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RESEARCH PAPER

Biodegradable Progesterone Microsphere Delivery System for Osteoporosis Therapy

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ABSTRACT

*The purpose of this study was to formulate and characterize a controlled-release biodegradable delivery system of progesterone for the treatment or prevention of osteoporosis. Microspheres of progesterone were formulated using copolymers of poly(glycolic acid-*co*-dl-lactic acid) (PGLA 50/50 and PGLA 15/85) and poly(*L*-lactic acid) (L-PLA) of similar molecular weight by the emulsion solvent evaporation technique. The effects of process variables, such as volume fraction, polyvinyl alcohol (PVA) concentration, polymer composition, and stir speed during preparation, on the yield, encapsulation efficiency (EEF), particle size distribution, in vitro release profiles of progesterone, and surface morphology of progesterone microspheres were investigated. Increasing the volume fraction from 9% to 22% increased the EEF without significantly increasing the yield; however, the rate of progesterone release from the microspheres decreased. Increasing the PVA concentration from 1% to 5% had no significant influence on the EEF, but the rate of progesterone release from microspheres increased. Polymer composition had no significant effect on the EEF, but had a significant effect on the particle size distribution, surface morphology, and release rate of progesterone from the microspheres. Stir speed did not have a significant influence on the EEF; however, stir speed influenced particle size distribution and the rate of progesterone release from microspheres of the same sieve-size range. The results suggest that controlled release of progesterone is possible by varying the different process variables, and that PGLA 50/50 provided the slowest release of progesterone. This should provide a means of delivering proges-*

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terone for months for the treatment or prevention of osteoporosis in postmenopausal women.
Key Words: *Biodegradable; Microspheres; Osteoporosis; Progesterone; Solvent evaporation.*

INTRODUCTION

Osteoporosis, a disease that affects 25 million people in the United States, primarily women of all races, is the cause of 1.5 million fractures annually, notably vertebral crush, hip, and distal fractures (1,2). Serious morbidity and mortality result from these fractures (1,2). The term *osteoporosis* is defined pathologically as a decreased amount of bone. Anatomically, the bones are of normal dimensions, but they contain less bone tissue per unit volume, although the bone tissue is histologically normal. The estimates of the cost of osteoporotic fractures in the United States are high, totalling about $10–20 billion annually (3).

The primary preventive modality for postmenopausal osteoporosis is hormone replacement therapy, and estrogen appears to be the best option at this time (4). It is known that estrogen prevents bone loss and reduces fracture risk among older postmenopausal women who began estrogen therapy 3 or more years after menopause (5). There is a 50% to 60% lower risk of fracture in women who used estrogen for 6 years or longer compared to those who had never used it (5,6). The decrease in risk is probably maximal only in women who continue to take estrogen (7). Although estrogen replacement therapy is contraindicated for those have or who have had breast cancer, endometrial cancer, undiagnosed genital bleeding, and so on, it is widely recommended for the reduction of osteoporosis in postmenopausal women (6,8–16).

The most common route of administration of estrogen to this class of patients in the United States is oral, although products delivered percutaneously or transdermally have been shown to be as effective as oral products (7,17). Estrogen replacement therapy involves the administration of the drug for a prolonged period of time, usually without interruption for about 6 years or more (5,6). Several epidemiologic studies found a decrease in the incidence of osteoporotic fractures was achieved only when the duration of estrogen therapy exceeded 7 years (6). Only 25% of postmenopausal women take some form of hormone replacement therapy in the United States, and current doses of estrogen may induce side effects, leading to noncompliance and loss of efficacy (18).

A combination of estradiol and progesterone has been shown to stimulate the mitogenesis and differentiation of normal adult human osteoblasts (19), which could lead to a reduction in the dose of estrogen administered. A combination of progesterone with estrogen appears to diminish adverse effects associated with estrogen (20). Current treatment regimens include progestins to protect women who have a uterus (11). Therefore, to overcome the problem of noncompliance due to the side effects of estrogen and the difficulty in taking the medication in prolonged therapy, an alternative approach is to formulate a low-dose estradiol and progesterone combination for subcutaneous administration for prolonged drug delivery for about 1 year using biodegradable biocompatible polymers.

The application of subcutaneous implants to deliver estradiol continuously for up to 1 year for treatment or prevention of osteoporosis has been officially available in Great Britain since 1981. The treatment has been very successful (21). The disadvantage is that nonbiodegradable polymers are used to prepare such implants, which requires surgical removal of the nonbiodegradable polymer after the polymer becomes exhausted of the drug or at the end of the duration of therapy (21). The application of biodegradable implants or microparticles of estradiol and progesterone that will not require surgical removal of the device after the implants become exhausted of the drug is a step in the right direction for the treatment or prevention of osteoporosis in postmenopausal women. Poly-dl-lactic acid (PDLA), poly(glycolic acid-*co*-dl-lactic acid) (PGLA), and poly-L-lactic acid (L-PLA) degrade to products that are normal metabolites of carbohydrate metabolism and therefore are eventually absorbed without any accumulation in the vital organs (22). The paper describes the formulation, characterization, and in vitro drug release of progesterone from biodegradable progesterone microspheres.

EXPERIMENTAL

Materials

PGLA 50/50, PGLA 15/85, and L-PLA of similar molecular weight ($M_w \sim 100{,}000$) were supplied by Birmingham Polymers (Birmingham, AL). Polyvinyl alcohol (PVA) (MW 30,000–70,000; degree of esterification 12) and progesterone were obtained from Sigma Chemi-

cal Company (St. Louis, MO). Methylene chloride and *n*-propanol were supplied by Fisher Scientific Company (Norcross, GA). Distilled demineralized water was used. All materials were used as supplied.

Preparation of Microspheres

Microspheres were prepared by the solvent evaporation procedure. PVA was dissolved in water to prepare a 1% w/v solution. Drug-polymer solution was prepared by dissolving PGLA or L-PLA (500 mg) and progesterone (300 mg) in methylene chloride (7 ml). Afterward, the drug-polymer solution was added to 25 ml of the PVA solution stirred at 400 rpm with a Lightnin Mixer (General Signal, New York, NY). The organic solvent was evaporated overnight at a continuous stirring rate of 400 rpm. The microspheres that were formed were harvested by filtration, washed with water, and dried under vacuum for 5 days to remove residual organic solvent. All products were sieve sized using a combination of U.S. standard sieve numbers 40, 60, 120, and 400. Fractions collected between 40 and 60 (250–425 μm), 60 and 120 (125–250 μm), and 120 and 400 (37–125 μm) were used for further studies. The effects of volume fraction at a constant PVA concentration, PVA concentration at a constant volume fraction, polymer composition, and stir speed during preparation on the kinetics of progesterone release were investigated. Each preparation was repeated twice to obtain three batches.

Morphology of Microspheres

The surface morphology, shape, and size of progesterone microspheres were obtained using an Electroscan model E-3 scanning electron microscope. The environmental scanning electron microscope allowed samples to be examined in their natural state without modification or preparation.

Determination of Progesterone Encapsulation Efficiency

Microspheres (10 mg) were dissolved in 40 ml of 1,4-dioxane. The amount of drug in the microspheres was determined using a UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) at 240 nm. The encapsulation efficiency (EEF) was determined as the ratio of the amount analyzed to the initial amount of the drug added during preparation. Experiments were conducted in triplicate.

In Vitro Release of Progesterone from Microspheres

The equivalent of 10 mg of the microspheres or free drug was used in all studies. The microspheres, or free drug, were placed in 900 ml of 25% *n*-propanol aqueous solution in dissolution beakers (USP apparatus 2) and stirred at 100 rpm using a VK 7000 dissolution testing station (VanKel Industries, Inc., Cary, NC). The VK 7000 was interfaced with the ADS 2000 sampling station (VanKel Industries) and a UV-1201 spectrophotometer. The dissolution system was programmed to collect samples automatically through full flow filters (VanKel Industries) at specified time intervals from the various beakers and circulated through the spectrophotometer for automatic recording of absorbances on a computer. Each sample was automatically returned to its original beaker after each measurement. The volume of the dissolution medium in each beaker was maintained at 900 ml throughout the dissolution studies. Absorbances of the dissolved drug were measured at 240 nm and concentrations of the drug were calculated from a standard curve. Release data for three batches of microspheres were combined in one plot.

RESULTS AND DISCUSSION

PGLA 15/85 was used to conduct all studies, except in experiments in which the effect of polymer composition was investigated. All of the microspheres produced were multidisperse and spherical in nature, as shown by the scanning electron micrographs of the microspheres. The encapsulation efficiency, morphology, particle size distribution, and release profiles varied with the formulation parameters, but the amount of microspheres harvested at the end of each preparation did not vary significantly. For the release studies, 25% *n*-propanol solution was used instead of phosphate buffer solution at pH 7.4 or saline solution because the release rate of progesterone from microspheres suspended in these media was too low for microsphere formulations to be differentiated in a shorter period of time.

Effect of Volume Fraction During Preparation on the Characteristics of Microspheres

The effect of volume fraction was investigated at a constant PVA concentration of 1% w/v. A PVA concentration of 1% w/v was used because we observed in a previous study that a PVA concentration between 0.5%

and 1% w/v during preparation provided optimum slowing of the release of ofloxacin from microspheres (23). The volume fraction was increased by decreasing the external aqueous phase, which contained a fixed amount of PVA, and by keeping the internal organic phase constant. Changing the volume fraction of the emulsion during preparation had no significant effect on the yield of microspheres harvested, but the EEF increased with increasing volume fractions for the range studied, that is, from 75% to 84% for volume fractions 9% and 22%, respectively (Table 1). This is attributed to a decrease in the amount of drug lost to the PVA solution at a higher volume fraction, in which the aqueous phase was decreased.

A light microscopic examination of microspheres prepared with different volume fractions indicated that the distribution of aggregates and the number of microspheres coming together to form an aggregate decreased with increasing volume fraction during preparation. Scanning electron micrographs of typical microspheres prepared with different volume fractions are shown in Fig. 1. Microspheres prepared with volume fraction 9% had the highest number of particles coming together to form aggregates (Fig. 1A). A slight increase in volume fraction from 9% to 12% did not result in a decrease in the number of microspheres coming together to form aggregates. However, a significant increase in volume fraction to 22% resulted in the production of microspheres with the least number of particles forming an aggregate (Fig. 1B), with most of the particles being discrete microspheres (Fig. 1C).

The effect of volume fraction during preparation on the release profiles of progesterone from microspheres of sieve size range 250–425 μm is shown in Fig. 2. An increase in the volume fraction from 9% to 12% did not affect the rate of release, but further increase in volume fraction to 22% resulted in a significant slowing of progesterone release from the microspheres. This could be attributed to the integrity of the microsphere wall, which is partly controlled by the rate of methylene chloride extraction into the aqueous phase and evaporation from the aqueous phase or microdroplets. At a low volume fraction (i.e., very high aqueous phase composition), the total amount of methylene chloride that dissolves in water is higher than at a high volume fraction. This results in faster precipitation of the microdroplets, which could reduce the smoothness of the surface of the microspheres. Higher magnification of the surfaces of the microspheres indicates that microspheres prepared with a volume fraction of 22% have smoother and less macroporous surfaces compared to those prepared with a volume fraction of 9% (Fig. 1D and 1E).

Table 1

Effect of Volume Fraction on the Yield and Encapsulation Efficiency (EEF) of Progesterone Microspheres

Volume Fraction (%)	Yield (%)	EEF (%)
9	80.5 ± 0.2	75.5 ± 1.8
12	85.3 ± 0.4	78.6 ± 0.6
22	84.7 ± 0.3	84.1 ± 1.2

Deviations are standard error of the mean.

Effect of Polyvinyl Alcohol Concentration During Preparation on the Characteristics of Microspheres

The effect of PVA concentration was investigated at a constant volume fraction of 22%. The change in PVA concentration from 1% to 3% to 5% w/v had no significant influence on the yield. However, EEF decreased with increasing PVA concentration from 1% to 3% w/v, but did not decrease with further increase in PVA concentration to 5% w/v (Table 2). This decrease in EEF is due to a higher loss of the drug to the aqueous phase with an increase in PVA concentration since the solubility of the drug was found to increase slightly with increasing PVA concentrations.

The sieve size distribution of the microspheres indicated that an increase in PVA concentration from 1% to 5% resulted in a significant increase in smaller size microspheres (Table 2). Approximately 4% and 93% of the microspheres were collected in the sieve size range 37–125 μm and 125–250 μm, respectively, for 1% w/v PVA compared to 21% and 73% for 5% w/v PVA. The change in size and sieve size distribution could be attributed to increasing emulsifier activity and viscosity of PVA solution. At high PVA concentration, smaller microdroplets are well stabilized by the emulsifier, and the tendency for microdroplets to coalesce to form bigger droplets is very low. Furthermore, the viscosity of the aqueous solution is relatively higher than at low PVA concentration, and microdroplets in close vicinity will have to overcome an energy barrier (Es) to separate from each other. This energy barrier gives rise to a tendency for microdroplets to aggregate together when they harden. Similarly, when small microdroplets separate, they also have to overcome an energy barrier (Ea) to aggregate together or recombine to form bigger microdroplets. It appears that the tendency for microdroplets to separate into discrete particles or form bigger microdroplets is smaller than that of aggrega-

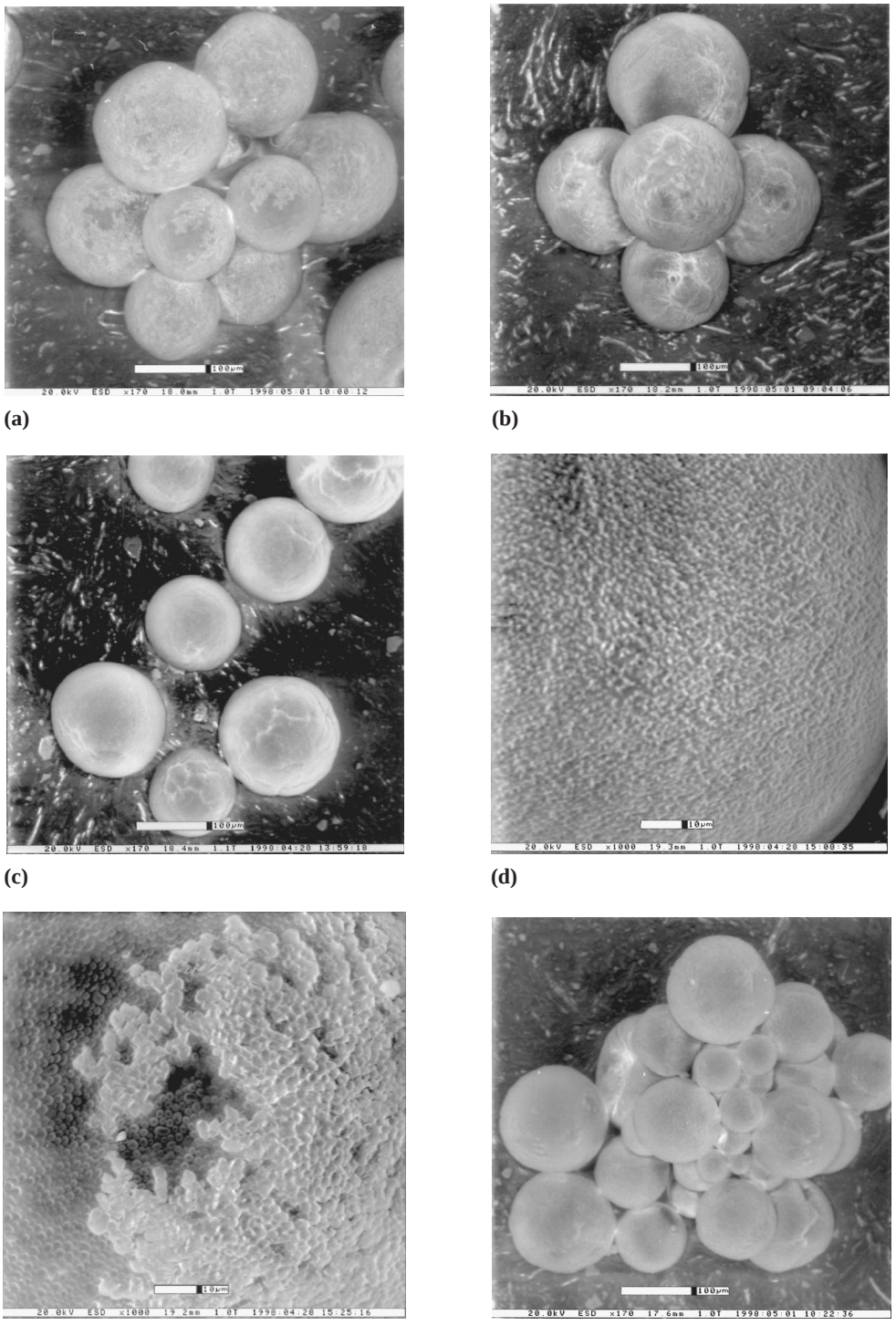

Figure 1. Scanning electron micrographs showing the effect of process variables on the morphology of progesterone microspheres: (A) aggregate of microsphere prepared at a volume fraction of 9%; (B) aggregate of microspheres prepared at a volume fraction of 22%; (C) discrete microspheres prepared at a volume fraction of 22%; (D) high magnification of B; (E) high magnification of A; (F) microspheres prepared with 5% w/v PVA; (G) microspheres prepared with PGLA 50/50; (H) microspheres prepared with L-PLA; (I) high magnification of H; (J) Microspheres prepared at 500 rpm; (K) microspheres prepared at 800 rpm.

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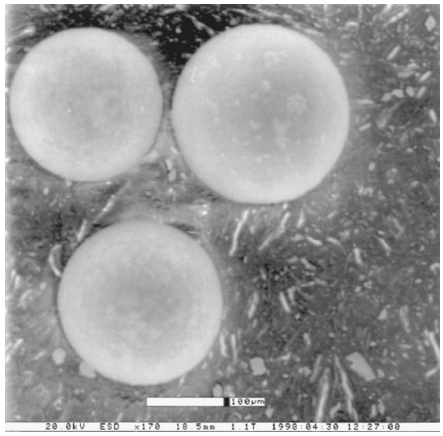

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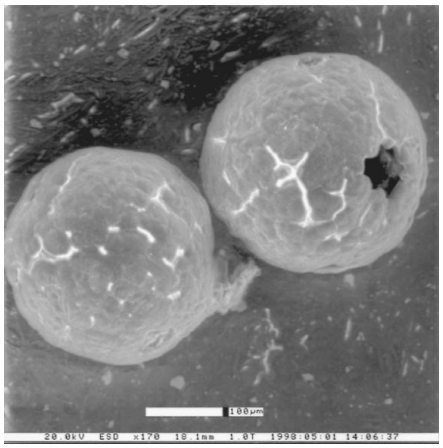

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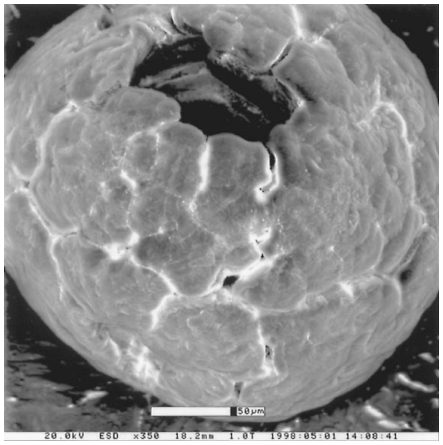

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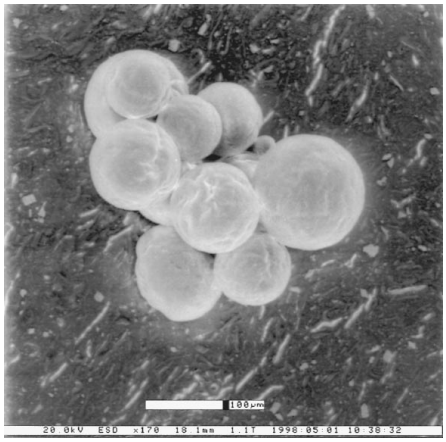

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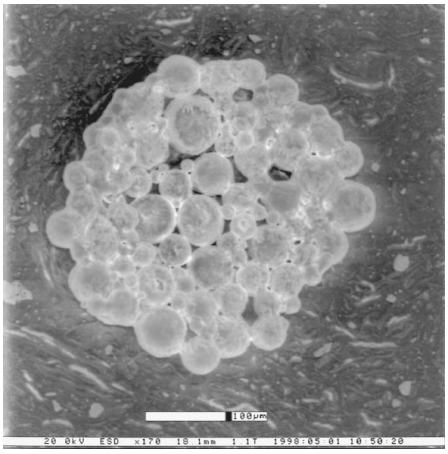


(k)

Figure 1. Continued

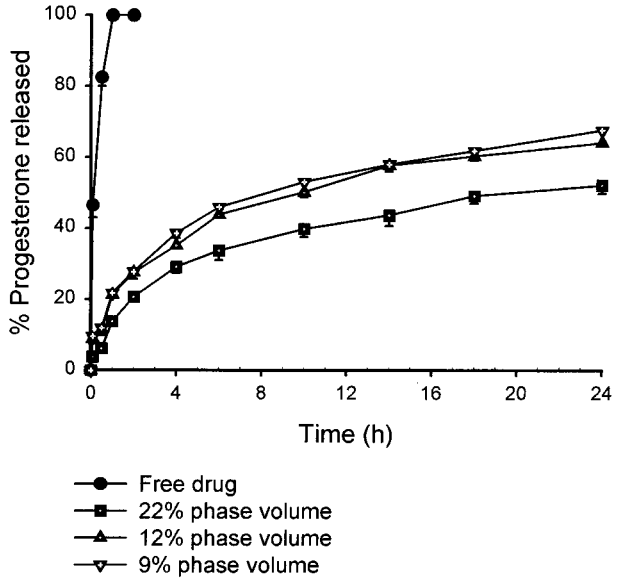
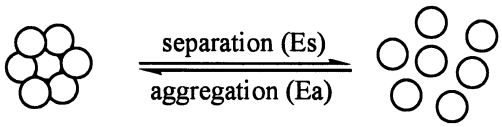


Figure 2. The effect of volume fraction at a constant PVA concentration of 1% w/v during preparation on the release profiles of progesterone microspheres.

tion under the experimental conditions, and aggregation was favored over coalescence or separation. Therefore, many smaller size aggregated microspheres were obtained when 5% w/v PVA was used compared to 1% w/v PVA. The size exclusion microscopy (SEM) pictures of an aggregate of microspheres prepared with 5% w/v PVA can be seen in Fig. 1F.



The profiles of progesterone release with increasing PVA concentration are shown in Fig. 3. Increasing PVA concentration during preparation resulted in an increase in the rate of progesterone release from the microspheres. The microspheres prepared with increasing PVA concentrations have increasing numbers of smaller microspheres, the total exposed surface area of which could have contributed significantly toward the overall rate of drug release.

Effect of Polymer Composition on the Characteristics of Microspheres

Although polymer composition had no significant effect on the EEF, the composition of polymer used for the preparation of microspheres significantly influenced the particle size distribution (Table 2). This influence is complicated and could be explained by the hydrophobicity or hydrophilicity of the polymer. PGLA and L-PLA obtained from the manufacturer were prepared from the monomer cyclic glycolide and lactide. The molecular struc-

Table 2

Effect of Polyvinyl Alcohol (PVA) Concentration, Polymer Type, and Stir Rate During Preparation on the Encapsulation Efficiency and Particle Size Distribution of Progesterone Microspheres

PVA Concentration (% w/v)	EEF (%)	Particle Size Range (%)		
		37–125 μm	125–250 μm	250–425 μm
1	84.1 ± 1.2	3.9 ± 0.6	93.1 ± 0.3	3.0 ± 0.3
1[a]	87.5 ± 0.5	2.9 ± 0.1	67.9 ± 0.3	29.2 ± 0.3
1[b]	87.5 ± 0.5	1.8 ± 0.0	50.2 ± 0.3	48.0 ± 0.3
1[c]	87.6 ± 0.9	32.1 ± 0.7	66.1 ± 0.9	1.8 ± 0.2
1[d]	88.7 ± 0.3	95.4 ± 1.3	3.5 ± 1.3	1.1 ± 0.0
3	77.3 ± 2.0	16.8 ± 2.5	76.6 ± 2.5	6.6 ± 0.1
5	78.7 ± 2.0	20.6 ± 2.3	73.2 ± 1.1	6.2 ± 0.6

Deviations are standard error of the mean.
[a]PGLA 50/50 polymer was used for the preparation.
[b]L-PLA polymer was used for the preparation.
[c]Microspheres were prepared at 500 rpm.
[d]Microspheres were prepared at 800 rpm.

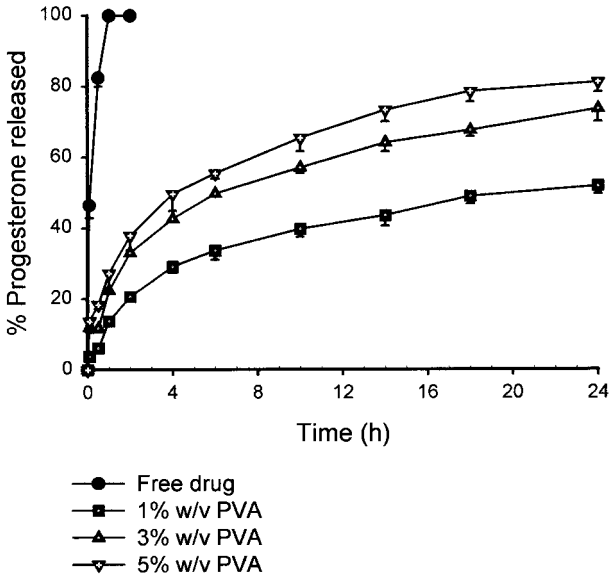
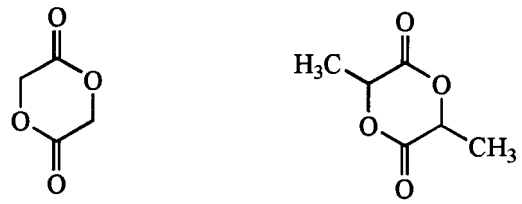


Figure 3. The effect of PVA concentration during preparation at a constant volume fraction of 22% on the release profiles of progesterone microspheres.

ture of the monomers indicates that the lactide, which has two extra methyl groups, is more hydrophobic than the glycolide.



Thus, the polymer that contains a higher fraction of lactic acid is relatively more hydrophobic and should precipitate out of an aqueous environment at a faster rate than those containing higher fractions of glycolic acid. Table 2 indicates that L-PLA, which is the polymer containing only lactic acid, has many microspheres in the larger sieve size range compared to either PGLA 50/50 or PGLA 15/85. L-PLA is the most hydrophobic of the three polymers, and a light microscopic examination of microdroplet formation and hardening indicated that most of the large microdroplets formed during the initial stages of emulsion formation were not reduced further to smaller size droplets before they hardened. At the same rate of stirring during preparation, a polymer that precipitates at a faster rate is likely to be composed of bigger size microspheres. It would be expected from Table 2 that microspheres of PGLA 50/50, which is more hydrophilic than PGLA 15/85 of the same molecular weight because of its high glycolic acid content, should have many more smaller size microspheres than PGLA 15/85. The discrepancy is not clearly understood, but may be attributed to the viscosity grade of the polymer. Notwithstanding, microspheres prepared with PGLA 50/50 were the most spherical with the smoothest surface (Fig. 1G). The nature of PGLA 50/50 microspheres suggests that solvent removal was the slowest of the three types of polymers used for preparation. In contrast, L-PLA that precipitates at a faster rate has the roughest surface, with some particles having holes on their surfaces (Fig. 1H). A higher magnification shows the presence of many macropores (Fig. 1I), similar to what was observed by Jalil and Nixon (24).

The type of polymer used during preparation plays a major role in the type of kinetics or the rate of release observed, which is also influenced by the surface characteristics of the microspheres. Figures 1B, 1G, and 1H clearly show that the type of polymer used during preparation determines the surface appearance of the microspheres, which in turn controls the rate of drug release. Consequently, the lowest rate of drug release was observed when PGLA 50/50 was used for the preparation of progesterone microspheres (Fig. 4). It is apparent from Fig. 4 that the type of polymer used to prepare the microspheres influences the rate of progesterone release. L-PLA is the most crystalline of the three polymers (22) and would be expected to release the drug at the slowest rate, but large and small pores contribute to the faster rate of drug release.

Effect of Stir Speed During Preparation on the Characteristics of Progesterone Microspheres

Stir speed during preparation had no significant effect on EEF; however, with an increase in stir speed, the percentage of small particles increased (Table 2). This is expected because high stir speeds provide the shearing force needed to break down the oil phase into smaller microdroplets. Drug release is faster from the microspheres prepared at higher stir speed than from those prepared at lower stir speed (Fig. 5). The morphology of the microspheres prepared at different stir speeds indicates that the higher the stir speed used for the preparation, the higher the level of aggregation and the smaller the size of the microspheres coming together to form aggregates

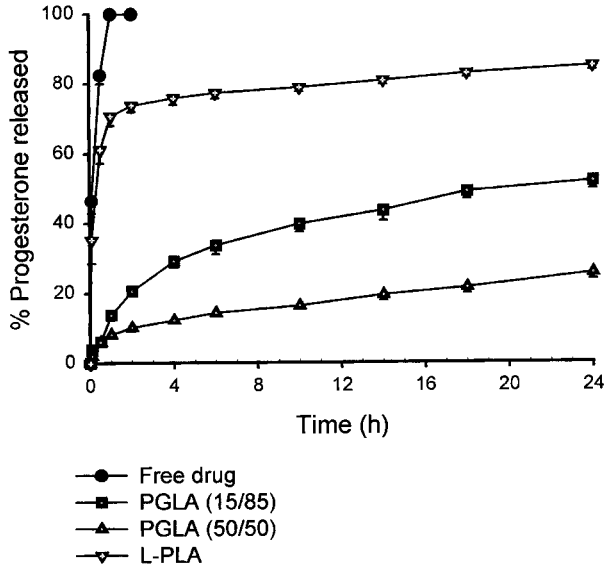


Figure 4. The effect of polymer composition at a constant PVA concentration of 1% w/v and volume fraction of 22% on the release profiles of progesterone microspheres.

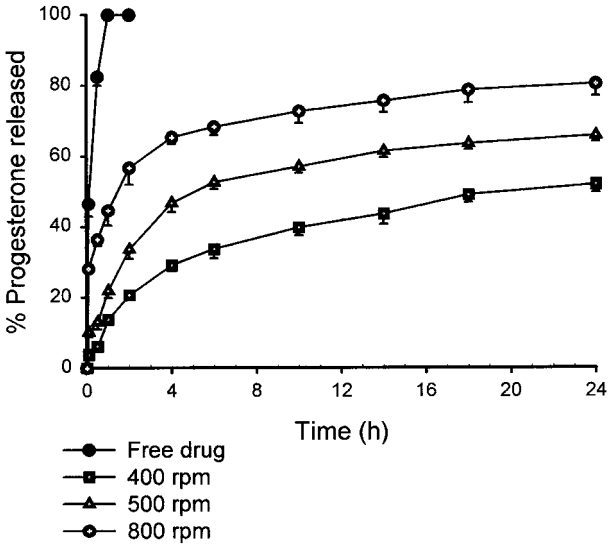


Figure 5. The effect of stir speed during preparation at a constant PVA concentration of 1% w/v and a volume fraction of 22% on the release profiles of progesterone microspheres.

(Figs. 1B, 1J, and 1K). The smaller the size of the particles in the sieve size range used for the release study, the greater the surface area exposed to the release medium, indicating that surface area played a role in the rate of progesterone release.

The results suggest that control of progesterone release is possible by selecting a suitable volume fraction, PVA concentration, type of polymer, and stir rate during preparation. Further slowing of progesterone release could be possible by choosing a pure aqueous release medium, decreasing the drug loading, and increasing the molecular weight of a suitable polymer, which in our study was found to be PGLA 50/50. It should be possible to deliver progesterone for months in postmenopausal women in combination with estradiol for the prevention of osteoporosis and to reduce the side effects associated with estradiol. It is hoped that this could improve compliance in postmenopausal women undergoing hormone replacement therapy.

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