Preparation, Characterization, and Pharmacodynamics of Exenatide-Loaded Poly(DL-lactic-co-glycolic acid) Microspheres

Bin Liu,[a,b,c] Qingguang Dong,[a,#] Mengshu Wang,[a] Lin Shi,[a] Yongge Wu,[a,b] Xianghui Yu,[a,b] Yanyu Shi,[c] Yaming Shan,[a] Chunlai Jiang,[a] Xizhen Zhang,[a] Tiejun Gu,[a] Yan Chen,*,[a] and Wei Kong*,[a,b]

[a] *The State Engineering Laboratory of AIDS Vaccine, Jilin University;* [b] *State Key Laboratory of Supramolecular Structure and Material, Jilin University; Changchun 130012, China: and* [c] *Jilin Product Quality Supervision Inspection; Changchun 130022, China.*   Received May 28, 2010; accepted August 10, 2010; published online August 16, 2010

Exenatide (synthetic exendin-4), a 39-amino acid peptide, was encapsulated in poly(DL-lactic-co-glycolic acid) (PLGA) microspheres as a sustained release delivery system for the therapy of type 2 diabetes mellitus. The microspheres were prepared by a double-emulsion solvent evaporation method and the particle size, surface morphology, drug encapsulation efficiency, *in vitro* release profiles and *in vivo* hypoglycemic activity were evaluated. The results indicated that the morphology of the exenatide PLGA microspheres presented as a spherical shape with smooth surface, and the particle sizes distributed from 5.8 to 13.6 μm. The drug encapsulation efficiency tested by micro-bicinchoninic acid (BCA) assay was influenced by certain parameters such as inner and outer aqueous phase volume, PLGA concentration in oil phase, polyvinyl alcohol (PVA) concentrations in outer aqueous phase. Moreover, *in vitro* release behaviors were also affected by some parameters such as polymer type, PLGA molecular, internal aqueous phase volume, PLGA concentration. The pharmacodynamics in streptozotocin (STZ)-induced diabetic mice suggested that, exenatide microspheres have a significant hypoglycemic activity within one month, and its controlling of plasma glucose was similar to that of exenatide solution injected twice daily with identical exenatide amount. In conclusion, this microsphere could be a well sustained delivery system for exenatide to treat type 2 diabetes mellitus.

Key words   exenatide; poly(DL-lactic-co-glycolic acid) microsphere; hypoglycemic effect; double-emulsion solvent evaporation method

Exenatide (synthetic exendin-4), an incretin mimetic agent, is a 39-amino acid peptide originally isolated from salivary gland venom of *Heloderma suspectum*, the Gila monster.[1] It is a glucagon-like peptide-1 (GLP-1) receptor agonist sharing 53% amino acid sequence identity with mammalian GLP-1.[2] Similar to GLP-1, exenatide has several bioactivities which account for its glycemic control effects, including glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying, and reduction of food intake.[3—5] Since these actions all contribute to the reduction in fasting and postprandial blood glucose in patients with type 2 diabetes mellitus without inducing hypoglycemia, exenatide has been approved by US FDA as an adjunctive treatment in patients unable to achieve adequate glucose control using metformin and/or sulfonylurea, with subcutaneous administration twice daily.[6] However, frequent injections are inconvenient for the patients, so the development of sustained-release delivery system would be beneficial.

During the past few decades, microspheres of biodegradable polymers such as poly(DL-lactic-co-glycolic acid) (PLGA) for sustained delivery of protein or peptide were widely developed, which could obviously improve patient compliance.[7,8] Drug burst release is a common problem in PLGA microspheres delivery system, which limited its application in diabetes therapy.[9] For drug of treating diabetes such as insulin, the burst release may induce the hypoglycemia of diabetic patients. Whereas, the hypoglycemic activity of exenatide is glucose-dependent. When the plasma glucose concentration is in the normal level, exenatide will no longer enhance the insulin secretion. This characteristic property of exenatide makes it suitable for preparing PLGA microspheres. Recently, the long-acting exenatide delivery systems are under development by Amylin Pharmaceuticals and Eli Lilly, and the activities *in vivo* has been reported.[10,11] Nevertheless, the investigation of the variable preparing parameters in formulations of exenatide microspheres has not been reported except Patent WO2004035762 to our knowledge. We have prepared exenatide microspheres following the formulation in above Patent, but the drug burst release was found to be high (45%), indicating that the formulation of exenatide microspheres should be further optimized. As is known, the properties of PLGA microspheres for different drug may have great disparity, especially for mid-length peptide such as exenatide. Therefore, to obtain an optimized formulation in the preparation of exenatide-loaded microspheres, multiple factors should be considered.

In this paper, we successfully prepared long-acting release (once-monthly) exenatide-loaded PLGA microspheres using water-in-oil-in-water (W/O/W) double-emulsion method and certain processing parameters influencing the properties of the microspheres were investigated for the first time. Furthermore, the pharmacodynamics of exenatide-loaded PLGA microspheres in streptozotocin (STZ)-induced diabetic mice was studied to investigate their release properties *in vivo*.

Experimental

Materials   Exenatide was synthesized in our laboratory by Peptide Synthesizer (PS3 Model, Protein Technologies Inc., U.S.A.) using the FMOC solid-phase peptide synthesis approach, and purified by reverse phase (RP)-HPLC to >95% purity. PLGA and poly lactic acid (PLA) were purchased from Birmingham Polymers (Birmingham, AL, U.S.A.). The micro-bicinchoninic acid (BCA) Assay Kit was supplied by Pierce. Polyvinyl alcohol (PVA) and STZ were purchased from Sigma. All other chemicals were of analytical grade.

Animals   Kun Ming (KM) mice with the body weight of 18—20 g were

supplied by Laboratory Teaching Center of Basic Medicine, Jilin University. Principles in good laboratory animal care were followed and all animal experimentation complied with the requirements of the National Act on the use of experimental animals (People's Republic of China).

Preparation of Exenatide-Loaded Microspheres Exenatide-loaded microspheres were prepared by a modified double-emulsion solvent evaporation method (W/O/W).[12] Briefly, 10 mg lyophilized exenatide powder was dissolved in 0.03 M sodium acetate buffer (pH 4.5). The aqueous exenatide solution was mixed with 5 ml dichloromethane (DCM) containing PLGA, and emulsified in a homogenizer (MICCRA D-8, Art Labortechnik, Germany) at high speed for 30 s. The primary emulsion was then added to distilled water containing PVA and emulsification continued at 19000 rpm for 30 s. The formed water-in oil-in water emulsion was stirred for 4 h at room temperature, allowing DCM to evaporate. The emulsion solidified gradually as the diffusion of the solvent from the emulsion droplets into the external phase. The resulting microspheres were washed three times in distilled water by centrifugation at 10000 g (Eppendorf centrifuge 5810R, Germany) and freeze-dried.

Particle Size Analysis The freeze-dried microspheres were dispersed in distilled water and the size distribution was measured by laser diffractometry using a Laser Sizer (MS2000, Malvern, England). The particle size was expressed as the volume mean diameter in micrometer. Size distribution was evaluated with span value, which was calculated as the ratio of ($D_{90\%} - D_{10\%}$) to $D_{50\%}$, where $D_{N\%}$ ($N=10, 50, 90$) means that the volume percentage of microspheres with diameters up to $D_{N\%}$ equals to $N\%$. The smaller the span value is the narrower the size distribution.

Surface Morphology of Microspheres The microspheres were evaluated by scanning electron microscopy (SEM, JSM-6700F, JEOL, Japan) to determine the shape and surface morphology. Microspheres were mounted onto metal stubs using double-sided adhesive tape. After being coated with a thin layer of gold under vacuum, the microspheres were examined by SEM at 3 kV.

Determination of Exenatide Loading and Entrapment Efficiency The entrapment efficiency was determined by a solvent dissolution method with DCM and acetone.[13] Briefly, 50 mg freeze-dried microspheres were placed in a 1.5 ml Eppendorf vial to which 0.3 ml DCM were added for dissolving, then 0.7 ml acetone were added and the vial was vortexed. The vial was centrifuged at 12000 rpm for 5 min, and the supernatant was discarded. The residue was washed with the mixed solvent of 3 : 1 acetone : DCM for three times by repeating the above process. After being allowed to stand at room temperature to evaporate the solvent, the residue was dissolved in pH 7.4, 10 mM phosphate buffered saline (PBS) (120 mM NaCl, 2.7 mM KCl), and the exenatide amount was measured by the micro-BCA Assay following dilution. The exenatide loading percentage (w/w, exenatide content per dry microspheres) was determined, and the entrapment efficiency was calculated by comparing the actual exenatide loading with the theoretical exenatide loading. Each sample was assayed in triplicate, and the data were presented as average with the standard deviations.

***In Vitro* Release Profiles** *In vitro* release studies of exenatide from microspheres were carried out according to the literature as follows[14]: 20 mg of microspheres were suspended in 1 ml of assay medium (10 mM pH 7.4 PBS with 2 mM sodium dodecyl sulfate (SDS) and 0.01% sodium azide) at 37 °C under continuous agitation at about 100 rpm. At predetermined time intervals, the suspension was centrifuged at 4000 rpm for 10 min. The supernatant was transferred to a 1.5 ml vial, and an equal volume of fresh medium was added into the precipitate for next assay. The transferred medium was centrifuged at 12000 rpm for 10 min, and the amount of exenatide in the su-

pernatant was determined by the micro-BCA Assay.[15] The results were calculated by comparing the cumulated released exenatide with the total exenatide amount in microspheres. Experiments were carried out in triplicate, and the data were presented as average with the standard deviations.

Glycemic Control in the Diabetes Mice KM mice, 8 weeks of age with equal number of female and male were used for experiments to evaluate the pharmacodymamics of exenatide-loaded microspheres. The mice were divided into four groups and each had eight mice. The first group was used as a negative control group with subcutaneous administration of saline twice daily. The second group was a diabetic control group which was injected subcutaneously with saline twice daily and administered intraperitoneally with STZ (30 mg/kg body weight) freshly dissolved in 0.1 M sodium citrate (pH 4.5) once daily for the initial 7 d.[16] The third group was exenatide solution testing group which was injected subcutaneously with exenatide solution (3 μg/kg body weight per day, the doses described hereafter have been converted from the actual drug dose by conversion factor between human and mice) twice daily and administered with STZ using the same way as for the positive control group. The fourth group was exenatide microspheres testing group which was given a single subcutaneous injection of exenatide-loaded microspheres suspension (containing 90 μg exenatide/kg body weight), with STZ. Blood samples were collected from tail vein at each time point after overnight fasting and the plasma glucose was determined by the glucose oxidase method using a One Touch Basic glucose meter (OneTouch Ultra 2, Lifescan, U.S.A.).

Statistical Analysis All the data were expressed as means±standard deviation (S.D.). The *in vivo* results were evaluated by one way analysis of variance (ANOVA). A comparison between two means was analyzed using *t* test with statistical significance set at $p < 0.05$.

Results and Discussion

Mean Particle Size of Microspheres Particle size is one of the important characters of microspheres, which may affect degradation rate, drug loading and initial burst release of microspheres. In this study, the mean particle diameters of exenatide microspheres were in the range from 5.8 to 13.6 μm as presented in Table 1, and the dispersity of all formulations was relatively uniform with the span values of 1.25—1.70. For each preparation parameter studied, batches of microspheres were prepared in triplicate. It was found that, the mean particle size of microspheres was influenced by several parameters in preparing process. An increase in the volume of the external aqueous phase resulted in a proportional increase in the particle size (Formulation A, D and E). This was attributed to a difference of shearing efficiency in the W/O/W emulsion process at different outer aqueous phase volume. At fixed shearing force, a larger water phase volume results in a lower mixing efficiency, which facilitates the formation of large emulsion droplets, and subsequently results in the larger particle size.[17] PLGA concentration in DCM also influences the particle size of microspheres to a certain extent. When PLGA concentration increased from 3

Table 1. Particle Size and Encapsulation Efficiency of Exenatide-Loaded Microspheres

Formulation ID	Volume of internal water phase (ml)	Volume of external water phase (ml)	PLGA concentration (%, w/v)	PVA concentration (%, w/v)	Mean particle size (μm)	Entrapment efficiency (%)
A	1	25	6	1	8.9±1.0	76.1±3.3
B	2	25	6	1	10.0±1.5	87.9±4.9
C	4	25	6	1	10.1±1.2	21.7±2.2
D	1	50	6	1	11.3±1.2	57.0±3.8
E	1	100	6	1	13.6±1.8	52.5±2.9
F	1	25	3	1	5.8±0.7	20.3±2.2
G	1	25	9	1	10.3±0.8	77.1±1.5
H	1	25	6	3	7.1±1.0	82.3±4.9
I	1	25	6	5	5.9±0.6	65.1±3.4

to 9% (Formulation F, A and G), the mean particle sizes of microspheres also changed from 5.8 to 10.3 μm. This fact arises from the effect of oil phase viscosity. Increased PLGA concentration elevates the oil viscosity, which induces the formation of larger emulsion droplets and thereby the larger particle size. The changes of particle size were also observed when changing PVA concentration in the external aqueous phase. The sizes of microspheres prepared at 1%, 3% and 5% PVA (Formulation A, H and I) were 8.9, 7.1 and 5.9 μm, respectively. A significant reduction in particle size was obtained as PVA concentration in the external water phase was increased. Since high PVA concentration could lead to the higher viscosity of the external water phase, stable smaller emulsion droplets could be formed in the secondary emulsion, which could result in the smaller particle size. This effect is considered to be related to enlargement in the steric hindrance of polymer molecules for high PVA concentration, which inhibits the coagulation of emulsions.[18]

Encapsulation Efficiency of Microspheres The encapsulation efficiency are usually influenced by various parameters, such as inner and outer aqueous phase volume, PLGA concentration in oil phase, PVA concentrations in outer aqueous phase. The encapsulation efficiency of PLGA microspheres was tested under different processing parameters and the results were shown in Table 1.

The encapsulation efficiency of exenatide microspheres was enormously influenced by volume of internal water phase. The encapsulation efficiency was increased from 76.1 to 87.9% when the internal water phase was changed from 1 to 2 ml (Formulation A and B). However, further increasing of internal phase volume to 4 ml brought up a sharp decrease of the encapsulation efficiency (Formulation C), indicating that too much internal phase made the primary emulsion unstable which in turn led to a large number of drug leaking from inner to outer water phase. As is known, the leaking process of hydrophilic drug in the microspheres preparation is a key factor leading to the pore formation of microspheres. More porous surface was consequently observed from microspheres prepared at larger internal phase (Fig. 1).

Effects of external water phase volume on encapsulation efficiency of exenatide microspheres were also investigated. As was shown, the encapsulation efficiency decreased from 76.1 to 52.5% when the external water phase was increased from 25 to 100 ml (Formulation A, D and E). The changes in microspheres structure also account for such results. Microspheres with smaller external water phase volume had a smooth, nonporous surface, whereas more porous surfaces were found in the microspheres with larger external aqueous phase. The porous surface may cause higher opportunity of hydrophilic drug leaking. Thus with the porous surface, the entrapment efficiency of exenatide in the microspheres prepared at 50 and 100 ml external aqueous phase was lower evidently than that of microspheres at 25 ml external aqueous phase. Parikh *et al.* also reported the similar results.[19]

PLGA concentration in the oil phase is also a key factor influencing the encapsulation efficiency of exenatide microspheres. Exenatide microspheres were prepared using PLGA by varying concentration as given in Table 1 (Formulation F, A and G). Increase in concentration of polymer resulted in an improvement in drug entrapment efficiency (from 20.3% for 3% PLGA to 77.1% for 9% PLGA). The increase in entrap-
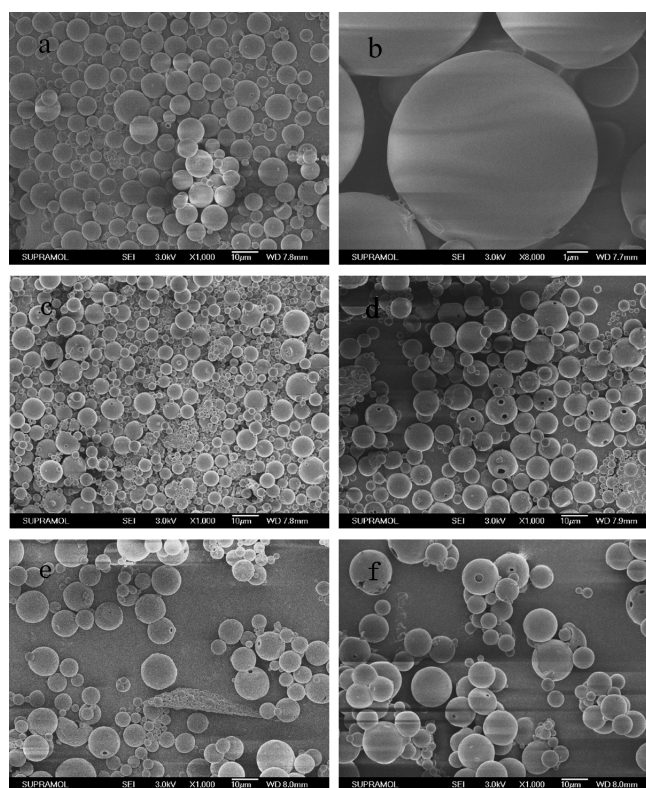

Fig. 1.　SEM Pictures of Exenatide-Loaded PLGA Microspheres at Different Inner and Outer Water Phase Volume

(a) and (b) Formulation A; (c) Formulation B; (d) Formulation C; (e) Formulation D; (f) Formulation E.

ment efficiency arises from the increase of the oil viscosity when elevating the PLGA concentration. More viscous solution of oil phase led to fewer leakage of hydrophilic drug from the inner aqueous phase into the outer phase. Similar effect was also observed by Zhu *et al.*[20]

The effects of PVA concentration in external aqueous phase on the encapsulation efficiency of exenatide microspheres were observed from Table 1 (Formulation A, H and I). The entrapment efficiency increased from 76.1 to 82.3% when the PVA concentration increased from 1 to 3%. This is due to that more viscous external aqueous phase caused by raising PVA concentration resulted in the reduction of exenatide diffusing rate from the inner water phase to the outer water phase, consequently improved the entrapment efficiency. However, further increasing of the PVA concentration to 5% led to decreased entrapment efficiency. Since most of the particle sizes of microspheres in our study were small and higher concentration of PVA lead to even smaller particle size, it could be considered that, when the PVA concentration increased to 5%, the emulsion droplets were too small to form thick and dense protective polymer layer well, which led to more internal exenatide diffusing to the external aqueous phase.

***In Vitro* Release** To obtain a best formulation with lower burst release and optimal accumulative release profile, we investigated the *in vitro* exenatide release profiles of microspheres. In some previous researches, the drug release of PLGA microspheres has been shown to be impacted by numerous parameters, such as polymer type, PLGA molecular, internal aqueous phase volume, PLGA concentration. To

study the effects of these parameters on *in vitro* release behaviors of exenatide microspheres, a series of formulation of exenatide PLGA microspheres were designed and prepared by the W/O/W double-emulsion method.

Figure 2 showed the release profiles of exenatide microspheres with different polymer type. The burst release of microspheres prepared with PLGA 50/50 was the lowest (11%), whereas that of 75/25 PLGA was the highest (27%). PLGA 50/50 microspheres displayed a typical three-phase release profile including burst release, lag period and erosion-controlled release,[21] which didn't present in the release profiles of 75/25 PLGA and PLA microspheres. This phenomenon was due to the slower degradation rate for higher lactic acid content, which led to the slower erosion-controlled release phase (the SEM pictures of microspheres with different PLGA type at various time points of *in vitro* release were shown in the supplementary document). Depending on the degradation rate, the cumulated exenatide release were 70%, 55% and 28% respectively for PLGA 50/50, PLGA 75/25 and PLA microspheres over 30 d.

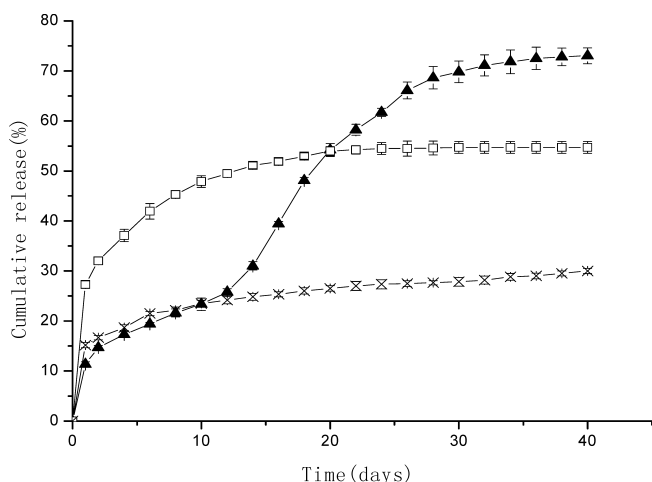The effects of PLGA molecular weight on *in vitro* release

of exenatide microspheres were shown in Fig. 3. PLGA 38400 microspheres presented the lowest initial burst release (11%), whereas PLGA 10000—20000 and PLGA 56000 microspheres had significantly higher burst release (65% and 39% respectively). This may be attributed to the formation of less stable primary emulsions for PLGA 10000—20000 and PLGA 56000 microspheres, which resulted in more drug locating in the surface of microspheres in the preparation process. Moreover, larger molecular weight would induce to higher oil phase viscosity, so the thicker polymer layer accordingly in the preparing process. Microspheres with larger molecular weight PLGA and thicker polymer layer had slower degradation, so the lower drug release rate. Not surprisingly, with the highest burst release and fast degradation rate, PLGA 10000—20000 released exenatide mostly within 6 d and had almost no extended exenatide release. PLGA 38400 microspheres presented a three-phase release profile within 30 d, whereas PLGA 56000 had no evident lag period and had a continuous release over at least 40 d. It can be attributed to the slower degradation of PLGA 56000 microspheres, as made no significant inflexion between lag period phase and erosion-controlled release phase.

The effect of internal aqueous phase volume to the *in vitro* release of exenatide microspheres was shown in Fig. 4. The burst drug release of microspheres was increased evidently from 9% for 1 ml internal phase to 42% for 4 ml internal phase. The significant rising of burst release was caused by the more porous surface structure in microspheres prepared with larger internal aqueous phase volume. Moreover, the larger internal phase volume would lead to the thinner polymer layer of microspheres, inducing a faster drug release rate within one month.

Figure 5 depicted the release profiles of microspheres fabricated at differentl PLGA concentrations. The initial burst release of microspheres was restricted from 49% for 3% PLGA to 3% for 9% PLGA. Since microspheres of lower PLGA concentration had smaller particle size, smaller microspheres with a greater surface area resulted in microspheres having more surface-bound exenatide. In addition, the viscosity of oil phase increased as the augment of PLGA

Fig. 2. Effect of the Polymer Type on the Release Profiles of Exenatide-Loaded PLGA Microspheres

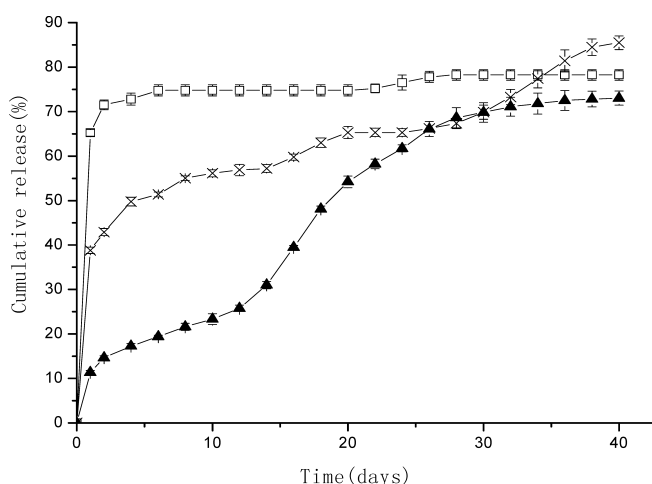PLGA 50/50 (▲), PLGA 75/25 (□) and PLA (×) (*n*=3).

Fig. 3. Effect of PLGA Molecular Weight on the Release Profiles of the Exenatide-Loaded PLGA Microspheres

PLGA 10000—20000 (□), PLGA 38400 (▲) and PLGA 56000kD (×) (*n*=3).
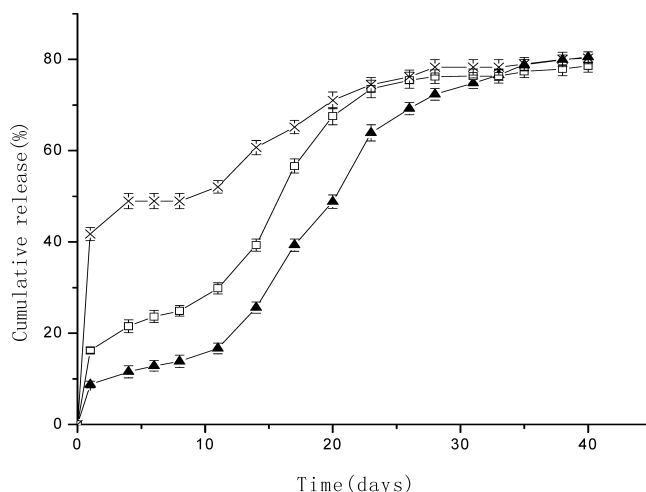

Fig. 4. Effect of Internal Aqueous Phase Volume on the Release Profiles of the Exenatide-Loaded Microspheres Prepared with PLGA 50/50, MW 38400

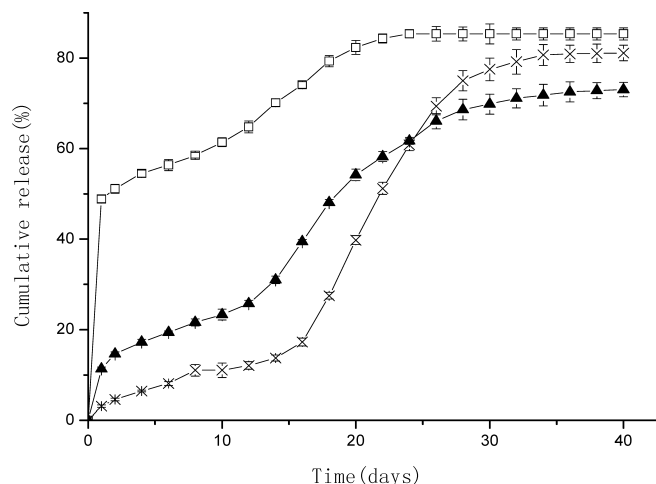Formulation A (▲), Formulation B (□) and Formulation C (×) in Table 1 (*n*=3).

Fig. 5. Effect of the PLGA Concentration on the Exenatide Release Profiles from Microspheres Prepared with PLGA 50/50, MW 38400

Formulation F (▲), Formulation A (□) and Formulation G (×) in Table 1 ($n=3$).
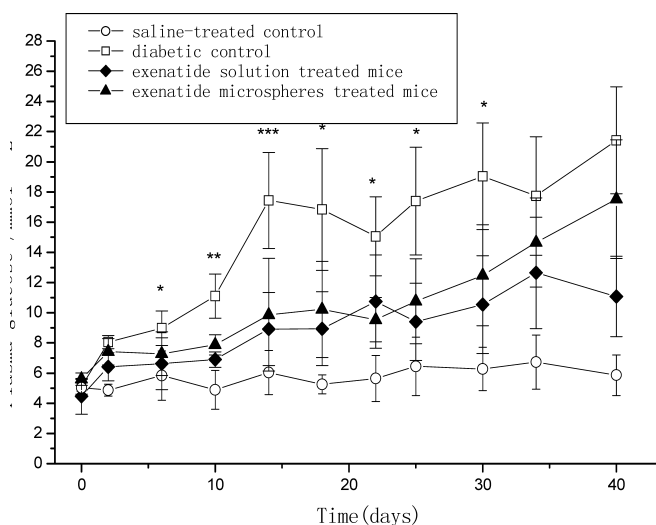

Fig. 6. Fasting Plasma Glucose Levels after One-Time Injection of Exenatide-Loaded PLGA Microspheres and Twice-Daily Injections of Exenatide Solution in Diabetic Mice

Date represent the mean±S.D., $n=8$ per group. Statistically significant difference of exenatide microspheres treated mice from diabetic control (∗$p<0.05$, ∗∗$p<0.01$, ∗∗∗$p<0.001$).

concentration. The higher viscosity causes less diffusion of hydrophilic drug through the oil phase and consequently less surface-conjugated exenatide of microspheres. Microspheres of all the PLGA concentrations had three-phase release profiles, but the length of lag time increased as the increase of PLGA concentration. This could be explained for the formation of the tighter and thicker polymer matrix structure with higher PLGA concentration, which decreased the degradation rate of microspheres.[22]

The Hypoglycemic Effect in Diabetes Mice To investigate the potential application of exenatide-loaded PLGA microspheres in therapy of diabetes mellitus, their hypoglycemic effect of in STZ-induced diabetic mice was evaluated. STZ is a reagent which can induce rapid and irreversible necrosis of B cells, and is widely used for making diabetic animal models.[23,24] Exenatide microspheres of Formulation H which has a lower initial burst, higher entrapment efficiency and sustained *in vitro* release within one month were chosen to observe the hypoglycemic behavior in diabetic mice. Mice of exenatide solution group were injected subcutaneously with exenatide solution twice daily repeatedly over 40 d, and the daily dose was 3 μg/kg body weight in corresponding drug amount. Mice of exenatide microspheres group were injected subcutaneously one-time with exenatide-loaded PLGA microspheres at the beginning of experiment, and the dose was 90 μg exenatide/kg body weight, which was equal to the total drug amount of exenatide solution group within one month.

Figure 6 demonstrated the changes in fasting plasma glucose of mice in each group after the administration within 40 d. The diabetic control mice were established by injection of multiple low-dose STZ (30 mg/kg body weight per day for 7 d). The results showed that, diabetic control mice had a continuous increase in fasting plasma glucose after the injection of STZ and the plasma glucose levels remained above 15 mmol·l^{-1} basically after day 14. The plasma glucose of exenatide solution treated mice and exenatide microspheres treated mice also increased to a certain extent compared with the saline-treated control. Although hyperglycemia developed in all mice treated with STZ, significant difference ($p<0.05$) in plasma glucose level was observed between the

exenatide microspheres group and the diabetic control group within 30 d. Furthermore, one-time injection of exenatide microspheres had a similar hypoglycemic effect to the exenatide solution injected twice daily in the diabetic mice within 30 d, indicating that the exenatide-loaded PLGA microspheres performed well slowed release of exenatide *in vivo*. In conclusion, the exenatide microspheres could effectively control the plasma glucose level of STZ-induced diabetic mice within 30 d.

Conclusion

In present study, an incretin mimetic, exenatide was successfully encapsulated into PLGA microspheres using the double-emulsion solvent evaporation method. Variable parameters affecting the properties of exenatide-loaded PLGA microspheres were studied, and an optimized formulation of exenatide microspheres with higher encapsulation efficiency and lower burst release was ascertained. As a preliminary research for the application of PLGA microspheres to the therapy of diabetes mellitus, its pharmacodynamic study in STZ-induced diabetic mice was investigated. The results showed that, the prepared exenatide microspheres could effectively control the plasma glucose within one month, and had a similar hypoglycemic effect to exenatide solution injected twice daily, which indicated *in vivo* slowed drug release of microspheres. Further studies are in progress, including the hypoglycemic effect evaluation and the plasma drug concentration determination of exenatide microspheres in the spontaneous diabetic mice.

References
1) Raufman J. P., *Regul. Pept.*, **61**, 1—18 (1996).
2) Eng J., Kleinman W. A., Singh L., Singh G., Raufman J. P., *J. Biol. Chem.*, **267**, 7402—7405 (1992).
3) Bray G. M., *Am. J. Health Syst. Pharm.*, **63**, 411—418 (2006).

4) Nielsen L. L., Young A. A., Parkes D. G., *Regul. Pept.*, **117**, 77—88 (2004).

5) Kolterman O. G., Buse J. B., Fineman M. S., Gaines E., Heintz S., Bicsak T. A., Taylor K., Kim D., Aisporna M., Wang Y., Baron A. D., *J. Clin. Endocrinol. Metab.*, **88**, 3082—3089 (2003).

6) Webb D. M., Wintle M., Malone J. K., *Drug Devel. Res.*, **67**, 666—676 (2006).

7) Ogawa Y., Yamamoto M., Okada H., Yashiki T., Shimamoto T., *Chem. Pharm. Bull.*, **36**, 1095—1103 (1988).

8) Esposito E., Cortesi R., Bortolotti F., Menegatti E., Nastruzzi C., *Int. J. Pharm.*, **129**, 263—273 (1996).

9) Wei G., Pettway G. J., McCauley L. K., Ma P. X., *Biomaterials*, **25**, 345—352 (2004).

10) Gedulin B. R., Smith P., Prickett K. S., Tryon M., Barnhill S., Reynolds J., Nielsen L. L., Parkes D. G., Young A. A., *Diabetologia*, **48**, 1380—1385 (2005).

11) Drucker D. J., Buse J. B., Taylor K., Kendall D. M., Trautmann M., Zhuang D., Porter L., *Lancet*, **2008**, 1240—1250 (2008).

12) Zhou X., Liu B., Yu X., Zha X., Zhang X., Wang X., Jin Y., Wu Y., Chen Y., Shan Y., Liu J., Kong W., Shen J., *Eur. J. Pharm. Biopharm.*, **68**, 589—595 (2008).

13) Meinel L., Illi O. E., Zapf J., Malfanti M., Peter Merkle H., Gander B.,

J. Controlled Release, **70**, 193—202 (2001).

14) Geng Y., Yuan W., Wu F., Chen J., He M., Jin T., *J. Controlled Release*, **130**, 259—265 (2008).

15) Chang A. C., Gupta R. K., *J. Pharm. Sci.*, **85**, 129—132 (1996).

16) Ito M., Kondo Y., Nakatani A., Hayashi K., Naruse A., *Environ. Toxicol. Pharmacol.*, **9**, 71—78 (2001).

17) Jeffery H., Davis S. S., O'Hagan D. T., *Pharm. Res.*, **10**, 362—368 (1993).

18) Ito F., Fujimori H., Makino K., *Colloids Surf. B Biointerfaces*, **54**, 173—178 (2007).

19) Parikh R. H., Parikh J. R., Dubey R. R., Soni H. N., Kapadia K. N., *AAPS PharmSciTech*, **4**, E13 (2003).

20) Zhu K. J., Jiang H. L., Du X. Y., Wang J., Xu W. X., Liu S. F., *J. Microencapsul.*, **18**, 247—260 (2001).

21) Cui F., Cun D., Tao A., Yang M., Shi K., Zhao M., Guan Y., *J. Controlled Release*, **107**, 310—319 (2005).

22) Yang Y. Y., Chung T. S., Ng N. P., *Biomaterials*, **22**, 231—241 (2001).

23) Junod A., Lambert A. E., Stauffacher W., Renold A. E., *J. Clin. Invest.*, **48**, 2129—2139 (1969).

24) Morgan N. G., Cable H. C., Newcombe N. R., Williams G. T., *Biosci. Rep.*, **14**, 243—250 (1994).