

Exenatide-loaded inside-porous poly(lactic-co-glycolic acid) microspheres as a long-acting drug delivery system with improved release characteristics

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ABSTRACT

The glucagon-like peptide-1 receptor agonist exenatide (EXT) is an effective treatment for type 2 diabetes. However, this peptide has a short biological half-life and the delayed release characteristic of current formulations limit its clinical application. Herein, we prepared EXT-loaded inside-porous poly(d,l-lactic-co-glycolic acid) (PLGA) microspheres with outside layers (EXT-PMS) using a $W_1/O/W_2$ emulsion method with a microfluidic technique and its fabrication and formulation conditions were systematically investigated. *In vitro* dissolution experiments showed that the PLGA concentration, proportion of drug and oil phase, and the number and size of pores strongly affected the release behaviors of EXT-PMS. *In vitro*, the optimized EXT-PMS with large internal pores exhibited rapid and stable release without a lag phase. In a rat model, subcutaneous administration of the product yielded plasma concentrations of EXT that was sustained for 30 days with low burst and no delayed-release effect. The preparation of inside-porous microspheres is lighting up the development of long-acting drug delivery systems for other drugs with favorable release characteristics.

ARTICLE HISTORY

Received 29 September 2020
Revised 5 November 2020
Accepted 9 November 2020

KEYWORDS

Exenatide; inside-porous microspheres; long-acting; release behavior; type 2 diabetes

1. Introduction

Type 2 or noninsulin-dependent diabetes accounts for 90–95% of all patients with diabetes and is a chronic disease with substantial morbidity and mortality. Patients with type 2 diabetes have a life expectancy that is reduced by 10 years and the disease is estimated to affect about 550 million people worldwide by 2030 (Johnson et al., 2009; Chatterjee et al., 2017; Rowley et al., 2017). Exenatide (EXT), the synthetic exendin-4, is an outstanding incretin mimetic that is approved by the Food and Drug Administration and the European Medicines Agency as the drug for type 2 diabetes (Deacon, 2004; Davidson et al., 2005; Song et al., 2019). It has multiple glucose regulatory therapeutic effects, such as enhancement of insulin secretion, reduction of food intake, and deceleration of gastric emptying (Li et al., 2015; Song et al., 2019). However, EXT only has a short plasma half-life of 2.4 h and an action time of about 8 h, thus the long-acting EXT formulations are of prime importance (DeYoung et al., 2011; Cai et al., 2013).


Poly(d,l-lactic-co-glycolic acid) (PLGA) microspheres are an outstanding material for implanting long-acting drug release characteristics safely, reliably, and efficiently (Danhier et al., 2012; Wu et al., 2017; Qi et al., 2019; Zhai et al., 2020). The only long-acting product of EXT on the market (Bydureon®,

once-weekly subcutaneous injection) is produced by using PLGA microspheres (Scheen, 2014). However, the side effects remained as the pharmacokinetic profile shows a delayed-release or lag phase (Nikou et al., 2005). After an initial burst in the first 2 days after administration of Bydureon®, the drug is not released again for 2 weeks and it then takes up to 7 weeks for complete drug release (Cai et al., 2013). These 2 weeks lag phase leads to poor pharmacokinetics and diminished efficacy which seriously limits its clinical applications. An optimal long-acting drug delivery system should have a reasonable release profile which exhibits a low initial release followed by a continuous release phase (Ruan et al., 2018; Wang et al., 2019b). New strategies are needed to improve the sustained-release characteristics of current EXT-loaded PLGA microspheres.

For water-soluble peptides loaded PLGA microspheres such as EXT (Li et al., 2013), water in oil (W/O) emulsions and water water-in-oil-in-water ($W_1/O/W_2$) emulsions are the most used preparation methods (Qi et al., 2014). The $W_1/O/W_2$ emulsion method has many advantages over the simple emulsion method for the microsphere preparation, such as entrapment of hydrophilic peptides, prevention of drug degradation, controllable rate, enhanced encapsulation efficiency (EE), and drug loading capacity (Mao et al., 2007; Mundargi et al., 2007; Wang et al., 2019a). Thus, $W_1/O/W_2$ method has

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 Supplemental data for this article can be accessed [here](#).

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been widely used to encapsulate hydrophilic protein and peptides in PLGA microspheres. Drug diffusion and polymer erosion are the critical factors in controlling drug release from PLGA microspheres (Ruan et al., 2018). Generally speaking, the initial phase of drug release is controlled by drug diffusion from the surface and the later phase (the lag and second drug release phase) occurs through erosion (Zolnik & Burgess, 2008; Gu et al., 2016). The peptide or protein are usually water soluble, which will lead to aggregates near the surface of microspheres that are prepared via $W_1/O/W_2$ emulsions (Leo et al., 1998). This solubility enables the rapid diffusion of drugs from the surface of microspheres in the initial phase. In the later phase, erosion leads to the formation of pores and drugs encapsulated inside the PLGA matrix begin to slowly diffuse through water-filled channels as polymer degradation (Fredenberg et al., 2011). Thus, this 'outside-in' pattern of porosity progression in the erosion process explains the lag phase observed in PLGA microspheres. The length of the lag phase depends on the time required for the entire microsphere to become porous. The occurrence of drug release after the lag phase appears to be the result of swelling of the microsphere following pore formation (Gasmi et al., 2016; Gu et al., 2016; Wang et al., 2019b). Therefore, porous microspheres minimize the lag phase by skipping the initial erosion phase which forming pores. However, incorporated drugs released rapidly during the initial phase caused by the highly porous of these microspheres (Kim et al., 2006).

Inner porous microspheres with an outer thin layer are expected to achieve the desirable release behavior of drugs, because the outside-layer blocks out routes of drug diffusion through pores in the initial phase, thereby reducing burst release. Besides, inside-porous microspheres owned larger porous surface and inter-sphere space rooms than nonporous microspheres that allow them to incorporate more drugs (Kim et al., 2012; Wei et al., 2016; Ni et al., 2017; Yang et al., 2019). Kim et al. (2011a,b) fabricated porous PLGA microspheres for

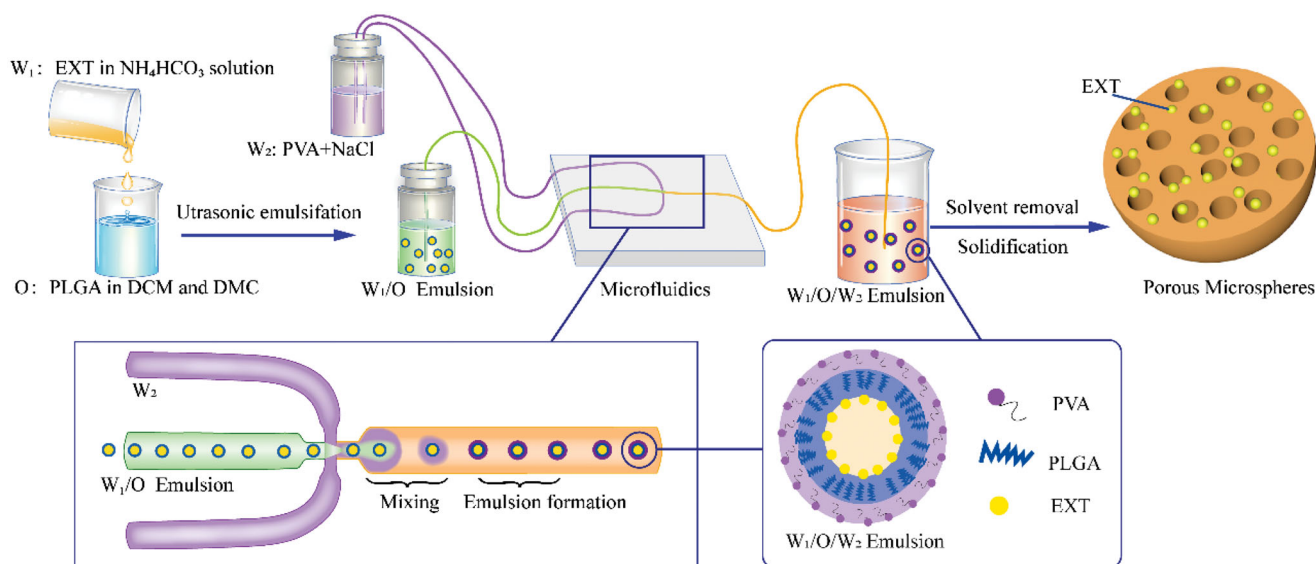
pulmonary delivery of EXT, they used palmitoyl-acylated EXT or albumin coated surface to reduce burst release caused by open-pores surfaces of porous microspheres.

In this study, we developed a microfluidic process for the preparation of EXT-loaded inside-porous PLGA microspheres with thin outside-layers based on $W_1/O/W_2$ emulsions (Scheme 1). We studied the release characteristics of EXT-PMS with various pore sizes which demonstrated that porosity is a determining factor of the later stage drug release. In addition, we investigated the potential influence of PLGA concentration, EXT concentration, and ratio of oil-to-water phase on the *in vitro* dissolution curves of EXT-PMS. *In vivo* pharmacokinetics studies in rats of EXT-PMS showed stable and sustained release of EXT with no delayed release and low burst release. With this approach, we seek to provide a simple and effective method for improving an existing long-acting dosage form that may potentially be used with other drugs.

2. Material and methods

2.1. Chemical materials

Exenatide was purchased from Jianyuan Pharmaceutical Technology Co., Ltd. (Shenzhen China). Poly(vinyl alcohol) 210 (PVA), sodium azide, and sodium dodecyl sulfate (SDS) were obtained from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). PLGA (85/15) (lactic acid (LA)/glycolic acid (GA) = 85/15, Mw = 50,000), PLGA (75/25) (LA/GA = 75/25, Mw = 50,000), and PLGA (50/50) (LA/GA = 50/50, Mw = 50,000) were all purchased from Shandong Academy of Pharmaceutical Sciences (Pilot Plant; Jinan, China). Micro-BCA kit was supplied by Shenggong Biological Engineering Co. Ltd. (Shanghai, China). Rhodamine 123 (Rh123) was supplied by Shanghai Yuanye Bio-technology Co., Ltd. (Shanghai, China). Dichloromethane (DCM), NH_4HCO_3 , and dimethyl carbonate (DMC) were all purchased from Damao Chemical Reagent Factory (Tianjin, China). All chemicals and reagents were analytical reagent grade.



Scheme 1. Schematic of the synthetic process for the EXT-loaded inside-porous PLGA microspheres.

2.2. Preparation of EXT-loaded PLGA microspheres

A water-in-oil-in-water ($W_1/O/W_2$) emulsion method was used to prepare EXT-PMS. The oil phase was a PLGA/DCM solution that was prepared by dissolving PLGA in DCM. The water phase 1 was prepared by dissolution of EXT and NH_4HCO_3 in an aqueous solution. The two phases were mixed and emulsified by sonication at 0 °C to form the W_1/O emulsion. Then, W_1/O emulsion internal phase was mixed with the external phase or water phase 2 containing PVA and NH_4HCO_3 in an aqueous solution through the microfluidic cross chip. PLGA microspheres were solidified after stirring to volatilize the organic solvent. The microspheres were centrifuged at 8000 r/min and harvested, thoroughly washed with distilled water and freeze-dried.

EXT-MS were also prepared using a $W_1/O/W_2$ emulsion method with a microfluidic technique. Specifically, the water phase 1 was prepared by dissolution of EXT in water. The oil phase was prepared by dissolving PLGA in DCM. The water phase 1 and oil phase were mixed and emulsified by sonication to form the W_1/O emulsion. Then, W_1/O emulsions were mixed with the water phase 2 which containing PVA through the microfluidic cross chip to form $W_1/O/W_2$ emulsions. PLGA microspheres were solidified after stirring to volatilize the organic solvent. The microspheres were centrifuged at 8000 r/min and harvested, thoroughly washed with distilled water and freeze-dried.

We used a single-factor approach to optimize the composition and synthesis process of the microspheres. The polymer type, PLGA concentration, drug concentration, W_1 to O ratio, and porogen concentration were each tested to obtain the optimal microsphere formulation. The drug-loading capacity (DLC), EE, and *in vitro* cumulative release curve were selected as the inspection index.

2.3. Size distribution measurement

The average diameter and Span value for the microspheres were calculated using data measured from 200 particles observed using a CX22 optical microscope (Olympus, Tokyo, Japan). The particle size distribution was evaluated by the Span value, which was calculated using the following equation:

$$\text{Span} = (D_{90} - D_{10})/D_{50}, \quad (1)$$

where D_{90} , D_{10} , and D_{50} are the average particle diameters of particles accounting for 90, 10, and 50% of all particles, respectively.

2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed using a G5 ProX-SE microscope (Carl Zeiss, Oberkochen, Germany). Microspheres with dried using infrared light, and then dry powders of microspheres were dispersed on carbon tape and coated with gold.

2.5. Confocal laser scanning microscopy

Features of the microspheres were observed using confocal laser scanning microscopy (CLSM) (Carl Zeiss, Oberkochen, Germany). Before observation, microspheres were suspended in water and fixed on a glass slide.

2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was carried out by using a DSC analyzer (PerkinElmer). Briefly, 10 mg samples were placed in an aluminum pan and heated from 20 °C to 200 °C at a rate 10 °C/min.

2.7. Encapsulated efficiency and drug loading

Ten milligrams of EXT-PMS or EXT-MS microspheres were added to 1 mL of 0.1 M NaOH solution (containing 5% SDS) and vibrated (100 r/min, 37 °C) for 24 h to dissolve EXT. The supernatant fluid (containing EXT) was obtained by centrifugation (8000 rpm, 10 min) and the EXT concentration was measured by micro-BCA kit. The linear range was from 2 to 40 $\mu\text{g/mL}$ ($r = 0.9997$). Encapsulated efficiency and drug loading were calculated with the following equations:

$$\begin{aligned} \text{Encapsulated efficiency (\%)} \\ &= \frac{\text{Drug loading}}{\text{Theoretical drug loading}} \times 100\% \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Drug loading (\%)} \\ &= \frac{\text{Mass of drug in microspheres}}{\text{Mass of microspheres}} \\ &\quad \times 100\% \end{aligned} \quad (3)$$

2.8. In vitro dissolution testing

Twenty milligrams of EXT-PMS microspheres were dispersed in 500 mL dissolution medium composed of 10 mM phosphate-buffered saline buffer and 0.01% sodium azide at pH 7.4. The dissolution medium was shaken at a rate of 100 r/min (37 °C \pm 0.5 °C). One mL sample was collected at 1, 3, 5, 7, 14, 21, 28, and 35 days separately. At each collection, 1 mL blank dissolution medium was added back to maintain the volume at 500 mL.

2.9. Pharmacokinetic analysis

Male Sprague-Dawley (SD) rats weighing 200 \pm 20 g were purchased from the Experimental Animal Center of Southern Medical University (Guangzhou, China) and were housed in the Experimental Animal Center of Guangzhou University of Chinese Medicine under room temperature, with moderate humidity 50–60% humidity and a 12-h day/night cycle. All animal experimental procedures were approved by the Animal Ethics Committee at the Experimental Animal Center of Guangzhou University of Chinese Medicine. Rats were kept under fasting conditions with free access to water for 12 h prior to initiation of the experiment.

Nine rats were randomly divided into two groups ($n=3$). A group was administered subcutaneous injections of either EXT-PMS suspension (100 mg/kg) or EXT-MS suspension (100 mg/kg). Blood samples were collected at 0.5, 1, 2, 4, 6, 8 h, and 1, 4, 7, 10, 14, 18, 22, 26, 30, 34, 38 days after administration. Plasma samples were obtained by centrifuging at $3000 \times g$ for 15 min at $2-8^{\circ}\text{C}$ and were stored at -20°C before analysis. All pharmacokinetic parameters were analyzed by DAS3.2 software (Chinese Pharmacological Society, China). Plasma concentration at each time point was reported as the mean \pm standard deviation.

3. Results and discussion

We investigated the effects of polymer type, PLGA concentration, drug concentration, W_1 to O ratio, and porogen concentration on the DLC, EE, and *in vitro* dissolution curve of the EXT-PMS. These factors are thought to influence the uniformity and ideal *in vivo* release characteristics.

3.1. Effect of polymer

PLGA is a copolymer of LA and GA. As a consequence of the presence of methyl side groups in LA, PLGA with a higher content of GA are more hydrophilic, absorbs more water and subsequently degrades more quickly and thus may affect the release characteristic of EXT (Zheng & Liang, 2010; Zhang et al., 2016). Thus, to find a suitable polymer, we investigated the influence of PLGA composition on the properties of EXT-PMS. PLGA (50/50), PLGA (75/25), and PLGA (85/15) which consists of 50, 25, and 15% of GA, respectively, were used to synthesis EXT-PMS. Microspheres prepared by PLGA (50/50) showed slightly higher encapsulation efficiency (EE) and DLC than its 85/15 and 75/25 counterparts (Supplementary Figure S1). This difference may due to the enhanced hydrophilic interaction between PLGA (50/50) and the water-soluble EXT, compared to the other two polymers (Gentile et al., 2014). There is no obvious difference of *in vitro* dissolution curve between these microspheres prepared by different polymers (Supplementary Figure S2).

According to previous studies, PLGA concentration played a crucial role in EE and DLC properties. To further improve EE and DLC of microspheres, we increased the concentration of PLGA (50/50) from 20 to 100 mg/mL. As shown in Figure 1(A), the higher polymer concentration clearly enhanced EE from 73 to 89% and had negligible effects on DLC. This may be due to the low polymer concentration easily causing leakage of EXT (Qi et al., 2013). Meanwhile, the higher PLGA concentration had a significantly lower burst release and continued having drug available for release on day 35 (Figure 1(B)). These improvements are due to the increased density of the microspheres caused by higher PLGA concentration, which may help to incorporate more drug and slow down the degradation (Tinsley-Bown et al., 2000).

3.2. Effect of the proportion of drug and oil phase

To improve the drug content of porous microspheres, prolong the sustained-release period and reduce administration times, we increased the mass proportion of EXT in the preparation process of EXT-PMS. From Figure 1(A,B), we concluded that 100 mg/mL of PLGA is the optimal concentration. Under this optimized PLGA concentration, we investigated the effects of mass ratio of EXT to PLGA on DLC, EE, and *in vitro* dissolution curve of EXT-PMS. For mass ratio of EXT to PLGA under 1:20, 1:15, 1:10, 1:5, and 1:2.5, the corresponding EXT concentration is 5, 6.78, 10, 20, and 40 mg/mL, respectively. As shown in Figure 1(C), the DLC was three times higher and EE was 50% lower when the mass ratio of EXT to PLGA (EXT/PLGA) increased from 1:20 to 1:2.5 gradually. For *in vitro* release curve (Figure 1(D)), EXT-PMS prepared with the ratio of 1:2.5 released almost 90% EXT on the first day, which exhibited extremely high burst release than that of 1:20. When the ratio of EXT/PLGA was maintained between at 1:15 (corresponding to 6.78 mg/mL of EXT), the resulting EXT-PMS did not display any burst release, as shown by the "S-shaped" dissolution curve. This may be explained by the increased osmotic pressure in W_1 phase caused by the addition of large amounts of drugs. According to a previous study, the increased osmotic pressure between W_1 and W_2 could prevent the influx of the W_1 phase into the W_2 phase and reduced the formation of water channels which could have led to a high initial burst (Yeo & Park, 2004).

Furthermore, the proportion of oil phase also could change the density of microspheres and affecting the release behavior of drugs, so the O/W_1 volume ratio was investigated in the system. Compared with higher ratios of oil to W_1 phase, the 10:1 and 10:2 ratio of O/W_1 group exhibited a slightly higher DLC and EE. These two groups also released less than 20% of EXT on the first day and there was no delayed drug release over the next 4 weeks (Figure 1(E,F)). However, once the proportion of W_1 phase is higher than 10:3, almost half of EXT was released in the initial release period. The reason is that the increased W_1 phase ratio and reduced O phase ratio resulting in a relatively loose structure inside the microsphere and eventually a higher burst release.

We selected an optimal formulation with a 1:15 ratio of EXT/PLGA (50/50) and 10:1 ratio of O/W_1 . Results of subsequent analyses described below have these characteristics unless otherwise specified.

3.3. Effect of the porogen concentration

As mentioned earlier, the formation of pores in nonporous microspheres mainly controls the time of the lag phase. In porous microspheres, the size and number of pores are affected by the porogen concentration (Bae et al., 2009). NH_4HCO_3 is a commonly used porogen. NH_4HCO_3 breaks down during the emulsification process into NH_3 , CO_2 , and O_2 and as these gases volatilize, the space they occupied form pores in the drying and solidification process (Thanh et al., 2016). Thus, we carefully investigated how the

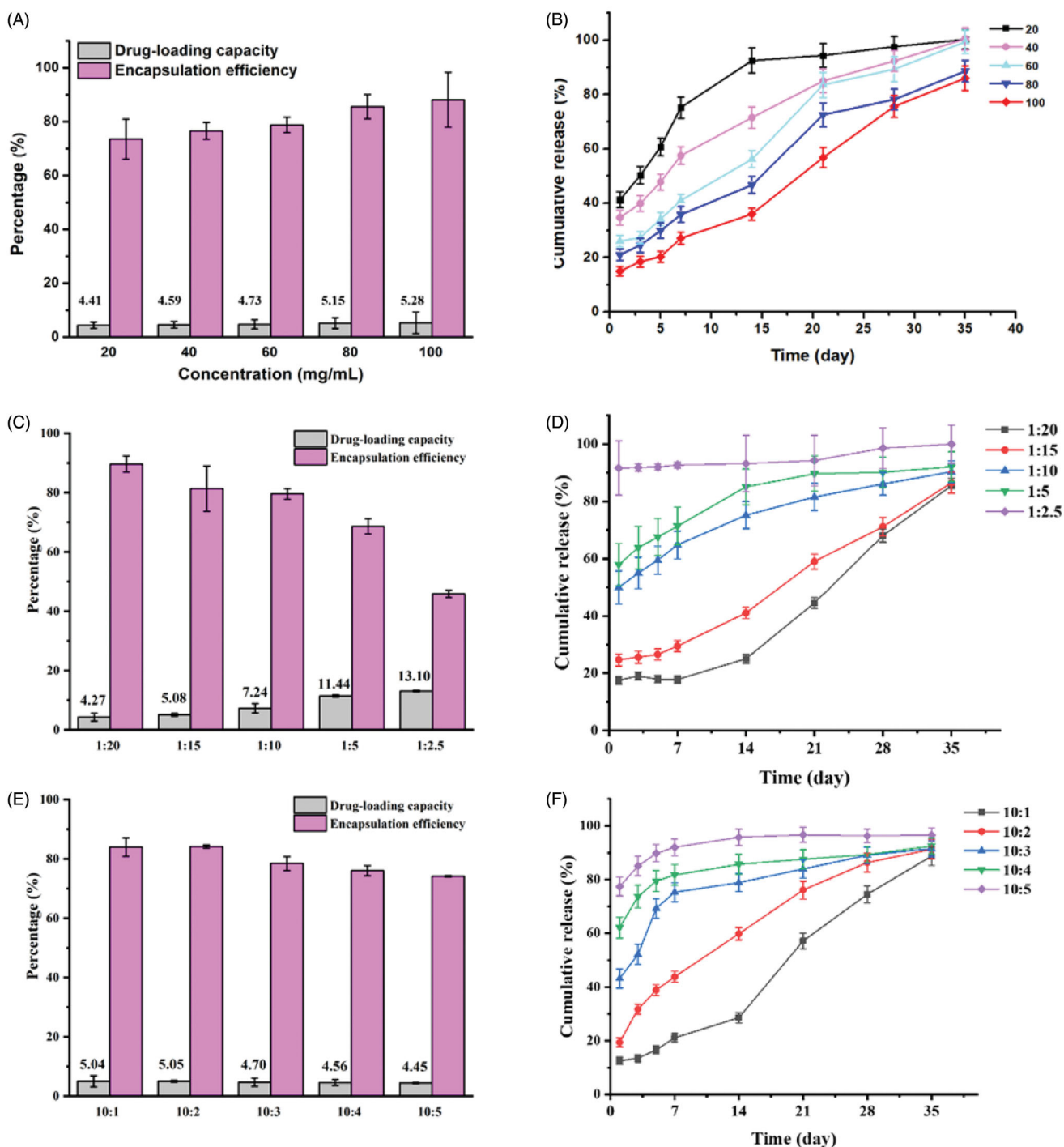


Figure 1. (A) Encapsulation efficiency and drug-loading capacity of EXT-PMS prepared by using different PLGA (50/50) concentrations. (B) Cumulative release of EXT from EXT-PMS prepared by using different PLGA (50/50) concentration (mg/mL). (C) Drug-loading capacity and encapsulation efficiency of EXT-PMS prepared by using different mass ratio of EXT to PLGA. (D) Cumulative release of EXT from EXT-PMS prepared by using different mass ratio of EXT to PLGA. (E) Drug-loading capacity and encapsulation efficiency of EXT-PMS prepared by using different volume ratio of oil to W_1 phase. (F) Cumulative release of EXT from EXT-PMS prepared by using different volume ratio of oil to W_1 phase.

concentration of NH_4NCO_3 affecting the characters of porous PLGA microspheres and release behaviors of drugs. As the proportion of NH_4HCO_3 increased to 10%, both the DLC and EE were reduced by about 21% compared with EXT-PMS using 2% (Figure 2(A)). Notably, EXT-PMS produced using 2% porogens continued exhibiting delayed-release characteristics (Figure 2(F)). However, EXT-PMS using 8 and 10% NH_4HCO_3 showed about 40% release on the first day. In particular, EXT-PMS produced with 6% NH_4HCO_3 displayed steady

release for 35 days and low burst release on the first day. These results strongly demonstrated that variations in the porogen concentration can accelerate the release of EXT or other drugs in the initial phase. The difference in drug release between these EXT-PMS produced from different NH_4HCO_3 concentrations can be explained by the different shortened diffusion pathways attributed by large pore sizes inside of the microspheres. Large pores which were caused by NH_4HCO_3 could shorten diffusion paths both in the initial

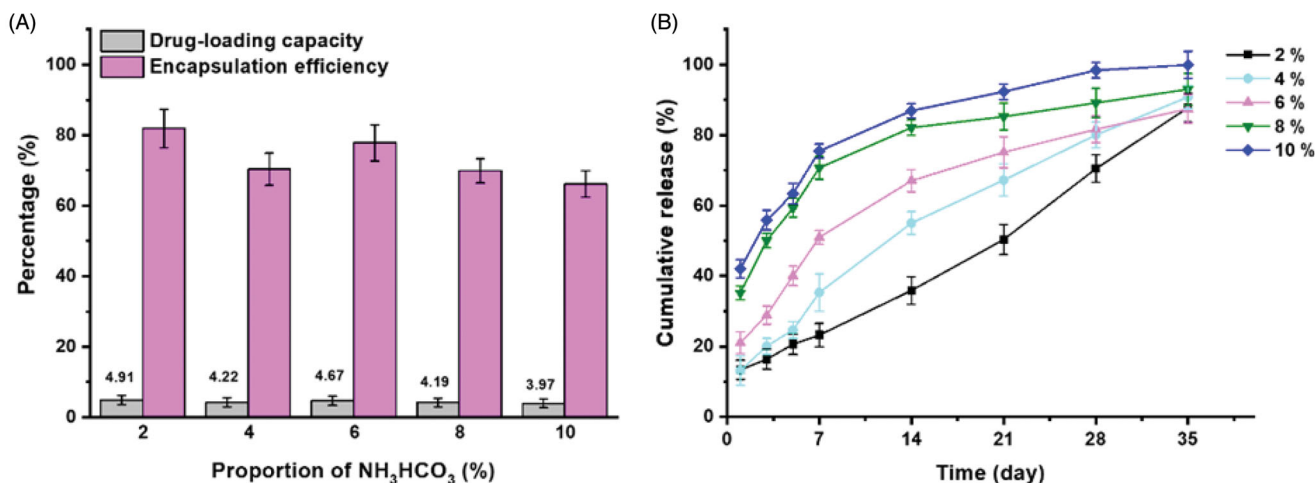


Figure 2. (A) Drug-loading capacity and encapsulation efficiency of EXT-PMS prepared by using different proportion of NH₄HCO₃ (%). (B) Cumulative release of EXT from EXT-PMS prepared by using different proportion of NH₄HCO₃ (%).

Table 1. The optimized formulation of EXT-PMS.

Polymer type (LA/GA)	Polymer concentration (mg/mL)	Mass ratio of drug to polymer	Volume ratio of oil to W ₁ phase	Proportion of NH ₄ HCO ₃ (%)
PLGA (50:50)	100	1:15	10:2	6

GA: glycolic acid; LA: lactic acid.

phase and later phase, thus EXT-PMS using 8 and 10% NH₄HCO₃ exhibited high initial release. After the initial release, the time needed to be released any drugs trapped inside the microspheres are also determined by the length of these pathways. EXT-PMS prepared with low concentration NH₄HCO₃ of 2% was not significantly improved the lag phase after the initial phase. As a result, EXT-PMS produced from 6% NH₄HCO₃ released EXT stable in the initial phase and more rapidly in the later phase.

Based on the results of our optimization tests, we concluded that 100 mg/mL of PLGA with LA/GA ratio of 50/50, a 1:15 ratio of EXT to PLGA, a 10:2 ratio of O/W₁ and 6% concentration of NH₄HCO₃ porogen, is the preferred formulations that can produce EXT-PMS with desirable *in vitro* dissolution characteristics (Table 1).

3.4. Characterization of microspheres

The crystallization information of EXT in microspheres was determined by DSC analysis (Figure 3(A)). Pure EXT had a broad endothermic peak at 68 °C and a narrow endothermic peak at 145 °C, which is consistent with the literature reports. PLGA (50/50) did not show any obvious absorption peak. EXT-PMS also showed an endothermic peak at 145 °C. These data proved the existence of EXT in microspheres. To certify the porous structure of EXT-PMS, the distribution of EXT within porous microspheres was observed by CLSM. Fluorescent EXT-loaded inside-porous PLGA microspheres (F-EXT-PMS) were obtained by encapsulated Rh123 with the same protocol of EXT-PMS. As illustrated in Figure 3(B), the green fluorescence only appeared at the surface of microspheres forming a thin circle outside, but not internally. In addition, the surface and inner morphologies of EXT-PMS were observed by SEM. Images revealed the smooth surface

and internal network structure. With statistical calculation, the average diameter of EXT-PMS was 85 μm. These microspheres exhibited 95 pores and a porosity of 1.5–10 μm with the median of 6 μm (Figure 3(C,D)). These data demonstrated that the obtained EXT-PMS has numerous pores inside and smooth surfaces.

3.5. Pharmacokinetics study

Inspired by the superior *in vitro* dissolution results of EXT-PMS, we further carried out pharmacokinetic experiments of them. Twelve SD rats were divided into two groups and were subcutaneously injected with either EXT-PMS or EXT-MS. The EXT plasma concentration after administration is shown in Figure 4 and a summary of the main pharmacokinetics parameters is provided in Table 2. EXT plasma concentration of rats treated with EXT-PMS showed similar burst release level with EXT-MS. Especially, EXT could be continuous release without a lag phase in 14 days. However, EXT-MS treated group showed obviously lag phase that only released EXT with effective concentration after day 15. In addition, The AUC₀₋₁ and AUC₀₋₃₈ values of EXT-PMS were 27.14 and 293.4 ng/mL·h. For EXT-MS, the AUC₀₋₁ and AUC₀₋₃₈ values were 23.23 and 326.17 ng/mL·h, respectively. Furthermore, we performed a significant test for data in Table 2. As shown, the parameters of C_{max} and T_{max} showed significant difference (*p* < 0.01) and the AUC₀₋₃₈ and AUC₀₋₁ showed no obvious difference. The significant differences between T_{max} and C_{max}, and plasma concentration–time curves of EXT-PMS and EXT-MS both demonstrated the rapid and sustained release property of EXT-PMS without delayed-release *in vivo*. In addition, the no significant difference between AUC₀₋₃₈ and AUC₀₋₁ illustrated that the bioavailability of EXT-PMS is as good as EXT-MS. These data demonstrated that the

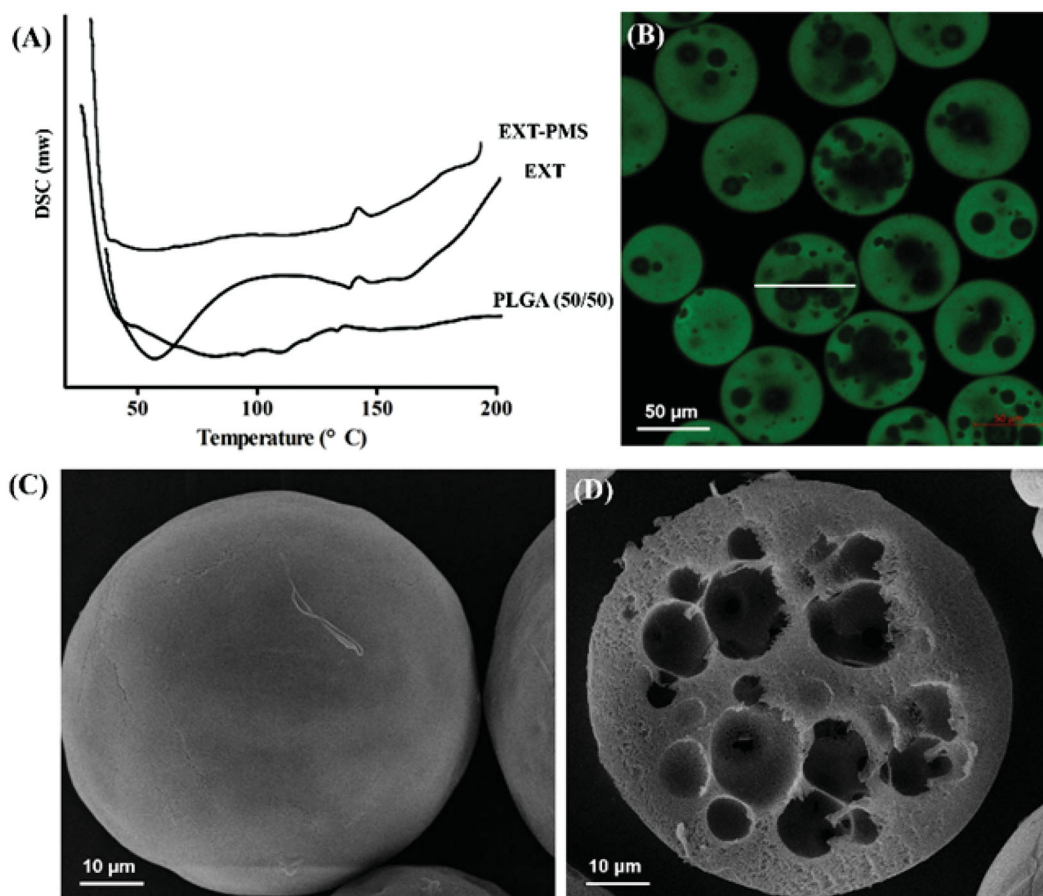


Figure 3. (A) DSC thermograms of EXT-PMS, PLGA (50/50) and EXT. (B) CLSM images of F-EXT-PMS. (C) SEM images of surface of EXT-PMS. (D) SEM images of internal structure of EXT-PMS.

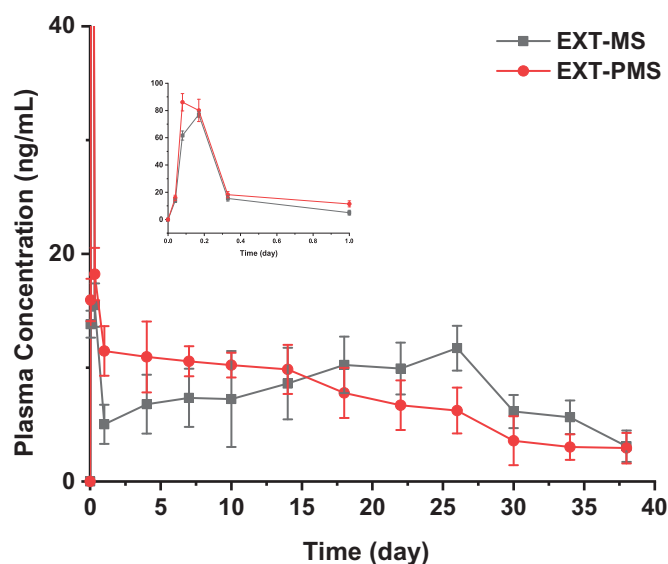


Figure 4. Plasma concentration–time curves of EXT-MS or EXT-PMS following subcutaneous administration in SD rats.

prepared EXT-PMS showed improved release characteristics without a lag phase, compared to EXT-MS. Long-term therapeutic effects were also observed which are consistent with *in vitro* result of EXT-PMS.

Generally, the effect of a drug is determined directly by the drug concentration at the site of action (more

Table 2. The pharmacokinetic parameters of EXT-PMS.

Parameter	EXT-PMS	EXT-MS
AUC _{0–38} (ng·mL ⁻¹ ·day)	293.4 ± 28.12	326.17 ± 50.84
AUC _{0–1} (ng·mL ⁻¹ ·day)	27.14 ± 1.92	23.23 ± 2.96
C _{max} (ng·mL ⁻¹)	46.05 ± 6.39**	37.00 ± 1.98
T _{max} (h)	2**	4

***p* < .01.

specifically, by the drug's binding with a receptor) (Holford & Sheiner, 1981; Sheiner & Steimer, 2000; Tozer & Rowland, 2006). Due to the importance of the concentration and its decisive effect on drug efficacy, many pharmacokinetics studies can focus on improving the release behaviors without further testing the pharmacodynamic properties. Thus, for a specific drug, like EXT in this study, the blood concentration is a quite important factor which can also reflect the drug effect. The EXT-PMS showed the rapid release of the drugs in the initial phase and could maintain effective concentration, which is the biggest advantage over other EXT-MS dosage forms with delay-release properties.

4. Conclusion

In this study, the EXT-loaded PLGA microspheres were effectively fabricated by W₁/O/W₂ emulsion microfluidic method. The effects of various polymer type, PLGA concentration, proportion of drug and oil phase, and especially the porogen

concentration on the release behaviors of EXT-PMS were studied in the system. Characterizations demonstrated the internal large pores and the external thin layer structure of EXT-PMS. Both *in vitro* and *in vivo* experiments validated the ability of our formulation of EXT-PMS to release drugs rapidly in the initial phase and to maintain effective concentration without delay-release for 30 days. The resulting inside-porous microspheres could sustain the release of EXT and offer the dual-advantage of (i) absence of a lag phase and (ii) low burst release. To the best of our knowledge, this is the first work to improve the release profile of EXT by inside-porous PLGA microspheres. Our method indicates the great potential for these inside-porous microspheres in the development of long-acting drug delivery systems with reasonable release profiles for various drugs.

Supporting information

Supporting Information is available free of charge. Drug-loading capacity and encapsulation efficiency of EXT-PMS prepared by different PLGA polymer; *in vitro* dissolution of EXT-PMS prepared by different PLGA polymer.

Disclosure statement

There are no conflicts of interest to declare.

Funding

This work was financially supported by the Science and Technology Program of Guangzhou, China [201604020166].

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