Original Article

# Preparation and Characterization of Estradiol Valerate Microspheres Using Biodegradable Polymers

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## Abstract

In this study, microspheres containing estradiol valerate were prepared by solvent evaporation method using poly (glycolide-co-lactide) (PLGA 50:50) and poly (lactide). The effect of different process variables such as polymer type, drug-polymer ratio, stirring rate, volume of internal phase and temperature of external phase on the morphology, particle size distribution, encapsulation efficiency and *in vitro* release profile were investigated. All microspheres had spherical shape with smooth surface. Increasing the internal phase volume and decreasing the external phase temperature resulted in smaller particles. At low drug loadings, PLGA microspheres were larger than PLA ones. Changing the theoretical drug loading from 5 to 20% decreased the drug release rate from PLGA microspheres whereas the result for PLA microspheres was the opposite.

## 1. Introduction

Estradiol valerate is an analog of estrogen that is used for the management of primary hypogonadism, ovarian failure and post menopausal syndrome. It is also used as contraceptive agent [1, 2]. Much attention has recently been directed toward the use of injectable polymeric drug delivery systems. Application of such systems to control the release of contraceptive drugs has also been investigated. In order to avoid the inconvenient surgical insertion of large implants, injectable biodegradable and biocompatible polymeric particles (microspheres, microcapsules, nanocapsules, and nanospheres) could be employed for controlled release dosage forms [3]. The polymers selected for the parentral administration must meet several requirements like biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties, and ease of processing [4]. These polymers degrade *in vivo* by hydrolytic reactions and produce nontoxic metabolites. Notable among these polymers are poly lactic acid and poly glycolic acid and their copolymers. They have a long history

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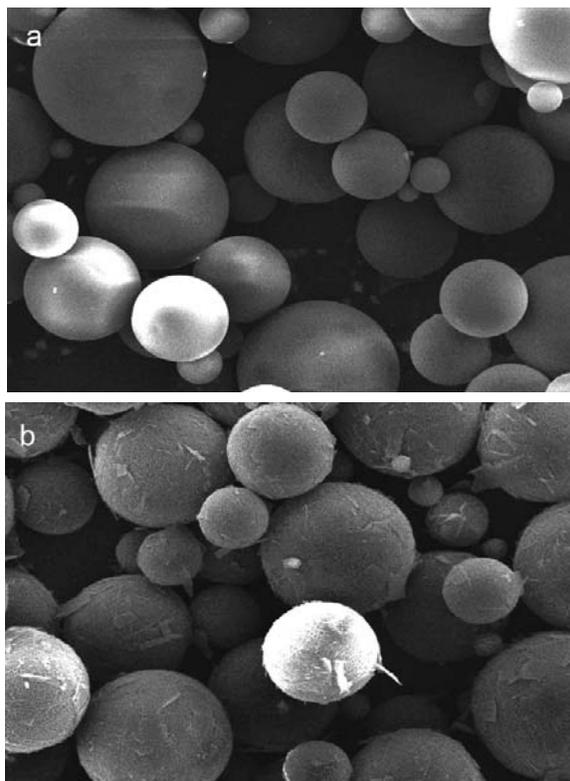E-mail: dinarvand@sina.tums.ac.ir

**Figure 1.** Scanning electron micrograph of PLGA microspheres with 2.5% (a) and 20% (b) drug loading.

of safe use in human for a wide variety of clinical applications. These polymers can also be administered orally, parenterally or via aerosols for site-specific delivery [5].

Injectable methods of contraception have been developed after oral contraception. These substances are released in the blood circle gradually through i.m. injection, therefore, they have prolonged action. It is estimated that almost 12 million women use injectable steroidal formulations all over the world.

Studies have shown that monthly contraception had been affected and they are tolerated well [6].

The aim of this study was to prepare biodegradable microspheres containing estradiol valerate and to evaluate the effect of different formulation parameters on microspheres characteristics.

## 2. Experimental
### 2.1. Materials

Estradiol valerate (USP) was purchased from Abureihan Co (Iran). Poly lactide-co-glicolide 50:50 (PLGA) (Resomer RG503H) (50:50) i.v. 0.2 dl/g and poly (L-lactide-co-D,L-lactide) (PLA) (Resomer LR 708) i.v. 6 dl/g were purchased from Boehinger Ingelheim, Germany. Poly vinyl alcohol (PVA) (MW 72000 g/mol, 87–89% hydrolyzed), dichloromethane (DCM), dodecyl sulfate sodium salt (SLS) and tween 20 were purchased from Merck, Germany. Ethanol 96 was supplied by Ettehadieh Co, Iran.

### 2.2. Preparation of PLGA microspheres

The emulsification solvent evaporation method was used to prepare microspheres of estradiol valerate. Known amounts of estradiol valerate and polymer (PLA or PLGA) were dissolved in DCM which was used as the internal phase. The external phase was prepared by dissolving 500 mg PVA in 100 ml distilled water, then the internal phase solution

**Table 1.** Effect of theoretical drug loading on encapsulation efficiency of estradiol valerate microspheres.

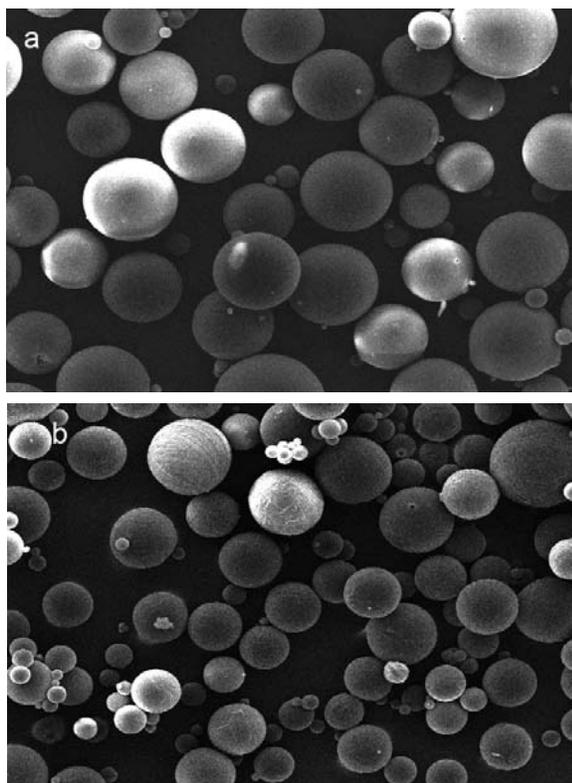| Polymer type | Theoretical drug loading (%) | Actual drug loading (%) | Loading efficiency (%) |
|---|---|---|---|
| PLGA | 2.5 | 2.70 | 108.0 |
| PLGA | 5.0 | 5.25 | 105.0 |
| PLGA | 10.0 | 9.92 | 99.2 |
| PLGA | 15.0 | 13.85 | 92.3 |
| PLGA | 20.0 | 16.97 | 84.8 |
| PLA | 2.5 | 2.70 | 108.0 |
| PLA | 5.0 | 5.11 | 102.0 |
| PLA | 10.0 | 9.25 | 92.5 |
| PLA | 15.0 | 12.95 | 86.3 |
| PLA | 20.0 | 17.07 | 85.3 |

**Figure 2.** Scanning electron micrograph of PLA microspheres with 2.5 (a) and 20% (b) drug loading.

was added drop wise in a rate of 2 ml/min to the external phase (which was cooled with ice bath) to form an emulsion. The mixture was stirred for 3-5 h at a constant rate by a mechanical mixer to complete evaporation of DCM and formation of microspheres. The microspheres were then filtered, washed with distilled water and dried at the room temperature for 24 h. The effect of different variables such as the amount of estradiol valerate (2.5, 5, 10 or 20% w/w in comparison with polymer), mixing rate (500 or 800 rpm), temperature of the external phase (room temperature or cooled in the ice bath) and the external phase volume (10 or 15 ml) were also studied.

### 2.3. SEM study

Morphology of microspheres was examined by scanning electron microscope (SEM) (DSM 960A, Zeniss, Germany). Samples were mounted on metal stubs and sputter-coated with gold for 4 min prior to examination under SEM.

### 2.4. Particle size analysis

Particle size analysis of microspheres was performed using sieve analysis with mesh sizes of 150 and 300 μm. All of the samples (500 mg) were sieved for 3 min and the weight percentage of the microspheres remained on each sieve was determined.

### 2.5. Encapsulation efficiency

An accurately weighed amount of estradiol valerate microspheres were dissolved in 10 ml DCM. The solution was filtered through a 0.22 μm membrane filter and analyzed at 280 nm wavelength with a UV spectrophotometer (Scinco 3100, Korea). PLA showed no absorbance at this wavelength. The encapsulation efficiency was calculated as follows:

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) × 100

### 2.6. In vitro drug release test

Estradiol release from microspheres was investigated by static method [5, 7]. Briefly, exactly weighed amounts of PLGA microspheres (50-100 mg) were added to the vials containing 25 ml dissolution medium (3% SLS, 10% ethanol and 0.06% tween 20 in distilled water to maintain sink condition) maintained at thermostatic water bath at 37±0.5 °C. Vials were shaken slowly at 8 h time intervals. At predetermined time intervals 5 ml of the release medium was withdrawn and filtered through 0.22 μm membrane filter and analyzed spectrophotometerically at 280 nm. The sample was replaced by 5 ml fresh dissolution medium.

## 3. Results and discussion

PLA and PLGA microspheres containing estradiol valerate were prepared using emulsification-solvent evaporation method. Figures 1a and 1b show the SEM photographs of PLGA microspheres with 2.5% and 20%
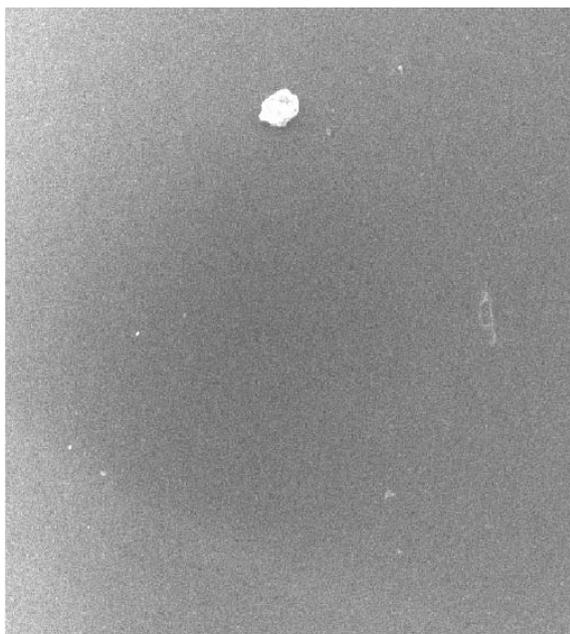
**Figure 3.** Scanning electron micrograph of the surface of a PLA microsphere after drug release.

drug loading, respectively. PLGA microspheres with 2.5% drug loading were spherical with smooth surface whereas microspheres with 20% estradiol were not quite smooth in surface. PLA microspheres with both 2.5 and 20% drug loading were spherical with fairly smooth surface too (Figure 2a and 2b). Figure 3 depicts the SEM photograph of PLA microspheres after 60 days of drug release in the dissolution medium. As it can be seen, the surface of PLA microspheres remained smooth and no change was observed after the drug release period. It is due to long biodegradation period

of the PLA polymer which led to drug release only through diffusion mechanism.

The effect of internal phase volume on particle size distribution of PLA microspheres with 10% drug loading is shown in Figure 4. As shown if Figure 4, by increasing the volume of the internal phase from 10 ml to 15 ml, the microspheres size decreases. This may be attributed to a decrease in the internal phase viscosity which makes the coalescnece of emulsified dispersed droplet less probable [8, 9].

The effect of polymer type on particle size distribution in the presence of different amounts of estradiol valerate in the microspheres is shown in Figures 5 and 6. According to Figure 5, PLGA microspheres with low drug loading (2.5%) were larger than PLA microspheres with the same drug content. However, at higher drug loading (20%), there was no significant difference between particle size distribution of PLGA and PLA microspheres (Figure 6).

The effect of external phase condition on particle size distribution of PLGA microspheres is shown in Figure 7. According to this figure, cooling the external phase by using an ice bath resulted in smaller particle size. Lowering the temperature of the external phase prevents the early evaporation of dichloromethane during the process of microencapsulation and gives enough time to the emulsion droplets to be broken to the
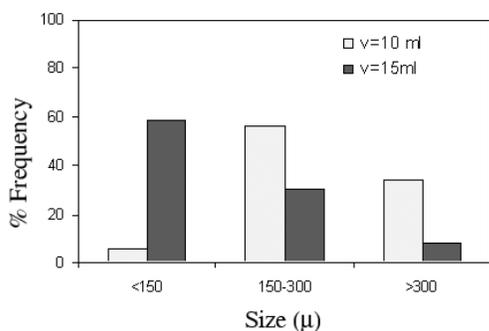


**Figure 4.** Effect of internal phase volume on particle size distribution of PLA microspheres with 20% drug loading.
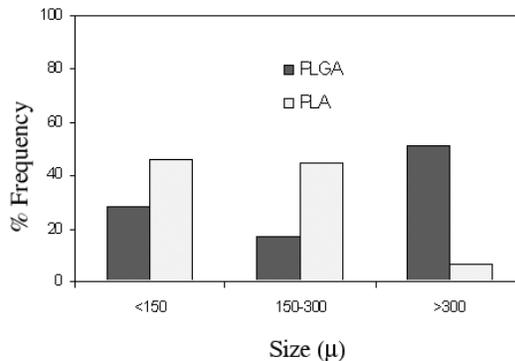


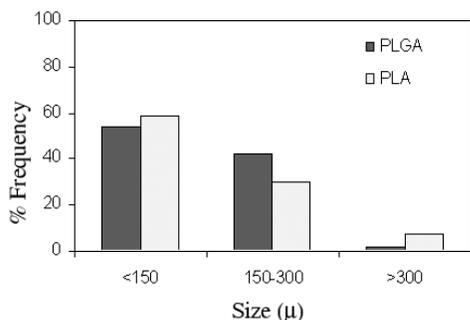**Figure 5.** Effect of polymer type on particle size distribution of microspheres containing 2.5% drug.

**Figure 6.** Effect of polymer type on particle size distribution of microspheres containing 20% drug.



**Figure 7.** Effect of non-cooled (A) or cooled (B) external phase on particle size distribution of PLGA microspheres.

smaller ones under shearing force caused by stirring propeller.

By increasing the rate of stirring from 500 to 800 rpm, particle size of microspheres decreased (Figure 8). This was expected because high stirring rates provide the shearing force needed to separate the internal oil phase to smaller droplets [10, 11].

Table 1 shows the effect of theoretical drug loading on encapsulation efficiency of different formulations. By increasing the theoretical drug loading from 2.5 to 20%, encapsulation efficiency decreased both for PLA and PLGA microspheres. Higher polymer to drug ratio protects the drug molecules from leaching out toward the external phase during microencapsulation process which leads to higher encapsulation efficiency.

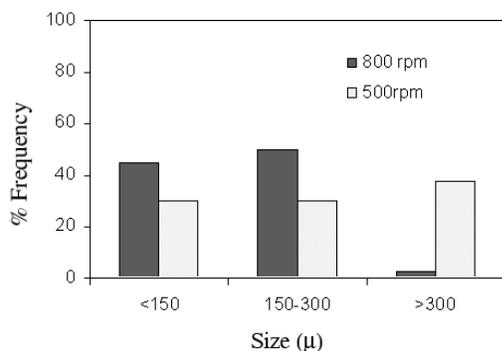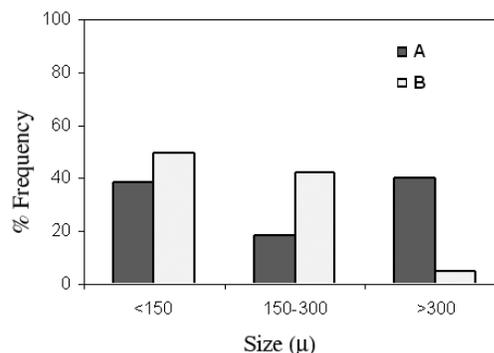Drug release rate from PLGA microspheres with different amounts of drug loading is

shown in Figure 9. Increasing the theoretical drug loading from 5 to 20% decreased the percentage of the released drug from 97 to 65% during a 15 days period. In the case of PLA microspheres, the effect of drug loading on the release rate was opposite (Figure 10). This difference could be attributed to the different drug release mechanism from PLA and PLGA microspheres. In PLGA microspheres, biodegradation controls drug release rate which was evident from the decrease of pH of dissolution medium from 6.5 to 4.5 during the experiment period. Degradation of PLGA produces some acidic products which in turn facilitates the degradation of remaining polymer chains [12]. Lower esterdadiol content in the microsphere means higher PLGA content and thus higher acidic degradation products. On the contrary, low degradation rate of PLA leads to drug release through diffusion
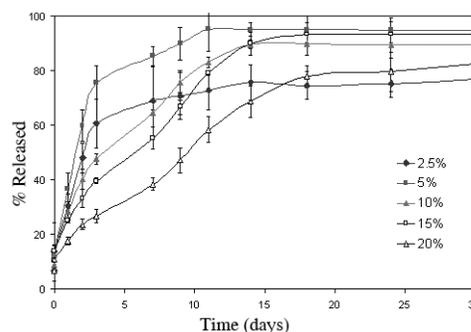


**Figure 8.** Effect of stirring rate on particle size distribution of PLGA microspheres.



**Figure 9.** Effect of drug loading on estradiol release rate from PLGA microspheres.
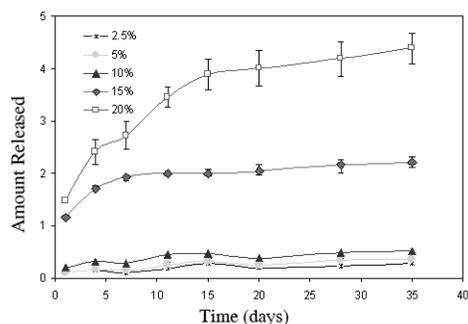
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**Figure 10.** Effect of drug loading on estradiol release rate from PLA microspheres.

mechanism which was supported by lack of pH change during the dissolution period. In this case, with higher drug loading, more drug molecules are available at the surface of microspheres, leading to higher initial release. Also, by increasing the amount of drug loading, a point will be reached when the solid drug particles will begin to form continuous pores or channels within the matrix. Under these circumstances, the path of least resistance for drug molecules will be diffusion within the channels formed from areas where drug has previously leached out from the matrix [13].

## 4. Conclusion

Estradiol valerate containing microspheres were prepared using PLA and PLGA. Increasing the volume of the internal phase and cooling the external phase decreased the particle size. At low drug loading, PLGA microspheres were larger than PLA microspheres but at higher drug loadings, there was no significant difference between the size of PLA and PLGA microparticles. Higher polymer to drug ratio improved the encapsulation efficiency. For PLGA microspheres, increasing the theoretical drug loading from 5 to 20% decreased the drug release rate. Whereas, in the case of PLA microspheres, the effect of drug loading on the release rate was opposite to the PLGA result.

## References

[1] Mitchell DS, Stancel GM. Estrogens and progestins. In: Hardman G, Limbird LE (editors). *Goodman-Gilmans: the pharmacological basis of therapeutics*. 10th ed. Dalas: Mc Graw-Hill, 2001; pp. 1597-630.

[2] Anderso PO, Knoben JE, Troutman WG. *Hand book of clinical drug data.* 10th edition. New York: Mc Graw-Hill, 2002; p. 679.

[3] Jalil R, Nixon JR. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *J Microencap* 1990; 7: 297-325.

[4] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactid-co-glicolide) (PLGA) devices. *Biomaterials* 2000; 21: 2475-90.

[5] Khuller GK, Dutt M. Sustained release of isoniazid from a single injectable dose of poly (DL-lactid-co-glicolide) microparticles as a therapeutic approach toward tuberculosis. *Int J Antimicrob Agents* 2001; 17:115-22.

[6] Bulent M, Noyan V, Fener N. Effects of a monthly injectable steroidal contraceptive, Mesigyna, on menstrual pattern, lipoproteins and coagulation parameters. *Contraception* 2001; 63: 151-3.

[7] Kamijo A, Kamei S, Saikawa A, Igari Y, Ogawa Y. *In vitro* release test system of (lactic-glycolic) acid copolymer microcapsules for sustained release of LHRH agonist (leuprorelin). *J Control Rel* 1996; 40: 269-76.

[8] Watts PJ, Davies MC, Melia CD. Microencapsulation using emulsification solvent evaporation: An overview of techniques and application. *Crit Rev Ther Drug Carrier Syst* 1990; 7: 235-59.

[9] Arshady R. Microsphere and microcapsules, a survey of manufacturing technique. I. suspension cross-linking. *Polym Eng Sci* 1989; 29: 1746-68.

[10] Dinarvand R, Moghadam SH, Mohammadyari-Fard L, Attyabi F. Preparation of biodegradable microspheres and matrix devices containing naltrexone. *AAPS Pharm Sci Tech* 2003; 4.

[11] Yang Q, Owusu-Ababio G. Biodegradable progestron microsphere delivery systems for osteoporosis therapy. *Drug Dev Ind Pharm* 2000; 26: 61-70.

[12] Li SM, Garreau H, Vert M. Structure-property relationships in the case of the degradation of massive aliphatic poly (alpha-hydroxy acids) in aqueous media. *J Mater Sci Mater Med* 1990; 1: 123-30.

[13] Cardinal JR. Matrix systems. In: Langer RS,

Wise DL, (editors). *Medical applications of controlled release systems.* Vol. 1. FL: CRC Press Inc, 1984; pp. 43-4.