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RESEARCH ARTICLE

Effects of formulation parameters on encapsulation efficiency and release behavior of thienorphine loaded PLGA microspheres

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Abstract
To develop a long-acting injectable thienorphine biodegradable poly (D, L-lactide-co-glycolide) (PLGA) microsphere for the therapy of opioid addiction, the effects of formulation parameters on encapsulation efficiency and release behavior were studied. The thienorphine loaded PLGA microspheres were prepared by o/w solvent evaporation method and characterized by HPLC, SEM, laser particle size analysis, residual solvent content and sterility testing. The microspheres were sterilized by gamma irradiation (2.5 kGy). The results indicated that the morphology of the thienorphine PLGA microspheres presented a spherical shape with smooth surface, the particle size was distributed from 30.19 ± 1.17 to 59.15 ± 0.67μm and the drug encapsulation efficiency was influenced by drug/polymer ratio, homogeneous rotation speed, PVA concentration in the water phase and the polymer concentration in the oil phase. These changes were also reflected in drug release. The plasma drug concentration vs. time profiles were relatively smooth for about 25 days after injection of the thienorphine loaded PLGA microspheres to beagle dogs. *In vitro* and *in vivo* correlation was established.

Keywords: Thienorphine, poly (d, l-lactide-co-glycolide), microspheres, formulation parameter, release behavior

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Introduction

Opioid abuse and dependence remains a serious worldwide health problem. Relapse of addiction is often caused by poor compliance and lack of retention in programs (1). Therefore, it is of great importance to be able to reduce the level of involvement of the subject with medicinal treatments, particularly those treatments involving a specific regimen.

Many experts have adopted sustained release methods to reduce the involvement of subjects in compliance. Biodegradable injectable microspheres have been studied widely in the last 30 years. They have been extensively investigated as drug carriers in controlled drug delivery systems, which have several advantages (2): (a) less frequent administration and reduced total dose; (b) improved compliance of patients; (c) the extended duration of drug effect and more predictable absorption; (d) fewer extra pyramidal side-effects and reduced medical workload. Among many biodegradable polymers investigated, poly (D, L-lactide-co-glycolide) (PLGA) has attracted much attention due to its good biodegradable and biocompatible properties.

Thienorphine[N-cyclopropylmethyl-7(-[(R)-1-hydroxy-1-methyl-3-(thien-2-yl)-propyl]-6,14-endo-ethanotetrahydronororipavine] is a new compound (Figure 1), synthesized by our institute. As an analog of buprenorphine, thienorphine is a partial agonist of the μ-opioid receptor, as is buprenorphine, which has been widely used in the therapy of opioid addiction (3,4). The pharmacology studies showed that thienorphine is a potent, long-acting partial opioid agonist and may have a possible application in treating addiction (5–7). Inspired by the result, we prepared the thienorphine loaded PLGA microspheres. Our objective was to develop a controlled release system that was effective for a period of 1 month.

The o/w solvent evaporation method was chosen to prepare thienorphine loaded PLGA microspheres in this

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study, because thienorphine is a lipophilic compound. The effects of a series of formulation parameters of emulsion on the microencapsulation and release behavior of thienorphine PLGA microspheres were investigated. Besides the evaluation of physicochemical characteristics, the gamma radiation sterilization and residual dichloromethane (DCM) content of the microspheres were also investigated. The drug content of microspheres *in vivo* was determined by a LC-MS-MS. Finally, the correlation between *in vitro* and *in vivo* release was established.

Materials and methods

Materials

Thienorphine (99% purity) was supplied by Beijing Institute of Pharmacology and Toxicology. PLGA (W_η 8800, 15,000; lactide/glycolide ratio, 75/25) was generously donated by Prof. Shaobing Zhou of Southwest Jiaotong University. Polyvinyl alcohol (PVA-124) and dichloromethane (DCM) were obtained from Beijing chemical reagents company. All other materials or solvents were of analytical grade and were used as obtained commercially.

Microspheres preparation

The preparation formulations of thienorphine loaded PLGA microspheres are shown in Table 1. The thienorphine loaded PLGA microspheres were prepared using oil-in-water (o/w) emulsion solvent evaporation method. This study had been described in our previous report (8). Briefly, an amount of PLGA and thienorphine were added to 1 ml of DCM. After being completely dissolved, it was poured into aqueous solution PVA-124 and then the mixture was emulsified by using a propeller stirrer (SXJQ-1, Zhengzhou, China) at various rates for 10 min at room temperature (T). Stirring at 300 rpm was then continued for 8 h to evaporate DCM. The resulting microspheres were washed three times with distilled water and dried under vacuum.

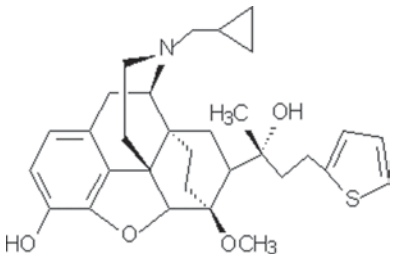

Figure 1. The chemical structures of thienorphine.

Particle size analysis

The mean size and size distribution of the prepared PLGA microspheres were analyzed by a light-scattering particle size analyzer (BT-9300, BETTER, China). The powder of microspheres was suspended in a large volume of distilled water.

Microscopic observations

The microspheres were mounted on metal stubs using a double-sided adhesive tape. After vacuum coating with a layer of gold, the surface of the thienorphine microspheres was observed by scanning electron microscopy (Hitachi S-450, Japan).

Residual DCM content

Gas chromatography (HP 5890, USA) was used to determine the residual DCM in the thienorphine loaded PLGA microspheres. 1 ml *N, N*- dimethyl formamide of 0.05 µl of ethyl acetate was used as internal standard. Approximately 25 mg of microspheres were dissolved in 1 ml of internal standard. GC conditions were as follows: DB-624 capillary column (30 m × 0.25 mm × 0.25 µm, HP, USA); high purity nitrogen (≥99.99%) as carrier gas with flow rate 30 ml/min; injection temperature at 270°C, with FLD detector.

Determination of the encapsulated thienorphine in the microspheres

Microspheres containing thienorphine were dissolved in 5 ml of DCM. The resulting solution was then diluted with ethanol. The media was filtered (0.22 µm, Fisher Scientific, USA) and analyzed by a reverse phase HPLC (LC-10AT VP, Shimadzu, Japan). HPLC conditions were as follows: C_{18} column (250 mm × 4.6 mm, 5 µm, Phenomenex, USA), a

Table 1. Formulations processing conditions of thienorphine loaded PLGA microspheres

Batch	Molecular weight of PLGA	PLGA concentration ($mg \cdot ml^{-1}$)	Drug/ polymer ratio	PVA concentration	Homogenization speed (rpm)	DCM (ml)
1	15,000	200	1/9	4%	800	10
2	8800	200	1/9	4%	800	10
3	15,000	200	1/19	4%	800	10
4	15,000	200	1/6	4%	800	10
5	15,000	100	1/9	4%	800	10
6	15,000	300	1/9	4%	800	10
7	15,000	200	1/9	2%	800	10
8	15,000	200	1/9	6%	800	10
9	15,000	200	1/9	4%	600	10
10	15,000	200	1/9	4%	1000	10
11	15,000	200	1/9	4%	800	5
12	15,000	200	1/9	4%	800	20

mixture of acetonitrile-methanol-0.02mol.l^{-1} phosphate (40:15:45) buffer containing 0.2% triethylamine (pH = 3) as eluant, detection at 220 nm. The thienorphine content was calculated with external standard method. Each measurement was performed in triplicate.

drug loading (%) = (weight of drug in microspheres/ weight of microspheres) × 100%

entrapment efficiency (%) = (drug loading (%)/ theoretical drug loading (%)) × 100%

In vitro release assays

About 25 mg of microspheres were suspended in 30 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 0.02% sodium azide and stirred at 72 rpm in an air chamber thermostated at 37 ± 1°C (9,10). At predetermined intervals, 1 ml of medium was drawn out and replenished with the same volume of fresh medium. Then the sample was centrifuged at 10,000 rpm for 10 min (11). The amount of thienorphine in the collected sample was measured by the HPLC method. Each measurement was performed in triplicate.

The similarity factor (f_2) was calculated to compare release profiles of thienorphine from microspheres. It is noted in the FDA guidance document that generally, f_2 values greater than 50 suggest equivalence of the two profiles (12).

$$f_2 = 50 \times \log_{10}\left\{\left[1+\left(\frac{1}{n}\right)\sum_{t=1}^{n}\left(R_t - T_t\right)^2\right]^{-\frac{1}{2}} \times 100\right\}$$

where n is the number of time points, R_t is the experimental data at time t, and T_t is the predicted *in vitro* percent cumulative release at time t.

Gamma radiation sterilization

The thienorphine loaded PLGA microspheres (batch 1) were carried out at room temperature with a ^{60}Co chamber GC-900 (Beijing Institute of Pharmacology and Toxicology) at dose values of 25 kGy (13). The dose rate (measured with the Fricke dosimeter) was 1.3 kGy/h. Uncertainty in dose value was less than 2%. Non-irradiated samples were kept as reference.

Sterility testing

The test for sterility was done on the thienorphine loaded PLGA microspheres (batch 1) which were sterilized by gamma radiation. Briefly, the sample was added to fluid thioglycollate medium and incubated at 30–35°C for 14 days according to the China Pharmacopoeia monograph (14). The contents were observed for any microorganism growth.

In vivo release studies in beagle dogs

Animal experiments were conducted according to the National Act on the use of experimental animals (PR China). The beagle dogs (adult male, 10 ± 1.0 kg, n = 3, Beijing Institute of Pharmacology and Toxicology) were used for the pharmacokinetic studies. Each animal was injected intramuscularly (i.m.) with the thienorphine loaded PLGA microspheres (batch 1) which were sterilized by gamma radiation, the doses administered to beagle dogs was 2.5 mg/kg. At designed time point, blood samples was collected and centrifuged at 8000 rpm for 10 min within 2 h. Then plasma was obtained and stored at–20°C before measurement. The drug content of microspheres *in vivo* was determined by a LC-MS-MS (Agilent1200, Agilent, USA). The method had been described in our previous report (8).

Results and discussion

Morphology and particle size

The surface morphology of the microspheres was examined visually by scanning electron microscopy. Photomicrographs of PLGA microspheres loaded with thienorphine produced by solvent evaporation method (batch 1) are shown in Figure 2A and B. It was observed that the drug loaded microspheres were spherical in shape with a smooth surface.

Particle size is one of the important characteristics of microspheres, because of its effects on degradation rate, drug loading and initial burst release of microspheres (15). The average particle diameters of thienorphine PLGA microspheres were from 30.19 ± 1.17 to 62.15 ± 0.67μm with a good dispersibility in Table 2.

Residual DCM contents

The residual DCM contents in all thienorphine loaded PLGA microspheres were below 600 ppm, which was in accord with the requirements of the ICH standard (16).

Effects of formulation parameters on encapsulation efficiency

It was reported that the encapsulation efficiency of microspheres using an oil-in-water (o/w) method was mainly dependent on drug partition coefficient in the internal and external phases (17). The acceleration of microsphere solidification may reduce the drug partitioning into the external aqueous phase and increase

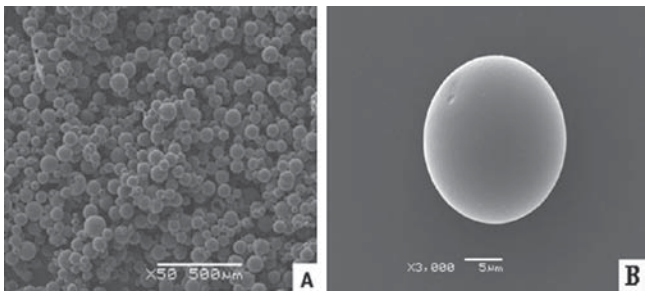

Figure 2. Scanning electron microscopy photograph ((A) magnification of 50, and (B) of 3000 of thienorphine loaded PLGA microspheres.

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Table 2. Characteristics of thienorphine loaded PLGA microspheres

Batch	Actual drug loading (%)	Encapsulation efficiency (%)	Particle size(μm)
1	7.16	71.60	37.84 ± 1.32
2	5.94	59.40	42.57 ± 0.86
3	4.42	88.40	55.38 ± 0.17
4	10.10	67.33	49.79 ± 0.07
5	6.45	64.50	42.69 ± 0.15
6	8.31	83.10	55.61 ± 0.27
7	7.54	75.40	52.37 ± 0.63
8	5.19	51.90	30.19 ± 1.17
9	7.24	72.40	53.74 ± 0.50
10	4.64	46.40	38.45 ± 0.11
11	6.83	68.30	47.19 ± 0.14
12	7.08	70.80	62.15 ± 0.67

the encapsulation percentage (18). The extracted rate of organic solvent from the oil phase and its evaporation rate from the aqueous phase were proved to be important factors (10).

As shown in Table 2, the encapsulation efficiency was highly dependent on the molecular weight of PLGA. The encapsulation efficiency increased significantly from 59.40% with PLGA 8800 microspheres to 71.60% with PLGA 15000 microspheres. This may contribute to the weaker compatibility of thienorphine with the lower molecular weight of PLGA.

The concentration of PLGA in oil phase had an impact on encapsulation efficiency. Higher concentration of PLGA increases the drug encapsulation of PLGA microspheres, because an increase in the viscosity of the oil phase prevents thienorphine from diffusion (19,20).

However, an increase in the concentration of PVA in the external phase leaded to a decrease in the encapsulation efficiency. It might be explained by that the solubility of thienorphine is increased in aqueous PVA solution.

Encapsulation efficiency of microspheres was also affected by the drug/polymer ratio. As the amount of polymer decreased, encapsulation efficiency decreased; this is due to the fact that less amount of polymer would produce small size droplets with increased surface area, such that diffusion of drug from such microspheres will be fast, resulting in the loss of drug with a consequent lowering in encapsulation efficiency.

Other preparation parameters which have significant influence on encapsulation efficiency are shown in Table 2.

Effects of formulation parameters on *in vitro* release of thienorphine from microspheres

The drug release of PLGA microspheres is impacted by numerous parameters, such as the polymer molecular weight, the physicochemical properties of drug, the drug/polymer ratio, particle size of microspheres, preparation process, etc. (21,22).

As shown in Figure 3, the molecular weight had significant influence on the *in vitro* release behavior of

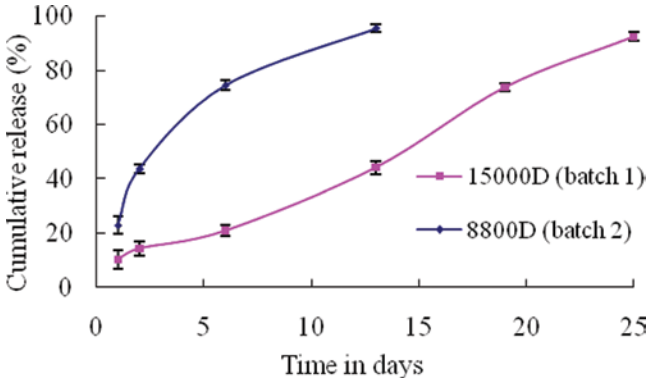

Figure 3. Effect of the molecular weight of PLGA on *in vitro* release behavior of thienorphine PLGA microspheres ($n = 3$).

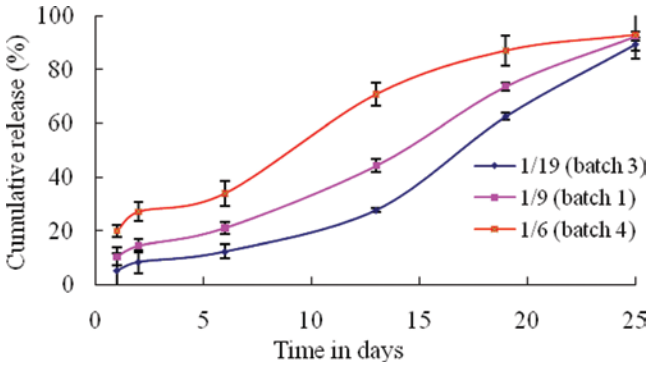

Figure 4. Effect of drug/polymer ratio on *in vitro* release behavior of thienorphine PLGA microspheres ($n = 3$).

thienorphine PLGA microspheres. The drug release rate of PLGA 8800 microspheres was much faster than PLGA 15000. The release of PLGA 8800 and PLGA 15000 microspheres during the first day were 22.96% and 10.46%, respectively. This situation is due to the fact that the low molecular weight of PLGA may increase water permeation and the diffusion of the drug, and also accelerate the erosion of PLGA (23).

As shown in Figure 4, the drug/polymer ratio showed significant influence on the drug release. The polymer amounts increased in the formulations the *in vitro* release became lower. This situation is due to the fact that scarcity of polymer augmented release of the drug and also the thin polymer wall of microspheres as diffusion path led the drug to be easily released in the dissolution medium.

Release profiles of thienorphine loaded microspheres prepared from PLGA at 2%, 4%, and 6% PVA concentrations are shown in Figure 5. Microspheres prepared using 6% PVA concentration exhibited a burst effect amounting to 25.41% released in the first day. The burst release is considered to be due to surface localized thienorphine. It appears that greater surface area of smaller particles formed as a result of increased viscosity of 6% PVA solution, results in microspheres having more surface-bound thienorphine. Microspheres prepared using 2% and 4% PVA exhibited a relatively slow release rate compared to microspheres prepared using 6% PVA. The release from

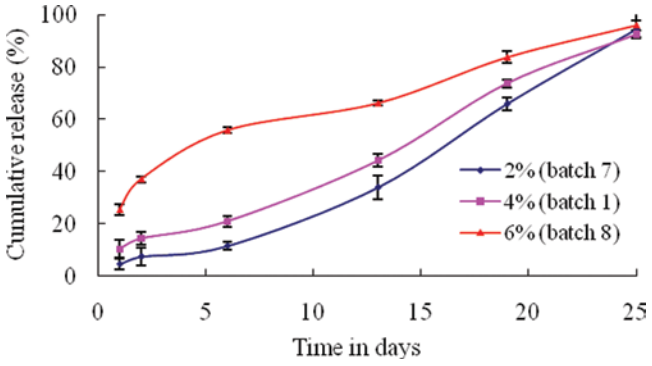

Figure 5. Effect of the PVA concentration on *in vitro* release behavior of thienorphine PLGA microspheres ($n=3$).

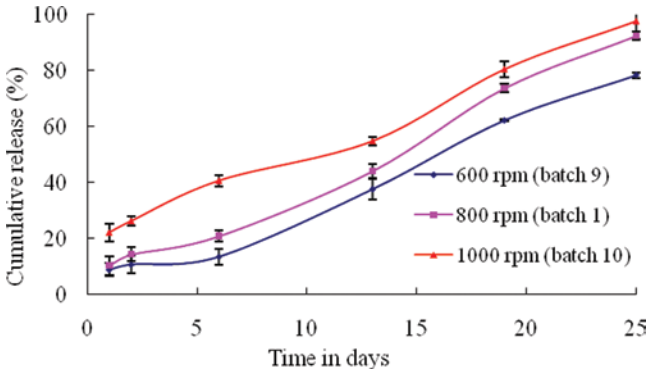

Figure 6. Effect of homogenization speed on *in vitro* release behavior of thienorphine loaded PLGA microspheres ($n=3$).

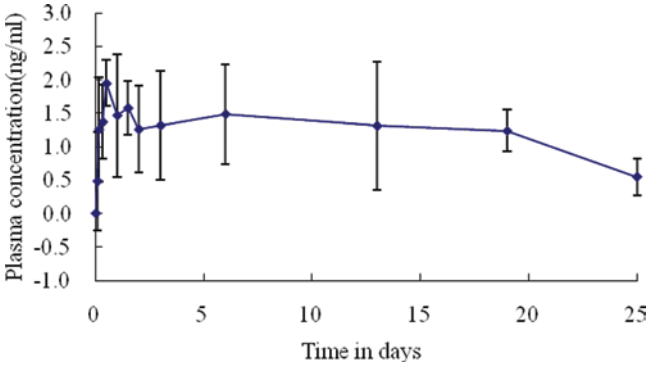

Figure 7. Drug plasma concentration versus time profile after i.m. of thienorphine loaded PLGA microspheres (batch 1) to beagle dogs (mean ± SD, $n=3$).

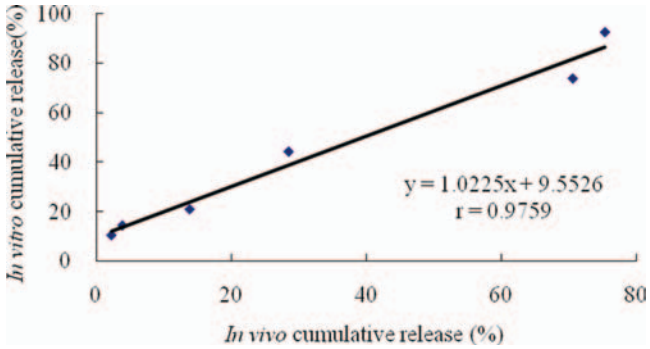

Figure 8. Linear regression plots of cumulative absorption *vs.* percent dissolution of thienorphine from PLGA microspheres.

microspheres prepared using 4% PVA was higher than microspheres prepared using 2% PVA due to smaller particle size and higher surface area.

Effect of the homogenization speed on *in vitro* release behavior of thienorphine PLGA microspheres was investigated (Figure 6). The particle size was affected with homogenization speed. The particle size of higher homogenization speed was smaller. The release of smaller particle size of microspheres was significantly faster, because smaller particle size has much larger ratio of surface/volume, which increases the diffusion of drug to the release medium.

However, the batch 2 was optimized by a high initial burst phase with a secondary first-order release phase until 14 days, and the coefficients of determination (r) was 0.9917. Among the formulations, thienorphine cumulative release of the batch 8 vs. time profile was fitted to Higuchi model which mean the drug diffusion and release from the polymer matrix follow fickian diffusion, and the coefficients of determination (r) was 0.9904.

Gamma radiation studies

The thienorphine loaded PLGA microspheres irradiated (batch 1) were of good morphological characteristics, spherical shape and smooth surface, as the non-irradiated ones. The color did not alter after exposure to the irradiation. The mean diameters of irradiated at 25 kGy (37.13 ± 9.87 μm) and non-irradiated (37.84 ± 1.32 μm) microspheres were not significantly different. The encapsulation efficiency was not affected by the irradiation process with values 71.85%.and 71.60%, for irradiated and non-irradiated microspheres, respectively. The f_2 value was greater than 50 between non-irradiated and 25 kGy, so both *in vitro* release profiles can be considered similar.

Sterility testing

The thienorphine loaded PLGA microspheres which were sterilized by gamma radiation complied with the test for sterility.

In vivo release studies in beagle dogs

Plasma concentration vs. time profiles of thienorphine after i.m. administration of thienorphine loaded PLGA microspheres was shown in Figure 7. Drug plasma concentration reached its maximum values within the first 0.5 days, about 1.95 ng/ml. Drug concentration decreased markedly after 2 days. The relative steady state concentrations for thienorphine reached from day 3 to day 19. As shown in Figure 8, a good linear regression correlation was demonstrated between the percentage of drug released in PBS at 37°C and the percentage of drug absorbed in beagle dogs of drug for the microspheres.

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Conclusions

The thienorphine loaded PLGA microspheres were designed and prepared successfully through the o/w emulsion solvent evaporation method. The formulations described in this study released thienorphine constantly for 25 days. The encapsulation efficiency is highly depended on the molecular weight of PLGA, polymer concentration in the oil phase, drug/polymer, and PVA concentration in the water phase. Higher drug encapsulation efficiency can be obtained by increasing the concentration of the PLGA in inner oil phase, decreasing the PVA concentration in the external phase, and lowering the homogenization speed during the preparation process. The molecular weight of PLGA and the parameters of the preparation show significant influence on *in vitro* release of the drug. *In vitro* and *in vivo* studies demonstrate that the microspheres can release the drug steadily within 25 days.

Declaration of Interest

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