Division of Pharmaceutics, Department of pharmacy, Shenyang Pharmaceutical University, Shenyang, P. R. China

Preparation and evaluation of poly (D, L-lactic acid) (PLA) or D, L-lactide/glycolide copolymer (PLGA) microspheres with estradiol

Z. XINTENG, P. WEISAN, Z. RUHUA and Z. FENG

PLA/PLGA was used for biodegradable and biocompatible carriers to achieve sustained release of estradiol. Microspheres were formed by an emulsification-solvent evaporation method, and then their properties and *in vitro* drug release behavior were examined including amongst others the effects of the concentration of PVA in the aqueous phase, the concentration of PLA in the organic phase, the stirring speed, the volume ratio of O/W, the weight ratio of E_2/PLA fed, and the type and molecular weight of the polymers.

1. Introduction

The aim of the present work was to investigate an emulsification–solvent evaporation method to prepare PLA/PLGA/estradiol microspheres. Any effects of drug entrapment, size distribution and *in vitro* drug release were investigated.

2. Investigations, results and discussion

2.1. Drug entrapment efficiency

The microparticle formulation used for investigation of the drug entrapment efficiency was as follows: the organic phase was 2% PLA methylene chloride solution, the aqueous phase was 2% PVA aqueous solution (or 0.5%, 1.0% and 2.0% PVA, to study the effect of the concentration of PVA on the drug entrapment efficiency, results shown in Table 1), the oil/water phase volume ratio was 1 : 4, the ratio of the weight of E_2/PLA fed was 1 : 13 (1 : 20,1 : 16 and 1 : 10, to study the effect of the ratio of the weight of E_2/PLA fed on the drug entrapment efficiency, results shown in Table 2) and the stirring speed was 400 rpm. After the organic solvent was evaporated, the microspheres were sieved and desiccated under reduced pressure. The drug entrapment efficiency of microspheres with particle size between 80 mesh and 400 mesh was between 58 and 63%.

Methylene chloride was one of the most popular organic solvents used in the preparation of microspheres by a method of emulsification-evaporation because of its low toxicity, low boiling point and proper solubility in water [7, 8]. In the preparation process, after the form of an oil-in-water emulsion methylene chloride within the droplets diffused out and dissolved in the aqueous phase, then evaporated off from the interface between the water and the air. During this process E_2 was carried out by methylene chloride into the outer water phase and exited in the form of crystal on the surface of the microspheres or suspended in the water phase, especially when the weight ratio of E_2/PLA was high (e.g. >1 : 13,w/w). Under some certain conditions, the E_2-carrying ability of methylene chloride was limited, so the drug entrapment efficiency increased with proper increasing of the ratio of E_2/PLA fed. But when the ratio increased further, the efficiency decreased probably because of the low solubility of E_2 in methylene chloride and the saturation of E_2 in the microspheres thereafter.

When the particle size was smaller, the surface area of microspheres was greater, then the interface area between the oil phase and the water phase was larger, the rate and the magnitude of E_2's out-diffusion were greater, thereafter the drug entrapment efficiency was lower.

Table 1: Effect of the concentration of PVA on the drug entrapment efficiency

PVA% (w/v)	Entrapment efficiency (%)
0.5	87.25
1.0	86.97
2.0	62.93

Table 2: Effect of the mass ratio of E_2 to PLA on the drug entrapment efficiency

E_2 : PLA (w/w)	Entrapment efficiency (%)
1 : 20	70.69
1 : 16	72.48
1 : 13	62.93
1 : 10	56.40

2.2. Particle size distribution

The microparticles formation formula used for investigation of the size distribution was as the following: the organic phase was 2% PLA methylene chloride solution (or 0.5%, 1.0% and 2.0% PLA, to to study the effect of the concentration of PLA on the particle size distribution, results were shown in Fig. 1), the aqueous phase was 2%

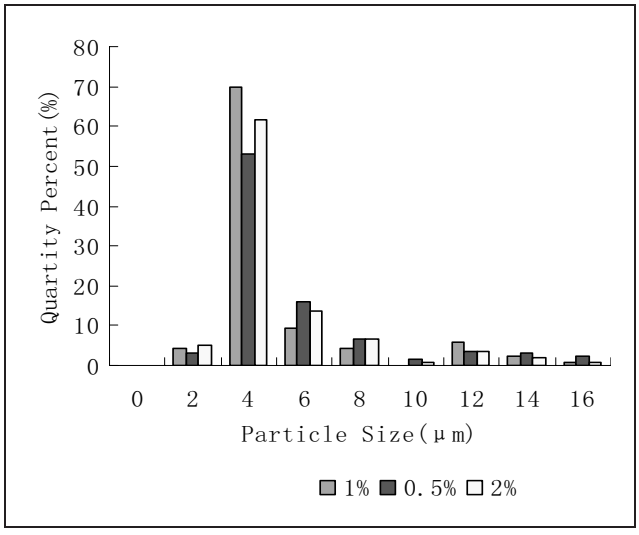


Fig. 1: Effect of the concentration of PLA on the particle size

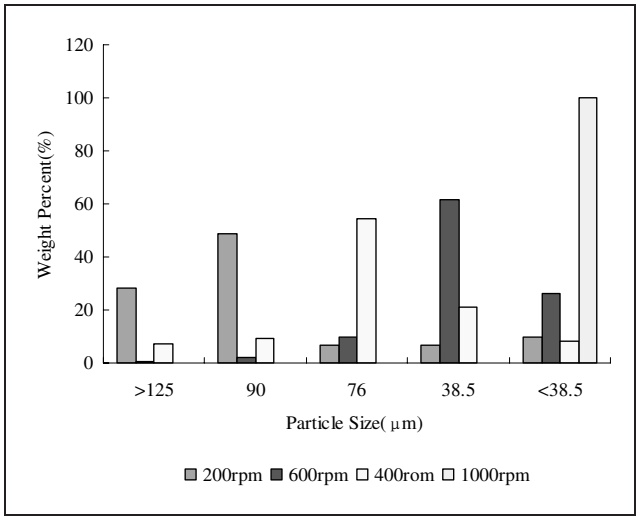


Fig. 2: Effect of the stirring speed on the particle size

PVA aqueous solution (or 0.5%, 1.0% and 2.0% PVA, to study the effect of the concentration of PVA on the particle size distribution, results were not shown) the oil/water phase volume ratio was 1 : 4 (or 1 : 6, and 1 : 8, to study the effect of the oil/water phase volume ratio on the particle size distribution, results analogous to Fig. 1), the ratio of the weight of E_2/PLA fed was 1 : 13, and the stirring speed was 1000 rpm (or 200 rpm, 400 rpm and 600 rpm, to study the effect of the stirring speed on the particle size distribution, (results shown in Fig. 2). After the organic solvent was evaporated off, the microspheres were sieved and desiccated under reduced pressure, then microspheres with different size range were weighed, and the particle size weight percent distribution graph was constructed. When the most of the particles were less than 400 mesh, a microscope was used to measure the particle size with the micrometer, and the particle size quantity percent distribution graph was constructed ($n > 500$).

From the figures we can see that over a certain range the particle size increased with an decrease of the stirring speed, decreased as the concentration of PVA in the aqueous phase and PLA in the organic phase decreased, and there appeared to be no volume ratio of oil/water dependency in the range studied.

2.3. In vitro release behavior of E_2 in PLA/PLGA microspheres

Microspheres were formed by the method described below, in which the organic phase was 2% PLA methylene chloride solution, the aqueous phase was 2% PVA aqueous solution, the oil/water phase volume ratio was 1 : 4, and the ratio of the weight of E_2/PLA fed was 1 : 13. After the microspheres were dried, *in vitro* release was studied with the dialysis method to investigate the effect of the type, molecular weight and particle size on the in vitro release of E_2 from the microspheres, as shown in Figs. 3–5.

From the figures above we can see that for the various particle size ranges of microspheres of various polymers E_2 was completely released during in vitro studies, and the smaller the microsphere size, the more rapidly was the drug re-

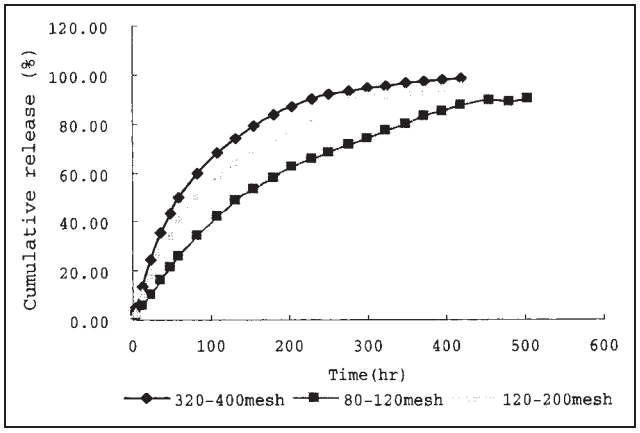


Fig. 3: Effect of particle size on drug dissolution of PLA_{9000}-E_2-MS

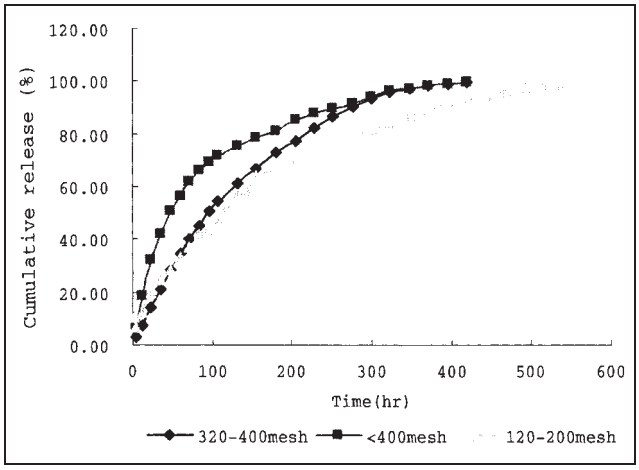
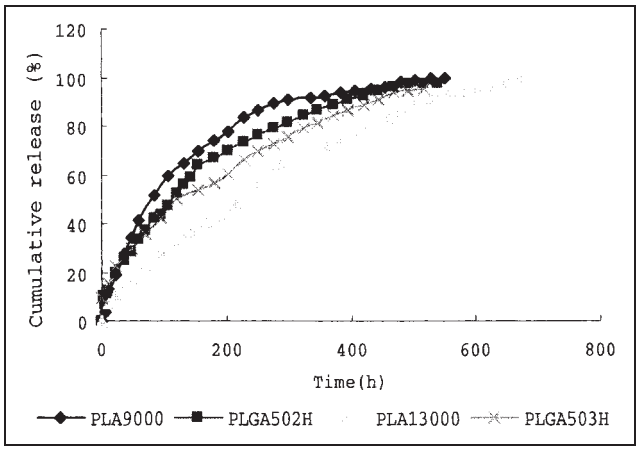
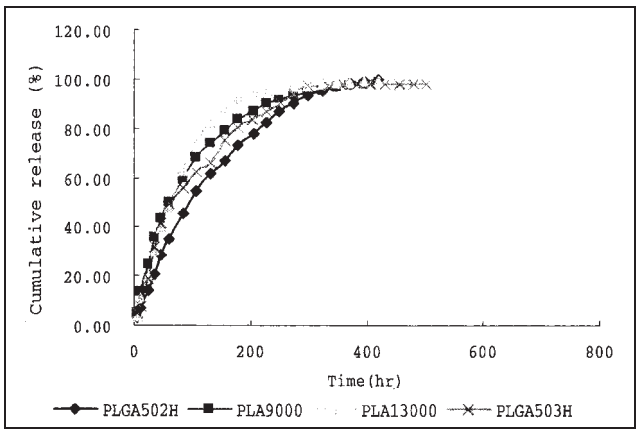


Fig. 4: Effect of particle size on drug dissolution of $PLGA_{502H}$-E_2-MS



(a) Size: 120–200 mesh



(b) Size: 320–400 mesh

Fig. 5: Effect of the type and molecular weight of polymers on drug dissolution

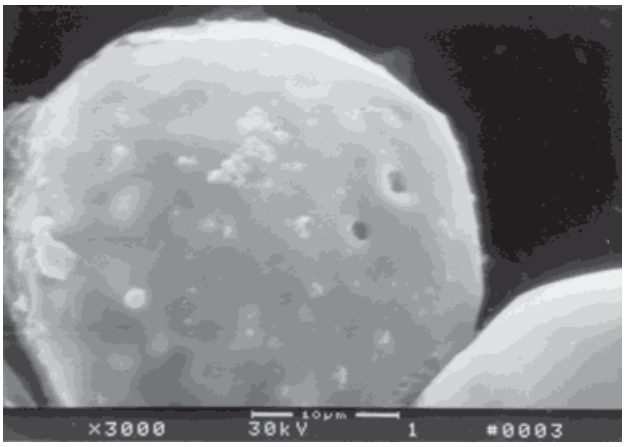


Fig. 6: Scanning electron photomicrograph of PLA_{13000}-E_2-MS before release

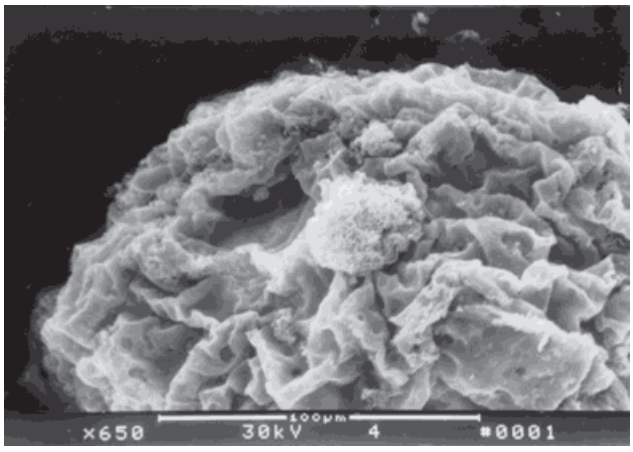


Fig. 7: Scanning electron photomicrographs of PLA_{13000}-E_2-MS after release for 900 h

leased, because the smaller the microsphere size, the greater was the surface area available for dissolution of the drug.

2.4. Observation of PLA/PLGA microspheres by SEM

Microspheres were formed by the method described below, in which the organic phase was 2% PLA methylene chloride solution, the aqueous phase was 2% PVA aqueous solution, the oil/water phase volume ratio was 1 : 4, and the ratio of the weight of E_2/PLA fed was 1 : 13. After desiccation, the microspheres were observed and photographed by SEM before release and for 900 h after release, and some of the microspheres were fractured under force to observe the inner morphology of the microspheres, as shown in Figs. 6–7.

From the photographs, we can see that the surface of the microspheres before release was smooth and had few holes, while the surface of microspheres 900 h after release was rough and the surface and the inner side were both porous, so it can be assumed that the drug release was mainly through diffusion.

3. Experimental

3.1. Materials

17β-Estradiol (E_2) was supplied by Zhejiang Xianju Pharmaceutical Corporation. PLA with an average molecular weight of 9000 and 13000 was supplied by Shandong Medical Machine Institute. The weight average molecular weight (Mw) was determined by gel permeation chromatography (GPC) by the supplier. PLGA (RESOMER®502H, 503H) was supplied by Boehringer Ingelheim, Germany. Polyvinyl alcohol (PVA) was supplied by Beijing Dajiaoting Organic Chemical Plant. Methylene chloride of analytic grade from Shenyang Chemical Agent Laboratory was used without further purification and water used was distilled.

3.2. Preparation of PLA/PLGA microspheres

Microspheres made of PLA/PLGA were prepared by an emulsion-solvent evaporation method [3–5]. PLA/PLGA and E_2 were dissolved in methylene chloride, and the resultant organic solution was added dropwise into the aqueous PVA solution under moderate mechanical stirring at room temperature to form an oil-in-water (O/W) emulsion. The stirring was continued until the organic solvent was evaporated off about 5 h later. The suspension formed containing microspheres was sieved through a series of standard sieves with the aid of water, and after washing with water three times, the microspheres were collected and desiccated under aspirator-reduced pressure. The microspheres with size less than 400 mesh were separated from the aqueous phase by centrifugation and then desiccated as mentioned above.

3.3. Measurement of size and entrapment of microspheres

A microscope was used to observe the process of formation of microspheres and their appearance. After desiccation, microspheres in different size ranges were weighed, and the particle size distribution graph was made. The resultant microspheres after desiccation were dissolved in 2% sodium hydroxide aqueous solution, which was analyzed at 280 nm by means of a HPLC technique after dilution with water with methanol-0.01M PBS (pH 7.0) (3.2 : 1,v/v) as the mobile phase, ODS as the fixed phase and 280 nm as the wavelength. The drug entrapment efficiency in the microspheres is represented by eq (1).

$$\text{Drug entrapment efficiency} = \frac{\text{Amount of drug in the microspheres}}{\text{Amount of drug fed into the systems}} \times 100 \quad (1)$$

3.4. In vitro release behavior of E_2 in PLA/PLGA microspheres

First, the effect of the dialyzer on the diffusion of E_2 was measured. A given volume of E_2-pH 7.4 PBS solution with known concentration was added accurately to the dialyzer, which was preprocessed. After sealing, the dialyzer was suspended in a glass bottle with pH7.4 PBS solution as the release medium from the dialyzer and this was maintained at 37 °C with magnetic stirring. At given intervals the medium outside was sampled and analyzed by HPLC. The outside/inside dialyzer concentration ratio vs time was graphed.

The microspheres (10 mg) were suspended in 5ml of pH7.4 PBS within the dialyzer which was suspended in the glass bottle. An appopriate volume of the same release medium was added outside the dialyzer and maintained at 37 °C with magnetic stirring. On the first day it was sampled at 1, 4, 8, 12 and 24 h intervals and on the following days it was sampled every 12 or 24 h. The medium out side of the dialyzer was all replaced when sampled on the following days and was filtered and assayed by the HPLC method mentioned above. After some days the drug content retained within the microspheres was assayed to further verify the release characteristics.

3.5. Observation of PLA/PLGA microspheres by SEM

The dried microspheres before release and 900 h after release were observed with a scanning electron microscope to examine their shapes, surface characteristics and inner structure.

References

1 Bodmeier, R.; McGinity, J. W.: Int. J. Pharm. **43**, 179 (1988)
2 Benoit, J. P.; Courteille, F.; Thies, C: Int. J. Pharm. **29**, 95 (1986)
3 Wakiyama, N.; Juni, K.; Nakano, M.: Chem. Pharm. Bull. **30**, 2621 (1982)
4 Corre, P. L.; Guevello, P. L.; Gajan, V.; Chevanne F.; Verge R. L.: Int. J. Pharm. **107**, 41 (1994)
5 Uchida, T.; Yoshida, K.; Nakada, Y.; Noriko N.; Konishi Y.; Nakai A.; Nishikata M.; Matsuyama K.: Chem. Pharm. Bull. **45**, 513 (1997)

Received March 14, 2002
Accepted May 2, 2002

Xinteng Zhou
Shen Yang Pharmaceutical University
Department of Pharmacy
Wen Hua RD 103
110016 Shen Yang
P. R. China
zhouxint@21cn.com