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# Bupivacaine-loaded biodegradable poly(lactic-*co*-glycolic) acid microspheres
# I. Optimization of the drug incorporation into the polymer matrix and modelling of drug release

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**Abstract**

Bupivacaine has been encapsulated by solvent evaporation method based on O/W emulsion, using poly(DL-lactic-*co*-glycolic) acid (PLGA) 50:50. The particle size can be controlled by changing stirring rate and polymer concentration. The encapsulation efficiency was affected by polymer concentration and burst effect of bupivacaine released from particles was affected by drug/polymer mass ratio. Orthogonal design was used to optimize the formulation according to drug content, encapsulation efficiency and burst effect. The dissolution profile and release model were evaluated with two different bupivacaine microspheres (bupi-MS) groups including low drug loading (6.41%) and high drug loading (28.92%). It was observed that drug release was affected by drug loading especially the amount of drug crystal attached on surface of bupi-MS. The drug release profile of low drug loaded bupi-MS agreed with Higuchi equation and that of high drug loaded bupi-MS agreed with first order equation.


*Keywords:* Bupivacaine; Microspheres; Poly(DL-lactic-*co*-glycolic) acid (PLGA); *In vitro* release

## 1. Introduction

In recent years, several approaches have been carried out to obtain controlled release drug delivery systems due to their potential advantages over the conventional drug therapy. These delivery systems can be localized at specific region in the body and make it possible to achieve prolonged pharmacological effects while lowering the systemic concentrations of drugs. Among the different approaches, drugs have been incorporated in biostable polymers as well as in biodegradable system. Several recent publications (Kreuter, 1994; Damge et al., 1988; Quong et al., 1998; Hyon, 2000) review the mostly applied natural or synthetic polymers, such as poly(lactic acid) (PLA), poly(DL-lactic-*co*-glycolic) acid (PLGA), acrylic polymers or copolymers, pluronic polys, alginates and so on. The most widely used polymers are PLA and PLGA because of their quite long history of biodegradation studies, fabrication techniques, application as suture materials and drug delivery system (Yang and Cleland, 1997; Bala et al., 2004). PLA and PLGA provide a wide range of degradation rate, from months to years, depending on their composition and molecular weight (Sah and Chien, 1995; Sah et al., 1994; Luan and Bodmeier, 2006).

The biodegradable microspheres can be prepared by different techniques recently (Jiang and Schwendeman, 2001; Schwendeman et al., 1998; Duarte et al., 2006; Seong et al., 2003), such as solvent evaporation or solvent extraction method, spray drying method, method using fluids under supercritical conditions without toxic residual solvents.

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Local anesthetics which are used for regional anesthesia and for regional control of major pain, are administered either via central routes, i.e. spinal and epidural routes, or via peripheral routes. Bupivacaine has been widely used for local anesthesia after surgery because of its rapid onset and relatively long-lasting anesthetic effect as compared to other commonly used local anesthetics (Arky and Charles, 2003). However, the frequent local administration of a low dose of bupivacaine hydrochloride solution is often required due to its fast local clearance and high systemic absorption which may lead to cardiovascular and central nervous system toxicity. Recent reports have shown that microspheres could be used as a drug delivery system to prolong the duration of action of bupivacaine and to reduce its systemic absorption (Corre et al., 2002).

The influences of drug loading and hydrophilic coating on lidocaine release from PLA and poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles have previously been demonstrated (Gref et al., 1994; Peracchia et al., 1997). It has been observed at the microspheres of this type that bupivacaine release curves comply with a simple square-root formula which serves as a general indicator of the dominant role of a diffusional mechanism (Corre et al., 1997). The process of drug release occurs by several, often simultaneous mechanisms. In the case of drug released from PLA and PLGA micro- or nanoparticles, the following mechanisms have been assumed (Li et al., 2001): (a) surface desorption, (b) diffusion through particle pole, (c) diffusion through an intact polymer, (d) diffusion through a water swollen polymer, (e) surface or bulk erosion of polymer matrix. Other effects are the changes of particle morphology which consequently influence the rate of diffusion release, swelling of the polymer network and solid drug dissolution (Jalil and Nixon, 1990). As has been mentioned above, the dissoution of drug crystals represents an important mechanism, which influences the rate of bupivacaine release from PLGA particles.

Our goal is to design a controlled release delivery system of bupivacaine-loaded PLGA microspheres for spinal anesthesia. In the present article we reported the optimization of microspheres fabrication procedure and drug encapsulation, followed by the characterization of microspheres and the study of drug release under *in vitro* conditions.

## 2. Materials and methods

### 2.1. Materials

Poly(DL-lactic-*co*-glycolic) acid named PLGA RG503 (50:50), $M_w$ 25,000–30,000 g/mol as indicated by the supplier (Boehringer Ingelheim Int. Cop., Germany), bupivacaine (Shanghai Sanwei Pharmaceutical Co. China), sodium alginate (AR Shanghai Reagent Supply Station), dialytic bag (Ø 15 mm, molecular weight of penetrants passing through is 10,000, 15,000, Shanghai Biochemical Reagents Store, China). The organic solvent was methylene chloride (Shanghai Reagent Factory, China).

### 2.2. Preparation of microspheres

The microspheres, loaded with bupivacaine were prepared by an emulsion–solvent evaporation technique (Corre et al., 1994). Typically the solution of polymer 240 mg in 1 mL of methylene chloride containing proper bupivacaine (from 20 to 120 mg) was mixed with 30 mL of 1% sodium alginate solution containing 0.2% Tween 20 (w/w). This mixture was stirred for 15 min by a constant speed stirrer and then removed into a water bath at proper temperature for 3 h to evaporate the organic phase. The microspheres were recovered by filtration and washed twice with 10 mL 0.02 mol/L sodium oxyhydride solution, then dried in vacuum drier. The amount of non-entrapped bupivacaine in the filtrated and washed solution was determined by UV/vis spectrophotometry as described later.

### 2.3. Characterization of microspheres

All microspheres size was measured with Coulter counter (Model TX-II, Coulter Electronic, UK). The microspheres were resuspended in saline together with 0.5% (v/v) Tween 80 and sonicated before counting. The morphology of microspheres was observed by scanning electron microscopy (SEM). SEM requires a previous coating with a palladium:gold (60:40) mixture, which was performed in a Fine coat Sputter at 1.2 kV, 5 mA for 5 min. The microspheres physical status characterization was performed by jointly measuring the differential scanning calorimetry (DSC) and X-ray diffractometry (XRD) patterns. To carry out DSC tests, 4 mg of sample was sealed in standard aluminum pans with lids. The sample was purged with pure dry nitrogen at a flow rate of 50 mL/min. The temperature ramp speed was set at 10 °C/min from 30 to 130 °C. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument. Crystalline state of bupivacaine was analyzed by powder XRD (D/MAX-III B, Rigaku, Japan). A Ni filter at 40 kV and 45 mA generated the radiation. The samples were placed in a quartz sample holder and scanned from 5° to 60° at a scanning rate of 3°/min.

### 2.4. Determination of the loading drugs in microspheres and the free drugs in water phase

The amount of non-entrapped bupivacaine was determined by spectrophotemetry at 263 nm (Warian, USA) .We used this method to determine the non-incorporated bupivacaine in the filtrated and washed solution. The filtrated and washed solution was filtered with Sartorius ultrafiltration system (Shanghai, China) equipped with a membrane filter (cut off 15,000 Da), then the obtained solution was centrifuged at 3000 × *g* for 1 h.

We also directly determined the amount of bupivacaine entrapped in the microspheres. The MS were dissolved in tetrahydrofuran and the solution was filtered by 0.45 μm Millipore filters (Kele, Shanghai, China), HPLC analysis can then be done as previously described (Corre et al., 1997). HPLC system was consisted of a Waters Model 515 pump and a PDA 996 Variable wavelength detector set at 205 nm. Analyses were performed with a Waters Model μBondapack $C_{18}$ column

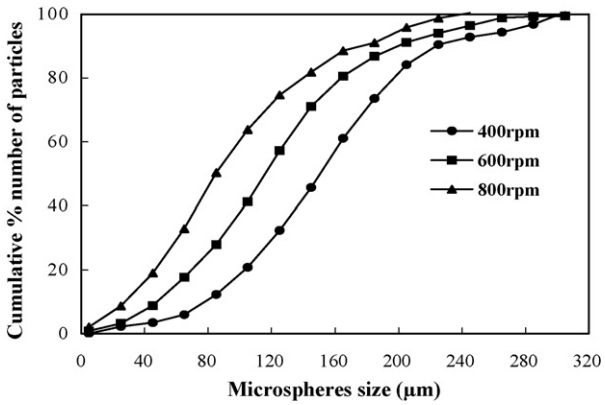


Fig. 1. Effect of stirring rate on bupi-MS size and distribution.

maintained at 30 °C. The mobile phase was acetonitrile–0.01 M pH 4.0 potassium dihydrogenophosphate mixture (40:60) at a flow rate of 1 mL/min.

### 2.5. *In vitro* release study

The *in vitro* release of bupi-MS were carried out with the USPXXII rotating paddle apparatus at 100 rpm and 37 °C under sink condition in 500 mL pH 7.4 PBS. Drug concentration was measured by HPLC method as previously described. Each determination was made in duplicate.

## 3. Results and discussion

### 3.1. Optimization of the particle size, encapsulation efficiency and burst effect of bupi-MS

In the formulation, sodium alginate instead of frequently used PVA, was used as the emulsifier. Compared with microspheres fabricated with PVA or Pluronic F-68, microspheres made with sodium alginate had more spherical shape, similar size and size distribution in our experiments. This suggested that sodium alginate could be a better emulsifier than PVA to fabricate bupi-MS in the emulsion–solvent evaporation technique.

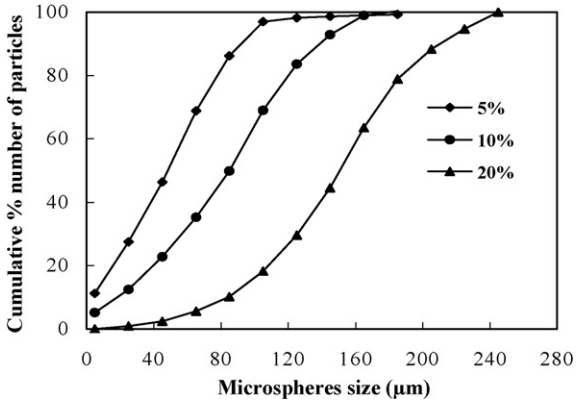


Fig. 2. Effect of PLGA concentration on bupi-MS size and distribution.

Table 1
Effect of stirring rate on bupivacaine content and encapsulation efficiency of bupi-MS

| Stirring rate (rpm) | Theoretical drug content (%) | Measured drug content (%) | Drug entrapment efficiency (%) |
|---|---|---|---|
| 400 | 33.33 | 29.41 | 88.24 |
| 600 | 33.33 | 30.05 | 90.16 |
| 800 | 33.33 | 30.46 | 91.38 |

Table 2
Effect of PLGA concentration on encapsulation efficiency of bupi-MS

| PLGA concentration (%) | Theoretical drug content (%) | Measured drug content (%) | Drug entrapment efficiency (%) |
|---|---|---|---|
| 5 | 33.33 | 26.20 | 78.62 |
| 10 | 33.33 | 27.81 | 83.43 |
| 20 | 33.33 | 30.32 | 90.98 |

In order to optimize the particle size, encapsulation efficiency and burst effect of drug release from bupi-MS, only one parameter was changed in each series of experiments and all other experimental conditions were left constant as described in Section 2.2. In all experiments described in this article, the mean particle size decreased along with the increasing of stirring rate (Fig. 1), probably because higher stirring rate provided more powerful shearing force to separate the oil phase into smaller droplets (Yang and Owusu, 2000). Fig. 2 shows the influence of polymer concentration on the microspheres size. It was noticeable that the size of the microspheres was significantly influenced by the polymer concentration. An increase in the polymer concentration resulted in a larger particle diameter. This might be explained by a greater probability of fusion of semi-formed particles when they ran into each other in the medium. In addition, increasing the concentration of dissolved polymer also increased the viscosity of the organic phase, which might prevent an optimal shearing of the emulsion when agitated (Jeffery et al., 1991; Sturesson et al., 1993). Table 1 reports the effect of stirring rate on encapsulation efficiency of bupi-MS. The results showed that encapsulation efficiency was not seriously affected by stirring rate. As shown in Table 2, the encapsulation efficiency increased along with increasing polymer concentration. This might because an increasing polymer concentration at a fixed internal phase volume resulted in a decreasing porosity of the particles with an increased volume weight mean diameter and encapsulation efficiency of drug (Edith et al., 1997). Table 3 shows the influence of drug/PLGA ratio on the burst effect and

Table 3
Effect of drug/PLGA ratio on burst effect of release of bupi-MS

| Drug/PLGA mass ratio | Mean particle diameter (μm) | Burst effect of release in 30 min (%) |
|---|---|---|
| 1:7 | 112 ± 42 | 12.20 |
| 1:4 | 121 ± 87 | 25.41 |
| 1:2 | 128 ± 99 | 39.46 |
| 1:1 | 119 ± 105 | 58.23 |

Table 4
Orthogonal design and results

| Test number | Drug/PLGA mass ratio | Temperature (°C) | $S_1$ | $S_2$ | $S_3$ | $S$ |
|---|---|---|---|---|---|---|
| 1 | 1:12 | 35 | 0.0641 | 0.8845 | 0.1523 | 0.7963 |
| 2 | 1:12 | 45 | 0.0563 | 0.8824 | 0.1541 | 0.7846 |
| 3 | 1:12 | 55 | 0.0578 | 0.8676 | 0.1634 | 0.7620 |
| 4 | 1:6 | 35 | 0.1224 | 0.8977 | 0.2116 | 0.8085 |
| 5 | 1:6 | 45 | 0.1088 | 0.8211 | 0.2241 | 0.6978 |
| 6 | 1:6 | 55 | 0.1002 | 0.6820 | 0.2268 | 0.5554 |
| 7 | 1:2 | 35 | 0.2892 | 0.8677 | 0.3452 | 0.8117 |
| 8 | 1:2 | 45 | 0.2737 | 0.8211 | 0.3646 | 0.7302 |
| 9 | 1:2 | 55 | 0.2273 | 0.6820 | 0.3753 | 0.5340 |

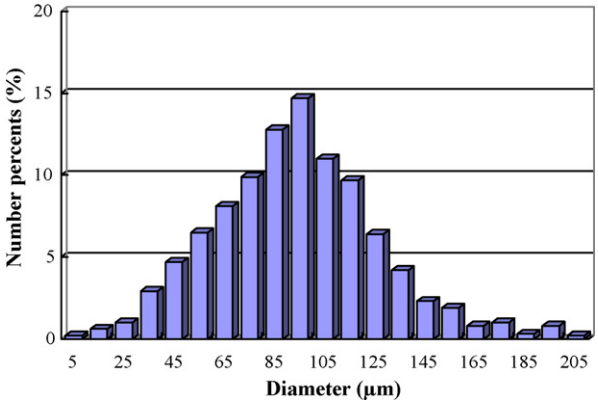


Fig. 3. Particle size distribution of bupi-MS estimated by Coulter counter.

mean particle size. It was remarkable that the burst effect was significantly influenced by the drug/PLGA ratio. An increase in drug/PLGA ratio resulted in increasing burst effect. Increase of initial drug loading amount at the same PLGA concentration means decrease of relative amount of PLGA that could encapsulate drug and hence increase of burst effect could be explained by relatively more drug could not be encapsulated in polymer microspheres or might be existed on surface of microspheres (Seong et al., 2003). The mean particle size was not affected by the drug/PLGA ratio.

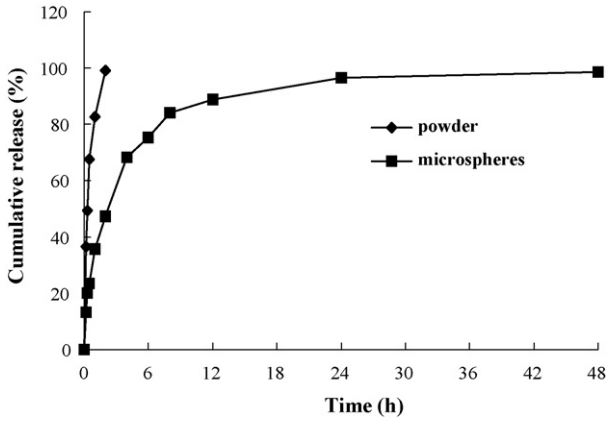


Fig. 5. Cumulative percent drug release as a function of time of bupivacaine from powder and low drug loaded bupi-MS.

### 3.2. Optimization of microspheres formulation

Orthogonal design was used to optimize the formulation according to drug content, encapsulation efficiency and burst effect (Table 4)

$$S_1 = \left(\frac{\text{content of drug}}{\text{weight of MS}}\right) \times 100\%$$

$$S_2 = \left(\frac{\text{determined content of drug}}{\text{theoretical content of drug}}\right) \times 100\%$$

$$S_3 = \left(\frac{\text{accumulative release amount in 30 min}}{\text{total amount of drug in MS}}\right) \times 100\%$$

$$S = S_1 + S_2 - S_3$$

### 3.3. Size distribution and morphology

Fig. 3 shows the particle size distribution of bupi-MS. Ninety-five percent particle size focus on the range from 45 to 135 μm. SEM observation showed that bupi-MS were spherical (Fig. 4). Low drug loaded bupi-MS possessed a smooth surface with

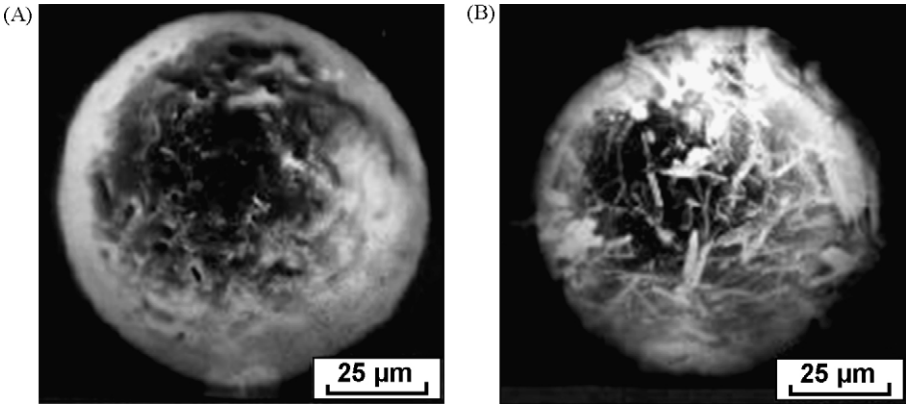


Fig. 4. Micrograph of bupi-MS by scanning electron microscopy. (A) Low drug loaded bupi-MS (6.41%), (B) high drug loaded bupi-MS (28.92%).

Table 5
Correlation coefficients corresponding to the given models of low drug loaded bupi-MS

| Test number | Zero order A | Higuchi B | First order C | Ritger-peppas D |
|---|---|---|---|---|
| 1 | 0.8720 | 0.9884 | 0.9731 | 0.9346 |
| 2 | 0.8728 | 0.9889 | 0.9755 | 0.9331 |
| 3 | 0.8785 | 0.9898 | 0.9758 | 0.9335 |
| 4 | 0.8731 | 0.9883 | 0.9774 | 0.9323 |
| 5 | 0.8780 | 0.9908 | 0.9787 | 0.9369 |
| 6 | 0.8813 | 0.9926 | 0.9826 | 0.9354 |
| Mean | 0.8760 | 0.9898 | 0.9772 | 0.9343 |
| S.D. | 0.0038 | 0.0016 | 0.0033 | 0.0017 |

Table 6
Correlation coefficients corresponding to the given models of high drug loaded bupi-MS

| Test number | Zero order A | Higuchi B | First order C | Ritger-peppas D |
|---|---|---|---|---|
| 1 | 0.8142 | 0.9610 | 0.9875 | 0.8786 |
| 2 | 0.7903 | 0.9556 | 0.9796 | 0.8824 |
| 3 | 0.7892 | 0.9534 | 0.9796 | 0.8775 |
| 4 | 0.7880 | 0.9539 | 0.9805 | 0.8820 |
| 5 | 0.7853 | 0.9526 | 0.9773 | 0.8744 |
| 6 | 0.7887 | 0.9520 | 0.9821 | 0.8756 |
| Mean | 0.7926 | 0.9548 | 0.9811 | 0.8784 |
| S.D. | 0.0107 | 0.0033 | 0.0035 | 0.0033 |

occasional pores and some drug crystals on the surface of high drug loaded bupi-MS were observed.

### 3.4. DSC and XRD analysis

In our work, differential scanning calorimetry and X-ray diffractometry techniques have been employed to study the physical characteristics of bupi-MS. Crystalline state of bupivacaine was analyzed by XRD and thermal characteristics were determined by DSC. The results indicated that bupivacaine trapped in the microspheres existed in crystallization status in the polymer matrix (figures were not presented within the paper).

### 3.5. In vitro drug release kinetics

In order to understand the release kinetics of bupi from bupi-MS, two different particle groups including low drug loaded bupi-MS (6.41%) and high drug loaded bupi-MS (28.92%) were prepared. The drug release was determined according to Section 2.5. Fig. 5 shows the in vitro release profile of bupivacaine from low drug loaded bupi-MS. Higher release rate was observed in the initial 8 h, which may correspond to release of drug on the surface of microspheres. From 8 to 48 h, the drug release rate was relatively constant, suggesting that the entrapped bupivacaine began to release. By fitting the observed data shown in Tables 5–7 to several release models (zero order, Higuchi, first order, Ritger-peppas). We could found that drug release model was affected by drug loading especially the quantity of drug crystal attached on surface of bupi-MS. The drug release from low drug loaded bupi-MS can be better agreed with Higuchi equation and the drug release from high drug loaded bupi-MS can be agreed with first order equation. This might be explained by the fact that the release rate and pattern of drug from the PLGA matrix is mainly dependent on not only degradation of PLGA but also diffusion of drug through the matrix (Batycky et al., 1997). The concentration of bupivacaine released from bupi-MS prepared with prescription 1 and from bupivacaine powder were studied as a function of time, respectively. The results over 48 h are shown in Fig. 5 and the former could be better agreed with Higuchi equation ($Q\% = 6.10 + 26.88t^{1/2}$, $r = 0.9850$) during the first 12 h (Fig. 6). Compared with bupivacaine powder, prolonged release of bupivacaine from microspheres was observed. In the case of our high drug loaded particles (28.92%), the drug exists in crystalline form on the surface (observed in Fig. 4) and probably inside the bupi-MS, so the burst effect is higher than that of low drug loaded microspheres. According to Section 3.2, the prescription 7 was selected to be the best prescription which was used for the pharmacokinetics and pharmacodynamics researches later.

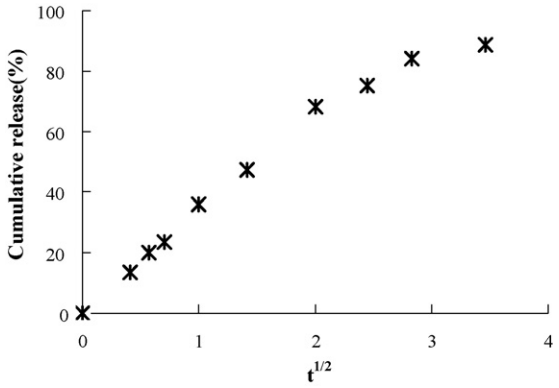


Fig. 6. The correlation between cumulative release and square root of release time.

Table 7
Results of paired *t*-test

| Groups | | A × B | A × C | A × D | B × C | B × D | C × D |
|---|---|---|---|---|---|---|---|
| Low drug loaded | *t*-Value | 13.88 | 32.03 | 13.87 | 6.89 | 6.05 | 23.44 |
| Bupi-MS(6.41%) | Test | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ |
| High drug loaded | *t*-Value | 16.22 | 44.27 | 20.93 | 2.85 | 8.36 | 76.07 |
| Bupi-MS(28.92%) | Test | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.05$ | $P < 0.01$ | $P < 0.01$ |

## 4. Conclusions

The emulsion–solvent evaporation procedure allowed us to prepare spherical bupivacaine-loaded biodegradable PLGA microspheres. The particles size can be controlled by changing stirring rate and polymer concentration. The drug/polymer mass ratio can dramatically affect the morphology of bupi-MS related to the drug release rate and release model. Effect of drug/polymer mass ratio on mean particle size was not observed. Drug/polymer mass ratio can also affect the drug content, encapsulation efficiency and burst effect. A novel observation has been made in this study that the drug release of low and high drug loaded MS were well agreed with Higuchi and first order model, respectively. With low loaded microspheres (6.41%), a long-lasting release over 48 h was observed, but the release rate was well agreed with Higuchi model only during the first 12 h. There is a negative deviation which is similar to the previous paper of Higuchi (Higuchi, 1963).

Generally, the duration of action of bupivacaine is 3–6 h with a single injection of drug. In our work, after an initial burst, a continuous drug release was observed for up to 24 h which meets the need of prolonging the duration of action of drug.

According to our original experimental design, the time of bupivacaine released from microspheres should be from about 12 to 24 h with suitable burst release which served as the first dose to achieve fast anesthetic effect. The release result we got in the paper can basically be matched with the purpose of our experiment.

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