A Novel Preparation Method for Octreotide Acetate–Loaded PLGA Microspheres with a High Drug-Loading Capacity and a Low Initial Burst Release, and Its Studies on Relations between In Vitro and In Vivo Release

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ABSTRACT: In this study, octreotide acetate (OTA)–loaded poly(lactide-*co*-glycolide) (PLGA) microspheres were prepared using an optimized double emulsion solvent evaporation method. The loading capacity (LC) was increased by raising the concentration of OTA in the inner aqueous phase (W1), and burst release was decreased (<10.0%) because the higher viscosity hindered the diffusion of OTA. The in vitro release profiles were closely correlated with in vivo release profiles calculated using the Wagner–Nelson method ($r = 0.9876$). To evaluate the drug release profiles rapidly, an accelerated release method was developed. In the accelerated release method, the acidic and alkaline buffers (the first 6 h, pH 4.0; the last 18 h, pH 9.6) were used as release media, respectively. Results indicated that the accelerated release profiles (24 h) were closely correlated with the in vivo release profiles ($r = 0.9623$). Therefore, through optimization of the experiment variables (buffer components and pH), an accelerated release method was developed to evaluate in vivo drug release from microspheres. © 2013 Wiley Periodicals, Inc. Adv Polym Technol 2013, 32, 21354; View this article online at wileyonlinelibrary.com. DOI 10.1002/adv.21354

KEY WORDS: Biodegradable, Biomaterials, Drug delivery systems, In vivo/in vitro release, Initial burst release

Introduction

An ideal poly(lactide-*co*-glycolide) (PLGA) microsphere formulation should have reasonably high drug encapsulation efficiency, high drug loading capacity, and sustained release of the loaded drug.[1,2] Increasing the loading capacity of drug, minimizing the initial burst release, and establishing an accelerated method of evaluating in vitro/in vivo release, all pose specific challenges.

There are several approaches to the reduction of initial burst release, including raising polymer concentration, coating the microsphere product with high-viscosity polymer, and incorporating additives, such as glycerol or glucose, into the microsphere formulation.[3–9] Researchers usually increase the in vitro release rate by regulating temperature or adjusting the pH of the release medium.[10]

In this study, microspheres with a low initial burst release and a high drug loading capacity were produced by significantly increasing the concentration of drug in the inner aqueous phase (W1). Furthermore, an accelerated release method was established for evaluating the correlation between the in vitro and in vivo releases, by controlling the pH of release medium.

The authors declare no conflict of interest.

Experimental

MATERIALS AND ANIMALS

Octreotide acetate (OTA) was purchased from Shanghai Taishi Biotechnology. PLGA (L/G 50/50, M_w 35,000) was purchased from Birmingham (Germany). Polyvinyl alcohol (PVA) (M_w 30,000–70,000) was purchased from Sigma (Acros, NJ). All other chemicals (Sinopharm chemical reagent Co., Ltd, Shanghai, People's Republic of China) of analytical reagent grade were used.

Six beagle dogs (three male and three female dogs), weighing 10–12 kg, were purchased from Natural Pharmatech (Jilin, People's Republic of China). 20 h before administration of drug,

dogs were placed in restriction cages without access to food but water.

MICROSPHERES' PREPARATION

The double emulsion solvent evaporation method was optimized for the preparation of OTA microspheres used in this study. In a typical procedure, 2.5 g of OTA powder was dissolved by vortexing in 5 mL of dichloromethane in a 10-mL distillation flask. This was followed by the addition of 1 mL of distilled water and removal of dichloromethane under reduced pressure 25°C for 10–15 min. PLGA (29% w/v in dichloromethane) was added to the distillation flask, and the solution inside the flask was stirred at 10,000 rpm with a high-speed stirrer (T18; IKA) at 15°C for 10 min to initiate the formation of emulsion. After an initial incubation at 4°C for 30 min, the initial emulsion was added dropwise to a 0.5% PVA aqueous solution to form the double emulsion. The mixture was allowed to stir at 800 rpm with a high-speed stirrer at 15°C for 10 min. The organic solvent was removed under reduced pressure (1 h) to form microspheres in a solidified state. The microspheres were collected by centrifugation at 3000 rpm for 5 min and were washed with acetate buffer (pH 4.0) (three times). After thorough washing, they were lyophilized to produce purified microspheres.

DETERMINATION OF IN VITRO INITIAL BURST RELEASE

OTA microspheres (15 mg) were weighed and placed in 20 mL of release medium (0.05 M sodium acetate buffer solution, pH 4.0). Here, the lower pH was employed because OTA is unstable at neutral pH.[8] Samples were slowly shaken in an incubator at 37°C for 24 h, and then the supernatant was carefully removed to avoid disturbing the microspheres, which had settled at the bottom of the container. The supernatant was then injected into a high-performance liquid chromatograph (HPLC) for quantification of OTA released from the microspheres.

DETERMINATION OF OTA INCORPORATED IN MICROSPHERES

First, 10 mg of microspheres was dissolved in 2 mL of tetrahydrofuran and then shaken and sonicated. After a clear solution was obtained, 8 mL of diluent containing 0.2% W/V sodium chloride was added. About 20 min later, the polymer precipitated out. The solution was filtered and injected into the HPLC.[8]

OTA was analyzed using a C18 column by the HPLC (1525; Waters). The mobile phase included components A (0.1% v/v trifluoroacetic acid in water) and B (0.1% v/v trifluoroacetic acid in acetonitrile). The gradient was 80:20 (A:B) to 40:60 (A:B) over 25 min, with a flow rate of 1.5 mL/min, UV at 220 nm.[11]

PARTICLE SIZE DISTRIBUTION

The particle size was determined with a laser particle size analyzer (Dandong Bettersize Instruments) using water as the circulating fluid. The particle size at 50% of the volume distribution (D_{50}) and the span were calculated using the software (BT-9300S, Bettersize) provided.

VISCOSITY MEASUREMENT

The viscosities were detected under the following conditions: size 3 spindle, 1.2 rpm, 20 ± 1°C for M1 and size 1 spindle, 60 rpm, 20 ± 1°C for M2 using a viscometer (NDJ-8S, Jingke, Shanghai, People's Republic of China). The spindle was immersed in the sample.

DETERMINATION OF RESIDUAL ORGANIC SOLVENTS

The trace amount of dichloromethane inside the microspheres was determined with a gas chromatograph (GC) (7890A, Agilent). Dichloromethane (0.1 mg/mL) was prepared by dilution with an internal standard (IS) solution (0.005% v/v ethyl acetate/dimethylformamide). Microspheres (40 mg) were dissolved in 2 mL of the IS solution, and the solution was filtered with a 0.45-μm filter to inject into the GC. The flow rate was 1.0 mL/min. The temperature for capillary chromatographic column (ZB-WAX) was maintained at 50°C, and the temperature for gasification was 200°C.

ASSESSMENT OF IN VIVO RELEASE

Administration and Dosage

Beagle dogs ($n = 6$), weighing 10–12 kg, were used. The dogs were fastened for 20 h before administration. The sites of administration of drug were marked by shaving the injection points on the skin. After disinfection, the drug solution was injected into left hindlimb biceps femoris with veterinary needles (1.2 mm) and syringe (5 mL). The dosage was 1.4 mg OTA/kg according to the result of preliminary experiments. After administration, blood samples (2 mL) were collected from the forelimb veins at predetermined points in time: 0, 0.3, 0.7, 1, 2, 5, 8, 10, 13, 16, 19, 22, 25, 28, 31, 34, and 36 days. The blood samples were placed in a centrifuge tube containing heparin sodium 45U, and centrifuge tubes were shaken and centrifuged for 20 min at 3500 rpm to gain plasma samples. Then the plasma samples were stored at –20°C until assay.[12,13] Because the microspheres could continue to release the drug for 1 month, the dogs were given only one dose and the plasma samples were collected for assay until the 36th day after administration.

Standard Solution Preparation

Stock solutions of OTA (2.0 mg/mL) for the preparation of standard samples were prepared in nanopure water. Stock solutions of IS (triptorelin, 2.0 mg/mL) were also prepared in nanopure water. OTA standard solutions (0.200, 0.600, 2.00, 6.00, 20.0, 60.0, and 200 ng/mL) were prepared by dilution of the OTA stock solution with methanol/water (50:50 v/v). A working IS solution (triptorelin, 500 ng/mL) was obtained by dilution of the triptorelin stock solution with acetonitrile/water (50:50 v/v). All solutions were stored at 4°C.

Plasma Sample Preparation

Exactly 400 μL of plasma, 50 μL of IS solution, 100 μL of methanol/water (50:50 v/v), and 800 μL of acetonitrile were

added to a 1.5-mL Eppendorf tube. The mixture was vortex-mixed for 30 s and then centrifuged at 15,000 rpm for 10 min. The supernatant was added to a tube containing 3.0 mL dichloromethane. After vortex-mixing for 1 min and centrifugation at 4000 rpm for 5 min, 40 μL of supernatant was injected into the liquid chromatography/mass spectrum/mass spectrum (LC/MS/MS).

Determination of the Concentration of OTA in Plasma by LC/MS/MS

The LC/MS/MS method for determining the concentration of OTA in plasma was developed as described.[14] The Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) coupled with an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) and incorporating electrospray ionization was used for this method. Chromatography was performed on a BEH C18 column (1.7 μm, 2.1×50 mm; Waters), maintaining the temperature at 40°C using gradient elution with 1% formic acid as solvent A and methanol as solvent B. The gradient was as follows: 40% B for 1.0 min; a linear increase from 40% to 95% B in 2 min, 95% B for 0.5 min; a linear decrease from 95% to 40% B in 1 min; and equilibration at 40% B for 2.5 min. The flow rate was 0.2 mL/min without a split. The detector was operated at low resolution in the multiple-reaction monitoring mode using the transitions of the protonated molecular ions of OTA at *m/z* 510.5 → 120.1 and 510.5 → 159.1, triptorelin at *m/z* 656.5 → 175.8.

IN VITRO RELEASE STUDIES BY THE FLOW-THROUGH METHOD (FT METHOD)

Microspheres (300 mg) were placed into the flow-through (FT) cell at 37°C. The buffer (0.05 M sodium acetate buffer solution, pH 4.0) was used as release medium, which was pumped by means of a syringe pump. An elute from the cell was collected at predetermined points in time: 0, 0.3, 0.7, 1, 2, 5, 8, 10, 13, 16, 19, 22, 25, 28, 31, 34 and 36 days. Then 100 μL of elute was loaded in the sample pool and the drug concentration was detected by HPLC.

ACCELERATED RELEASE STUDIES USING THE PADDLE METHOD

Accelerated release of OTA from microspheres was investigated according to the paddle method in which 800 mL of release medium was stirred at 37°C, 200 rpm. The acidic and alkaline buffers (acidic release medium for the first 4 h: 0.05 M sodium acetate buffer, pH 4.0; alkaline release medium after the acidic release medium for 18 h: 0.95% ammonium chloride/triethylamine (w/v), pH 9.6) were used as release media, respectively. Microspheres (300 mg) were loaded in a dialysis bag, which was placed in the above release media. To determine the drug release behavior accurately, the remaining microspheres were recovered by centrifugation at the specified points in time: 0, 2, 4, 6, 8, 10, 1215, 17, 18, 20, 22, and 24 h. The amount of OTA remaining in the microspheres was also determined by HPLC as mentioned above. The correlation between the long-term release (the FT method) and the accelerated release was established by plotting different levels of release in days versus hours.[15]

SCANNING ELECTRON MICROSCOPY

The scanning electron microscopy (SEM) studies were conducted on stents before and after exposure to the release media to indicate surface morphological changes. The stents exposed to the release media were rinsed three times with water and patted dry with a paper towel. All stents were examined using a field emission scanning electron microscope (Fei Sirion 200).

DATA ANALYSIS

The rate of drug absorption in the animal experiment was calculated by using the Wagner–Nelson method (Wagner and Nelson, 1964)[16]. In the Wagner–Nelson method, the mean terminal elimination rate constant of OTA (0.408 h^{-1}) was taken from the study of dogs.

Results and Discussion

OPTIMIZATION OF W1 PREPARATION

Lyophilized (freeze-drying) OTA appears as fleecy powder. The bulk of 2.5 g of OTA has the same volume as that of 20 mL of water. It is difficult to dissolve 2.5 g of OTA in 1 mL of water, because its surface has limited contact with water. The highest concentration of W1 prepared using the traditional methods can only reach about 1.0 g/mL, which results in poor viscosity. Based on that, the method of preparation of W1 was optimized. First, some dichloromethane was added to the distillation flask containing 2.5 g of OTA powder to immerse OTA, producing turbid liquid. Then small amount of water (1 mL) was added to the turbid liquid. The mixture was vortexed to evaporate dichloromethane. After evaporation, a highly viscous inner aqueous phase was produced. Dichloromethane was chosen as the additive because of its higher gravity than water and low boiling point.

MICROSPHERE CHARACTERIZATION

All the samples of OTA microspheres prepared using the optimized method and conventional W1/O/W2 method were characterized by analytical techniques. Characterizations are carried out on the basis of the OTA concentration in the inner aqueous phase (C_{W1}), the viscosity of the inner aqueous phase, particle size distribution (D_{50}), loading capacity (LC), initial burst release, and residual solvent (Table I and Fig. 1). Here, M_1 was prepared using the optimized method and M_2 was prepared using the conventional W1/O/W2 method. C_{W1} of M_1 (2.5 g/mL) was five times that of M_2 (0.5 g/mL) whereas LC of M_1 (12.5%) was approximately twice that of M_2 (6.3%) ($P < 0.05$). The initial burst release of M_1 (8.5%) was approximately 14% less than that of M_2 (22.3%) ($P < 0.05$). The particle size of M_1 and M_2 was similar, with D_{50} values of 59.19 and 57.81 μm ($P > 0.05$), respectively. The concentration of residual solvent in M_1 and M_2 was 486.8 and 462.5 ppm ($P > 0.05$), respectively, and both

TABLE I
Characterization of OTA Microspheres Prepared by Various Methods

Property	M1[a]	M2[b]
CW1 (g/mL)[c]	2.5	0.5
Viscosity (mPa·s)[d]	1025 ± 30	5.06 ± 0.26
*D*50 (μm)	59.19 ± 1.53	57.81 ± 1.74
LC (%)	12.5 ± 0.3	6.3 ± 0.4
Initial burst release (%)	8.5 ± 0.2	22.3 ± 0.3
Residual solvent (ppm)	486.8 ± 27.1	462.5 ± 42.2

[a]Microspheres made by the optimized method.
[b]Microspheres made by the primal method.
[c]OTA concentration of W1.
[d]The viscosity of W1; data are represented as mean ($n = 3$) ± *SD*.

were within 600 ppm, as stipulated in International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. The viscosity of inner aqueous phases of M_1 and M_2 was 1025 and 5.06 mPa·s ($P < 0.05$), respectively.

These results demonstrated that the higher concentration and viscosity of the inner aqueous phase are primarily governed by the total amount of drug added. There was no difference between M_1 and M_2 with respect to the particle size or residual solvent. The initial burst release and loading capacity of microspheres were found to be related to the higher concentration of OTA in W1. This may be why M_1 presented the pattern with low initial burst release. For most water-soluble drugs, higher LC means more drug on the surface of the microspheres. A higher concentration gradient was observed between W1 and W2. The easier the diffusion of OTA, the easier the formation of the channels, which in turn increases the absorption of water and burst release. In this study, the target LC was increased by raising the concentration of OTA in W1. However, owing to the high viscosity of W1, the diffusion of OTA was limited and the burst release of OTA on the surface of the microspheres was obstructed. The higher LC and the lower burst release were affected by increasing drug concentration in W1.

The influence of double emulsion time on initial burst releases of M_1 and M_2 was determined, and the results are shown in Fig. 2. During the double emulsion process, initial burst release of M_1 and M_2 samples was determined at 1, 2, 3, 4, and 5 h, as described above. At each point in time, OTA microspheres were collected by centrifugation at 3000 rpm, 4°C for 5 min. The results of initial burst release of both M_1 and M_2 showed that,

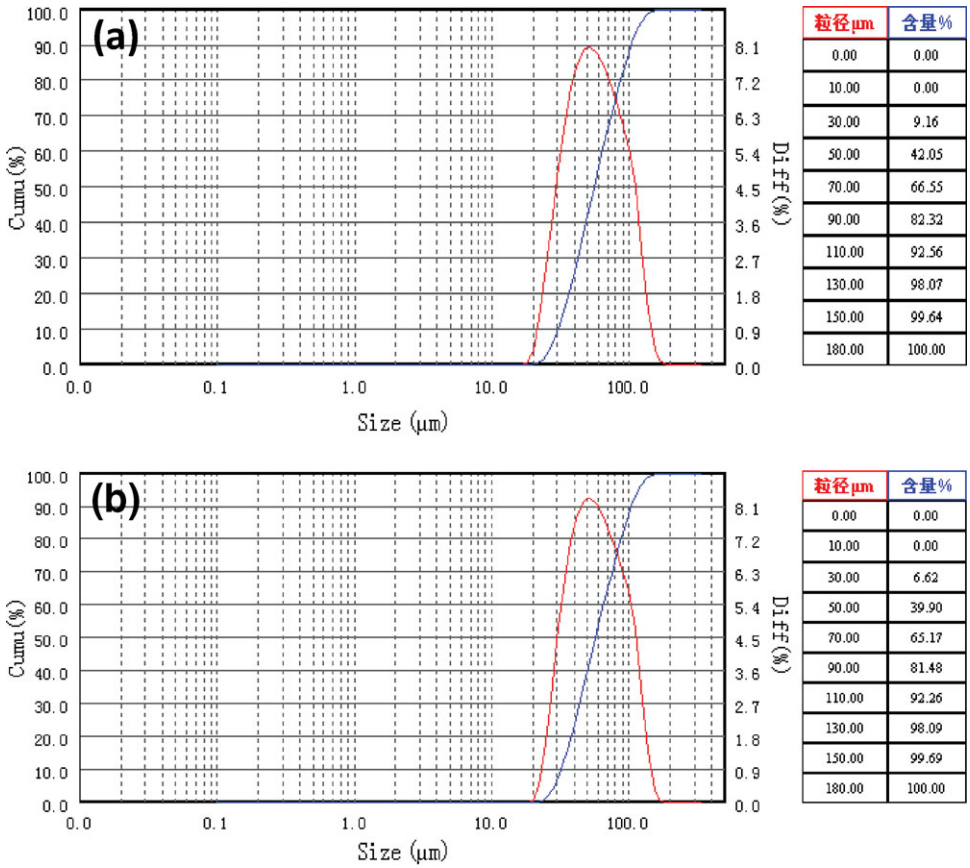

FIGURE 1. (a) The particle size determination of M1; (b) The particle size determination of M2. 粒径μm represents particle size (μm); 含量% represents content percentage (%). The abscissa axis represents particle size and the vertical axis (cumu) represents cumulative, and it means cumulative diffusion of particle size; the vertical axis (diff) represents diffusion, and it means diffusion of partial particle size. The red curve corresponds to the vertical axis of diffusion, and the blue curve corresponds to the vertical axis of cumulative. For example, subfigure 1 a shows that the top point (cumulative 100%) of the blue curve corresponds to the abscissa axis of 180 μm. So the particle sizes of all the microspheres were below 180 μm.

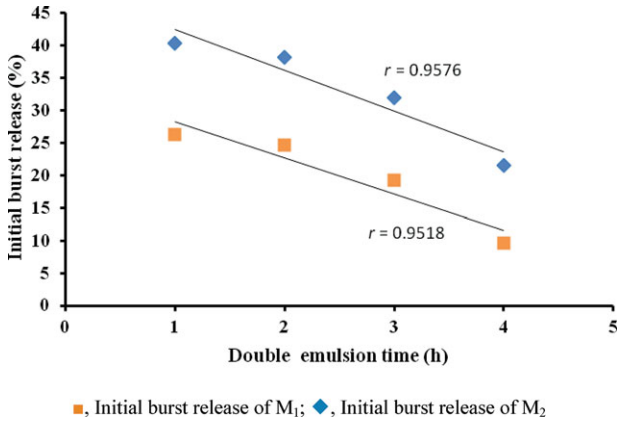

FIGURE 2. Relationship between burst release and double emulsion time. Symbols: ■, Initial burst release of M_1; ♦, initial burst release of M_2

as the duration of double emulsion increased, the amount of initial burst release decreased (M_1 26.31–9.56%; M_2 40.32–23.26%). These results may be attributable to the glassy state of the microspheres at the beginning of the double emulsion process. In this way, the drug was easily released from the microsphere because of its specific structure. As the double emulsion process continued, a solidified layer began to form on the surfaces of microspheres. This decreased drug release from the microspheres.[9] This demonstrated that the extent of initial burst release was correlated with the degree of solidification of the microspheres. The correlation curve of the first 4 h is shown in Fig. 2. When the double emulsion time extended beyond 4 h, the initial burst release did not increase in either M_1 or M_2 (M_1 9.62% vs. 9.56%; M_2 23.56% vs. 23.26%), indicating that the microspheres had solidified. Here, for initial burst release of each time point during double emulsion, the value of M_1 was lower than that of M_2, demonstrating that the optimized W1/O/W2 method for M_1 could be a superior method for producing OTA microspheres.

IN VIVO DRUG RELEASE

For water-soluble drugs, sustained release from microspheres could be divided into three phases according to the mechanism of drug diffusion and polymer degradation. The first phase is the initial burst release. Drug on or near the surface of microspheres is rapidly released by diffusion. The second phase is sustained release. The drug diffuses or migrates from microspheres through multichannel structures formed by the polymer skeleton degradation. The third phase is complete release. The skeleton is damaged with the polymer degradation, and then the drug is released completely.

To investigate the release behaviors of M_1, the in vivo release of M_1 was evaluated in beagle dogs. The plasma drug concentration at different points in time was determined, and the release curve was plotted (Fig. 3). At the first day of in vivo release, initial burst release was clearly visible (4.36 ng/mL). After that, the concentration of drug in plasma decreased to 0.18 ng/mL. This was followed by a gradual increase in the concentration. The amount of in vivo initial burst release determined using the Wagner–Nelson method was 9.6% (while in vitro it was 8.5%).

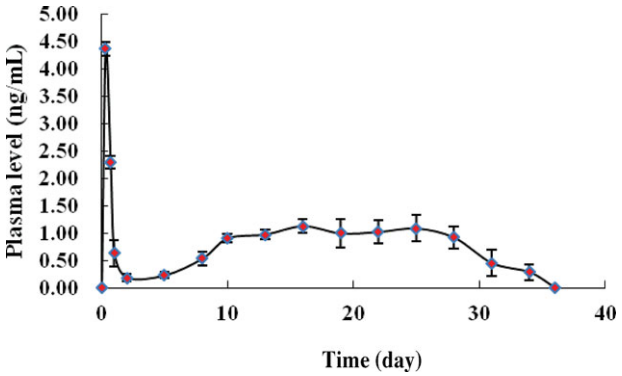

FIGURE 3. Mean plasma concentration–time profiles of OTA after intramuscular administration of OTA microspheres in beagle dogs (mean ± *SD*, $n = 6$).

The initial burst release of OTA microspheres determined by the optimized method was lower than that of the microspheres reported in the literature.[17] The plasma drug concentration was maintained at a stable level of 0.9 ng/mL from 10th to 27th day after drug administration, demonstrating the sustained release of the OTA microspheres. During this period, the release mechanism was diffusion of the drug from the microspheres into surrounding tissues. By day 36, the total amount of released drug had exceeded 85% of the total drug administered. The results show that most of the OTA drugs inside microspheres were released, as expected, indicating possible good therapeutic efficacy.

ESTABLISHMENT OF CORRELATION BETWEEN IN VIVO AND IN VITRO RELEASE

Microspheres prepared using the optimized method (M_1) were used for in vitro release testing with the United States pharmacopeia apparatus 4 (flow-through cell) method. To establish the conditions that would best reflect OTA release behavior in vivo, the drug-release experiments performed using the FT method were conducted under various flow rate conditions. The resultant release profiles of OTA are shown in Fig. 4.

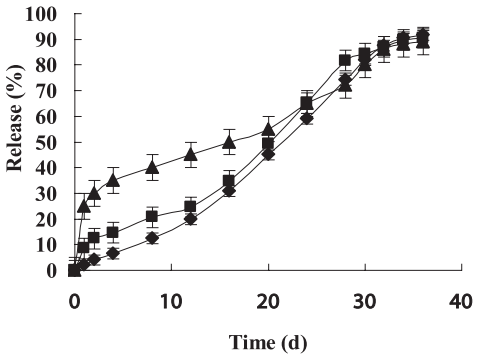

FIGURE 4. Release profiles of OTA from microspheres in buffer solution, pH 4.0. Symbols: ▲, in vitro (the FT method, flow rate 200 ml/min, 37°C); ■, in vitro (the FT method; flow rate 100 mL/min, 37°C); ♦, in vitro (the FT method, flow rate 30 ml/min, 37°C). (mean ± *SD*, $n = 3$).

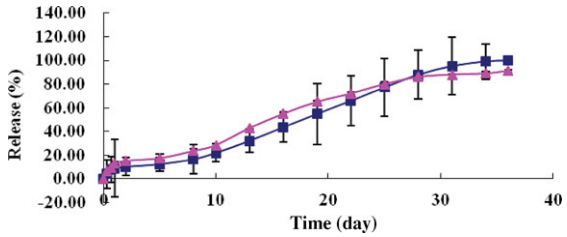

FIGURE 5. In vitro and in vivo release profiles of OTA from microspheres. Symbols: ▲, in vitro release (the FT method); ■, in vivo release (the Wagner–Nelson method) (mean ± *SD*, $n = 3$).

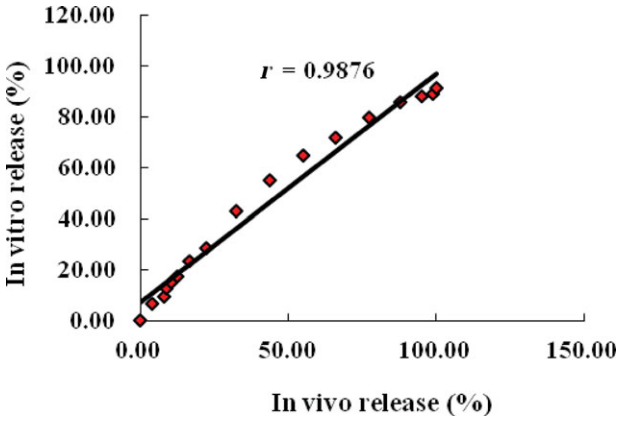

FIGURE 6. Relationship between in vitro and in vivo release of OTA from microspheres.

During the first 30 h of the experiments, the release rate under low flow rate conditions (30 and 100 mL/min) was considerably lower than that under a high flow rate (200 mL/min). During 50–600 h, the release profiles of OTA under various flow rate conditions were in a stable release phase. After that, the OTA remaining inside the microspheres was completely released into the media at the same release rate. To imitate in vivo release profiles, the flow rates of the flow-through cell were adjusted three times after the exposure of microspheres in the release media. The flow rates were as follows: 50.6 mL/h for the first day, 95.5 mL/h for 2–28 days, and 73.1 mL/h after 28 days (Fig. 5). Figure 5 shows the relationship between in vitro and in vivo release profiles. The latter was plotted as a release curve of OTA, which was calculated using the Wagner–Nelson method. The in vitro release rate was faster than the estimated in vivo release during the first 25 days and then slower than the in vivo release after 28 days. We attributed the faster in vitro release rate to the relatively high water solubility of drug and to the bulk aqueous solution surrounding the microspheres during in vitro release. A good correlation was observed between in vivo and in vitro release with a correlation coefficient of 0.9876 (Fig. 6). The results indicated that the in vitro release profiles obtained by the FT method were closely correlated with in vivo release profiles and were suitable for the prediction of in vivo release.

ACCELERATED RELEASE STUDIES BY REGULATION OF pH USING THE PADDLE METHOD

The effects of pH on the degradation of the most biodegradable polymers have been carefully investigated. Some studies reported that the degradation of PLGA can be affected by the pH of the release medium, suggesting that OH^- or H_3O^+ attacks the ester bonds and thus produces intermediate degradation products, followed by further division of the initial products into fractions with lower molecular weights.[8]

First, the first 24 h release profile of OTA microspheres was initial burst (8.5%; Fig. 4) in the long-term release, the bulk of which happened in the first 6 h (5.3%) in the acidic medium (pH 4.0). Thus in the accelerated method, the first 6 h release was used to represent the first 24 h initial release of the long-term release and the pH of release medium was also 4.0 in the first 6 h. Figure 7a shows that during the initial burst release the surface of the microspheres was porous, so the dominant release mechanism was concluded to be drug diffusion through the pores. Figure 8 shows that the pH of release medium rose a little in the first 6 h of the accelerated release procedure. This was attributed to the occupation of H^+ by the acetate of OTA. Figure 7b shows that the pores of microspheres surface disappeared gradually and the surface of microspheres became smooth during the burst release, as also observed by Wang et al.[18]

Second, some researchers reported that as the pH of the release medium increased the molecular weight loss of polymers accelerated.[19] This may be because ester bonds are sensitive to alkalis. On the one hand, the alkaline condition could promote breakage of ester bonds to form intermediate degradation products. On the other hand, the excess of alkali in the release medium

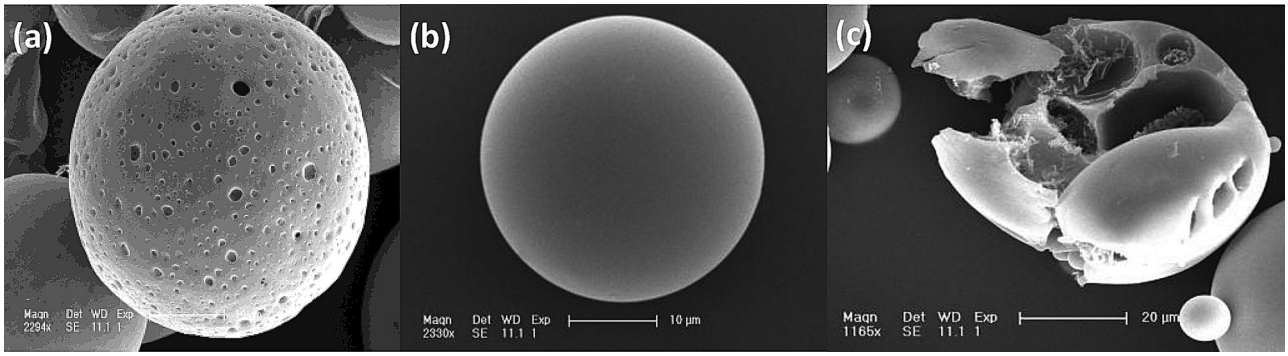

FIGURE 7. External morphology of OTA-loaded PLGA microspheres of M1 by SEM. (a) Exposure to the acidic release medium; (b) exposure to the alkaline release medium; (c) the last phase of accelerated release.

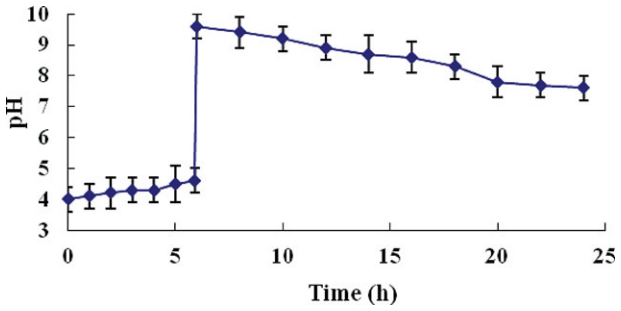

FIGURE 8. pH of the accelerated release medium.

could neutralize the acidic degradation products to form homologous salts, which made the hydrolysis reaction out of balance and promoted the reaction to end.[18] These facts suggested that the alkaline condition could promote degradation of PLGA rapidly and accelerate OTA release. Thus, after initial burst release (6 h), the acidic release medium (pH 4.0) was replaced with alkaline medium (pH 9.6) in the accelerated release method. Figure 9 shows that OTA was released rapidly from microspheres in the alkaline medium (pH 9.6) and the tendency was similar to the long-term release. As observed in Fig. 7, the microspheres size became smaller and smaller with the degradation of the outer layer of PLGA. Figure 8 shows that during the alkaline release procedure the pH of release medium decreased slowly as the accelerated release continued. This may be attributed to the fact that the excess alkali in the release medium could neutralize the acidic degradation products to form homologous salts.[18] During this process, the bulk of OTA (85%) was released into the release medium from the microspheres (Fig. 9).

Finally, as the intermediate degradation products continued to degrade, the molecular weights of the degradation products became smaller and smaller until the degradation products could dissolve in the release medium and OTA was released completely. As shown in Fig. 7c, the skeleton of PLGA was damaged with the polymer degradation and the microspheres were broken into slivers. When the pH fell below 8.0, the release of OTA gradually slowed down (Figs. 8 and 9). Under neutral condition (pH < 8.0), the release mechanism was similar to that at pH 4.0 (long-term release).[20] The last phase of accelerated release reflected the release degree of OTA from microspheres (>90%).

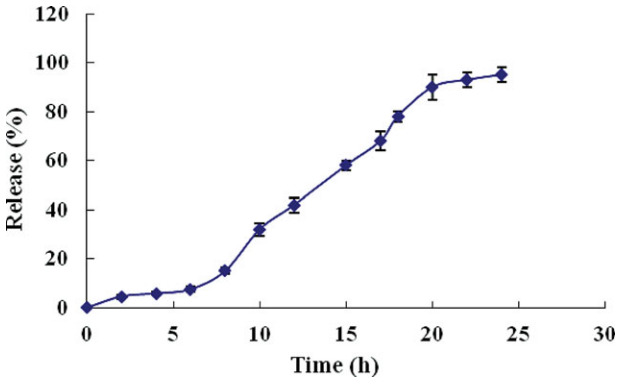

FIGURE 9. Accelerated release profiles of OTA from microspheres (the paddle method; pH 4.0–9.6).

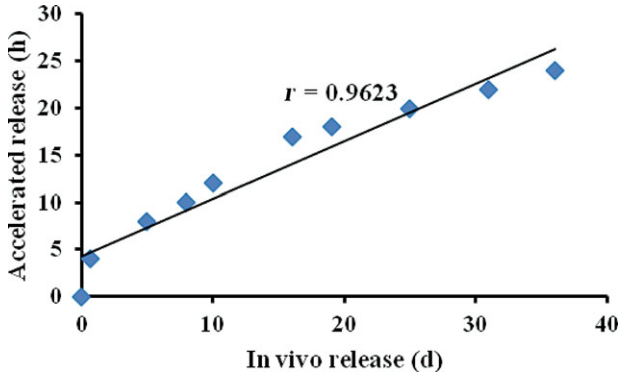

FIGURE 10. Correlation between in vivo release and accelerated release of OTA from microspheres. The accumulated release amount of each time point was approximately 0%, 6%, 15%, 32%, 42%, 69%, 75%, 86%, 93%, and 95% in turn.

Overall, through the regulation of pH, the rate of polymer hydrolysis reaction was changed in a controlled way. In the three release phases, pH was used to influence OTA diffusion from the surface of microspheres, the degradation rate of PLGA (through promoting breakage of ester bonds and neutralizing the acidic degradation products), and dissolving of degradation products, respectively. As shown in Figs. 5 and 9, the profiles of accelerated release coincided with those of in vivo and long-term release, which involved three phases.

CORRELATION BETWEEN IN VIVO RELEASE AND ACCELERATED RELEASE

The correlation between in vivo release and accelerated release was established in several studies by plotting different levels of release in days versus hours, as shown in Fig. 10. The correlation coefficient was 0.9623, indicating that accelerated release profiles were closely correlated with that of in vivo. In this way, an accelerated method (24 h) could be used to predict in vivo drug release from microspheres.

Conclusions

An optimized method with a low burst release and a high drug loading capacity was developed by increasing the concentration of OTA in W1. During the double emulsion process, the double emulsion time was closely correlated with OTA burst release. By modifying the flow rate of the FT cell, a FT method was developed. Using this method, good in vivo and in vitro correlations were observed. This long-term release method could be used to predict in vivo rates of release. On the basis of these findings, an accelerated release method was developed. This method involved modifying the pH of the release medium, which made the PLGA skeleton degrade rapidly. The accelerated release profiles were closely correlated with in vivo profiles. In this way, an accelerated release method was used to predict the rate of in vivo drug release from microspheres.

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