DLC of 5-Fu-loaded poly[lactic acid–4-hydroxyproline–poly(ethylene glycol)] reported by Duan et al.[25] varied from 1.32 to 4.92%, depending on the formulation varieties. In this study, the highest DLCs achieved in the 5-Fu-loaded PHBHHx NPs and MPs were 8.03 and 18.87%, respectively, which were higher than those reported by Duan et al.[25] This might have been because of the hydrophilic hydroxyl groups in the PHBHHx main chain, which provided a more hydrophilic environment for 5-Fu absorption compared with that in poly[lactic acid–4-hydroxyproline–poly(ethylene glycol)]. The DLC of 5-Fu in the PHBHHx NPs and MPs should be increased by further optimization of the preparation technique and modification of the PHBHHx copolymers.

### *In vitro* release of the 5-Fu-loaded PHBHHx NPs and MPs

The *in vitro* release behaviors of 5-Fu from the PHBHHx NPs and MPs in a phosphate buffer are shown in Figure 4(A,B). The release behavior of 5-Fu showed an early rapid release phase, followed by a slower phase. The initial rapid release was more obvious for the NPs and MPs with low DLCs. The 5-Fu-loaded MPs with a DLC of 4.83% released nearly 90% of the drug during the first 8 h, whereas MPs with a DLC of 18.87% released only about 45% of the drug during the same time. The same trend was observed for the 5-Fu-loaded NPs. A drug can be released from polymer NPs and MPs by the mechanisms of diffusion and/or polymer erosion. Because neither a significant decrease in PHBHHx molecular weight nor a decrease in the NP or MP diameter were observed after the *in vitro* release experiment (data not shown), the release mechanism of 5-Fu from the PHBHHx NPs and MPs was attributed predominantly to drug diffusion. The initial burst release of 5-Fu may have been the dissolution and diffusion of drug that was poorly entrapped in the polymer matrix, whereas the slower and continuous release may have been the diffusion of drug localized in the PHBHHx core of the NPs and MPs. With an increase in DLC, more 5-Fu was entrapped in the

**TABLE IV**
**Effects of the 5-Fu Feeding Amount on the DLC and EE**

| | NP | | | MP | | |
|---|---|---|---|---|---|---|
| 5-Fu (mg) | DLC (%) | EE (%) | Yield (%) | DLC (%) | EE (%) | Yield (%) |
| 5 | 3.53 ± 0.04 | 44.62 ± 3.12 | 24.80 ± 1.88 | 4.83 ± 0.04 | 71.69 ± 7.78 | 29.24 ± 2.88 |
| 10 | 5.17 ± 0.03 | 37.43 ± 1.56 | 27.86 ± 2.68 | 6.60 ± 0.06 | 69.26 ± 5.82 | 40.39 ± 3.10 |
| 25 | 6.97 ± 0.05 | 25.35 ± 2.29 | 33.05 ± 2.23 | 12.93 ± 0.04 | 65.06 ± 2.28 | 45.73 ± 1.38 |
| 75 | 8.03 ± 0.04 | 13.55 ± 0.81 | 38.91 ± 1.43 | 18.87 ± 0.04 | 38.54 ± 0.68 | 47.14 ± 2.36 |

The samples were prepared with a PHBHHx concentration of 25 g/L, an O/A solution ratio of 1/10, and a CTAB concentration of 2 m*M*.

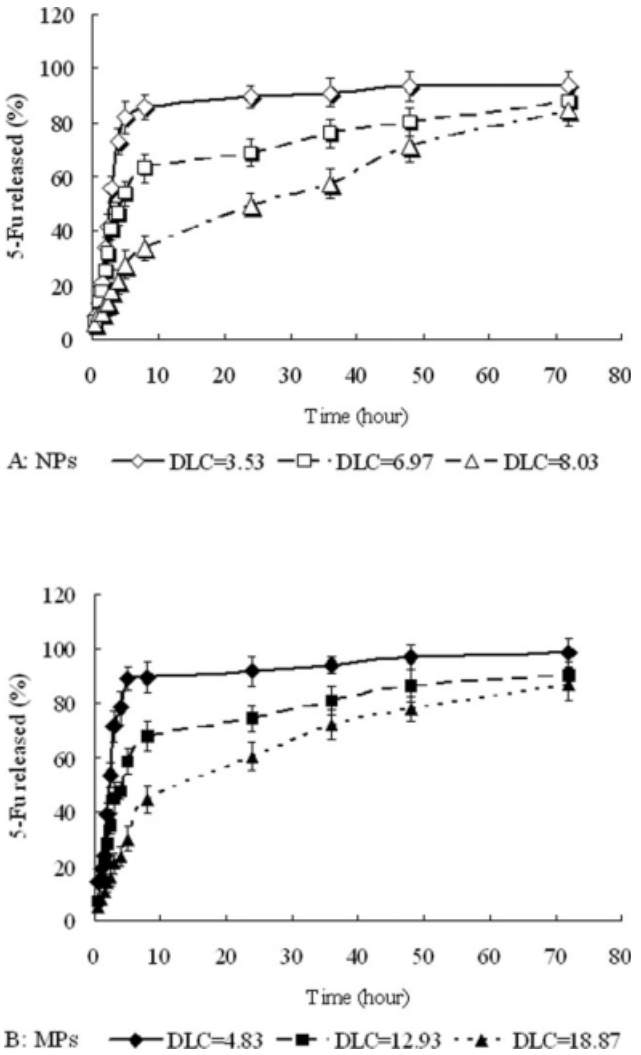


**Figure 4** *In vitro* release profile of entrapped 5-Fu from PHBHHx NPs and MPs with different DLCs: *in vitro* release profile of 5-Fu-loaded (A) NPs and (B) MPs.

core of polymer, which explained the less significant burst release of 5-Fu from the NPs and MPs with high DLCs. The release of 5-Fu from the NPs was relatively slower compared with that from the MPs. This might have been because of the sonication treatment, which attenuated the adsorption of 5-Fu on the surface of NPs. Therefore, the proportion of the drug entrapped in the polymer matrix was higher; this resulted in a slower release and a less obvious burst effect.

## CONCLUSIONS

Novel biodegradable PHBHHx copolymeric NPs and MPs were prepared and served as drug-delivery carriers for the controlled release of 5-Fu. The prepared particles appeared spherical on a micrometer or nanometer scale under transmission electron microscopy. The MP size depended mainly on the O/A phase ratio. A lower O/A phase ratio tended to produce smaller MPs. The NP size depended on both the O/A phase ratio and the ultrasonic dispersion time: a lower O/A phase ratio and a long ultrasonic dispersion time tended to produce smaller NPs.

The DLC and EE were affected by the 5-Fu feeding amount. The *in vitro* drug-release characteristics of the NPs and MPs appeared to have two phases, with an initial burst effect being more obvious for the particles with a low DLC. In general, the release of 5-Fu from the NPs was much slower compared with that of the MPs with the same DLC. The NPs with a higher DLC (8.03%) had the slowest release rate. Therefore, in conclusion, these results show that biodegradable PHBHHx with good biocompatibility can be applied as a novel drug-delivery carrier material for controlled drug release.

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