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Biodegradable Microparticulates of Beta-Estradiol: Preparation and In Vitro Characterization

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ABSTRACT Beta-estradiol has been recommended for the long-term therapy of osteoporosis and its oral formulations are subjected to intensive first pass metabolism. The present investigation was aimed at preparing and characterizing biodegradable microparticles of beta-estradiol with polymers such as PLA, PLGA 85/15, PLGA 75/25, and their mixtures. The microparticles were prepared by solvent evaporation method using methylene chloride as a solvent and polyvinyl alcohol as a surfactant. The drug-polymer ratios were 1:3, 1:5, and 1:7. The prepared microparticles (twelve formulations) were tested for encapsulation efficiency and in vitro drug release in 50% methyl alcohol/phosphate buffer pH 7.4. The results showed that the encapsulation efficiency varied from 81 to 100% and the formulation fabricated from PLGA 85/15 (1:3) showed less burst and consistent long time release. This formulation when further characterized displayed irregular spherical shape with an average particle size of 72 μm. The crystallinity of the drug was reduced when investigated using X-ray diffractometry. No chemical interaction between the drug and the polymer was observed as evidenced by FT-IR analysis. The results indicated that beta-estradiol biodegradable microparticles with PLGA 85/15 (1:3) could be a suitable approach for long term therapy of osteoporosis.

KEYWORDS Beta-estradiol, Controlled drug release, Biodegradable polymers, Osteoporosis, Microparticulates

INTRODUCTION

Injectable implants of biodegradable microparticles have been widely used as delivery systems to control the release of drugs. Although, currently, only a small number of commercially available products utilize this technology, the biodegradable polymers have great utility for the controlled release of several drugs e.g., vaccines, hormones, antitumors, anti-inflammatory, contraceptives, etc. (Carballido et al., 2004; Dhanaraju et al., 2003; Fournier et al., 2004; Schwach et al., 2004; Yeh & Chiang, 2004). The physical properties and FDA use allowance of poly-lactic acid (PLA), poly-glycolic acid (PGA), and copolymer poly (lactide-co-glycolide) (PLGA) make them the most extensively used commercially available biodegradable polymers.

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Osteoporosis, a disease that affects almost one quarter of the population in the United State primarily women of all races, is the cause of 1.5 million fractures annually that cost about 10–20 billion USD (Lindsay, 1992, 1995). Although there has been long-standing concern about breast cancer risk among women who take hormone replacement therapy (HRT), no effect on breast cancer mortality was observed (Rossouw et al., 2002). The primary preventive modality for postmenopausal osteoporosis is still HRT and estrogen appears to be the best option at this time (Seeman et al., 1995). Several epidemiological studies showed that a 50–60% lower risk of fracture in women who used estrogen for 6 years or longer compared to those who had never used it before (Cummings et al., 1985; Roux, 1997). The most common route of administration of estrogen to this class of patients is oral, although products delivered percutaneously or transdermally have been shown to be as effective as oral products (Riis et al., 1987; Stevenson et al., 1990). Estradiol and other estrogens are readily absorbed from the gastrointestinal tract and through the skin or mucous membranes. However, the natural unconjugated estrogens such as estradiol undergo extensive first-pass metabolism in the gastrointestinal tract and liver following oral administration (Sweerman, 2002). Therefore, in order to maintain an effective concentration of estradiol in blood serum, the doses delivered orally must exceed many times those delivered non-orally. The problems of side effects, noncompliance, first pass metabolism, and difficulty in taking the medication for long time results in only 25% of postmenopausal women taking some form of the HRT in the United States (de Lignieres, 1996). To overcome these problems, an alternative approach is to formulate a low dose of estradiol in biodegradable biocompatible polymers for subcutaneous or intramuscular administration to deliver the drug for a long period of time. The main advantage of this long acting delivery system is that it does not need to be removed after a drug is released because they are degraded to compounds that are metabolized and eliminated from the body (Heller, 1983; Linhardt, 1989).

Beta-estradiol has been formulated before in PLA or PLGA polymers (Birnbaum et al., 2000; Buntner et al., 1998; Xinteng et al., 2002). The prepared formulations showed long term release behavior but showed pulsatile drug release for about 50% or more in the first 24 h. PLA is known to have more hydrophobic properties than PGA (Chasin & Langer, 1990). The release from PLA and PLGA occurs through both diffusion and erosion as the polymer hydrolyzes. Therefore, by selecting the polymer type and monomer ratio with appropriate physical characteristics, the release kinetics of the drug from its polymer matrix can be controlled.

In this study, PLA, PLGA 85/15, PLGA 75/25, and mixture of these polymers were used to prepare beta-estradiol microparticles at drug-polymer ratios of 1:3, 1:5, and 1:7. The prepared formulations were characterized for their encapsulation efficiency and in vitro drug release. Based on these data, a selected formulation was further characterized using particle size analyzer, scanning electron microscopy (SEM), X-ray diffractometry (XRD), and FT-IR spectroscopy.

MATERIALS AND METHODS

Materials

Poly (dl-lactide), PLA (IV=0.26, average MW 12,000–24,000), copolymers poly(dl-lactide-co-glycolide), and PLGA 85:15 and 75:25 (IV=0.61, average MW 80,000) were obtained from Birmingham Polymers Inc. (Birmingham, AL), poly-vinyl alcohol, PVA (average MW 30,000–70,000), and beta-estradiol were obtained from Sigma Chemical Co. (St. Louis, MO); methylene chloride and methyl alcohol were obtained from Fischer Scientific Co. (Norcross, GA), and mono- and dibasic potassium phosphate were obtained from EM Science (Gibbstown, N.J.). Water used was distilled and deionized. Other solvents and excipients were of analytical grade and used without further purifications.

Preparation of Beta-Estradiol Microparticles

Microparticles of beta-estradiol were prepared by solvent evaporation method using PLA, PLGA 85/15, PLGA 75/25, and mixtures of these polymers (1:1:1) at different drug polymer ratios 1:3, 1:5, and 1:7 with initial drug loading of 25, 16.66, and 12.5%, respectively. Polymer solutions were prepared by dissolving the specified amount of polymer(s) in methylene chloride (20 ml) using magnetic stirrer (Fisher Co., Fair Lawn, NJ). After complete dissolution, the drug (200 mg) was added, thoroughly mixed with the polymer solution and stirred for further 15 min at

1400 rpm. The drug polymer mixture was added gradually to 180 ml of water containing 1% PVA and maintained at 4°C while homogenizing at 10,000 rpm for 10 min using PRO 250 homogenizer (Monroe, CT). The mixture of organic and aqueous phases created an oil-in-water emulsion. The organic phase was allowed to evaporate overnight at a continuous stirring speed of 1400 rpm. The resultant microparticles were filtered using Buchner funnel with Whatman #4 qualitative filter paper and vacuum pump if necessary. The particles remaining on the filter paper were washed with water twice, collected, and dried at 30°C for 24 h. The dried particles were then passed through #20 mesh sieve (U.S. standard sieves, Dual Mfg. Co., Chicago, IL) to disaggregate the particles (if necessary). The prepared microparticles (twelve formulations) were tested for encapsulation efficiency and in-vitro drug release. The formulation that showed the best release behavior and less burst release was further characterized for particle size distribution using particle size analyzer, shape, and surface of the particles using scanning electron microscopy, for crystallinity change using X-ray diffractometry, and for drug-polymer interaction using Fourier transform infrared spectroscopy.

Drug Encapsulation Efficiency

A known weight of prepared microparticles were dissolved into a 50 ml of 60/40 methylene chloride/methyl alcohol cosolvent. The solution was sonicated for 30 min (3510R-DTH sonicator, Branson Corp., Danbury, CT) to obtain a completely clear solution. Samples were then taken and assayed spectrophotometrically at 280 nm (GBC Scientific Equipment Pvt. Ltd., Dandenong, Australia). The polymer and other additives did not interfere at this wavelength. The drug encapsulation efficiency was calculated using the formula:

$$\text{Encapsulation efficiency \%} = \frac{\text{experimental drug loading}}{\text{theoretical drug loading}} \times 100$$

In Vitro Drug Release

Dissolution experiments were carried out according to Parikh et al. (1993) with slight modification. Briefly, amounts of microparticles containing equal amounts of estradiol (based on the data of encapsulation efficiency) were suspended in 5 ml of 50% methyl alcohol/phosphate buffer pH 7.4 in glass scintillation vials. The vials were placed in a shaker water bath maintained at 37°C and 50 rpm. At each sampling time, the entire release solution was removed with syringe fitted with filter (Vankel Technology Group, Cary, NC) to prevent any loss of particles. The withdrawn samples were properly diluted with the dissolution medium and analyzed spectrophotometrically at 280 nm and the corresponding drug concentration was calculated.

Particle Size Distribution

Particles were sized by laser diffractometry using a Nicomp® 380 Particle Sizing Systems (Santa Barbara, CA). The average particle size was expressed as intensity, volume, and number mean diameter in μm.

Scanning Electron Microscopy (SEM)

The shape and surface morphology of the particles as well as the drug were examined with SEM (JEM-100 CX, JOEL Inc., Japan). The dry particles were mounted on an adhesive stub and then coated with gold palladium under vacuum using an ion coater. The coated specimen was then examined under the microscope at 10 kV and photographed.

X-Ray Diffractometry (XRD)

X-ray diffractometry (XRD) for drug and drug-polymer microparticles were recorded using X-ray diffractometry (Philips Norelco, Philips Electronics, Eindhoven, Netherlands).

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) spectra of drug, polymer, and drug-polymer microparticles were obtained using FT-IR spectroscopy (Nexus 470 FT-IR Thermo Nicolet Corporation, Madison, WI).

TABLE 1 Composition and Encapsulation Efficiency of Beta-Estradiol Biodegradable Polymer Formulations

Drug/polymer ratio	Form. no.	Preparation drug loading %	Experimental drug loading %	Encapsulation efficiency %
(PLA 1:3)	F1	25.00	25.00	100.00
(PLA 1:5)	F2	16.66	14.29	85.79
(PLA 1:7)	F3	12.50	10.90	87.21
(PLGA 85/15 1:3)	F4	25.00	23.38	93.52
(PLGA 85/15 1:5)	F5	16.66	16.66	100.00
(PLGA 85/15 1:7)	F6	12.50	10.94	87.53
(PLGA 75/25 1:3)	F7	25.00	22.23	88.90
(PLGA 75/25 1:5)	F8	16.66	16.65	99.96
(PLGA 75/25 1:7)	F9	12.50	10.66	85.24
(Polymer mixture 1:3)	F10	25.00	20.45	81.78
(Polymer mixture 1:5)	F11	16.66	16.66	100.00
(Polymer mixture 1:7)	F12	12.50	10.97	87.77

RESULTS AND DISCUSSION

Encapsulation Efficiency

List of formulations and their encapsulation efficiencies are shown in Table 1. From the results it appears that, with few exceptions, there are correlation between the initial drug loading and encapsulation efficiency. All the 16.66% initial drug loading formulations (except F2) showed high encapsulation efficiencies (100, 99.96, and 100 for F5, F8, and F11, respectively). On the other hand, all the 12.5% initial drug loading showed lower encapsulation efficiencies compared to the previous ones (87.53, 85.24, and 87.77 for F6, F9, and F12, respectively). This correlation was evidenced by many authors (Mehta et al., 1996). Regarding the 25% drug loading formulations, there were some differences in the results of encapsulation efficiency, but it appears that formulations containing high percent of PLA showed higher encapsulation efficiency compared to others containing the same initial drug loading but with low PLA composition (F1<F4<F7<F10). This may be due to the hydrophobic nature of both PLA and beta-estradiol. PLA is known to have more hydrophobic character than PGA and PLGA. The drug will be more homogenous with polymer mixture containing high percentage of PLA.

In Vitro Drug Release

The in vitro release of beta-estradiol from the prepared microparticles is shown in Figs. 1 and 2. In this study, different amounts of microparticles containing equal amounts of beta-estradiol were used. This was based on the encapsulation efficiency of each formulation. Withdrawing the entire release solution and substituting with a new one prevented the pH change of the release medium on keeping in contact with polymer degradation products for long time. Also, we tried to avoid using the centrifuge in separation of the release solution from the solid powder. Instead, a syringe fitted with filter on its tip was used. This method eliminated 1) the undesired loss of microparticles during sample preparation and handling; 2) shear stresses due to the centrifugation for sample recovery.

A common problem in the dissolution testing of insoluble drugs is the attainment of sink condition. It has been demonstrated that cosolvents could be chosen to provide sink conditions as long as the drug release is diffusion-controlled and the matrix material is insoluble in the cosolvent (Corrigan, 1991). Since PLA and PLGA are insoluble in methanol and the release of beta-estradiol is diffusion controlled, the release solution chosen was 50% methanol/phosphate

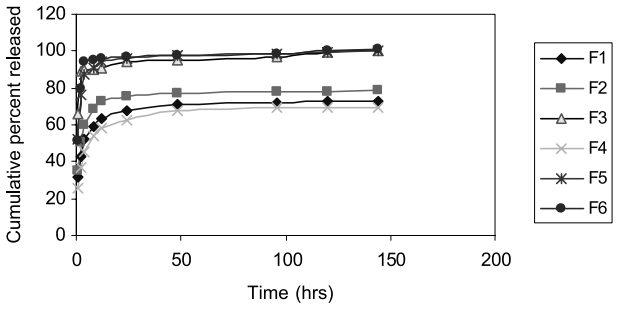

FIGURE 1 Release Profiles of Beta-Estradiol Microparticles in 50% Me OH/Phosphate Buffer pH 7.4 (F1:F6).

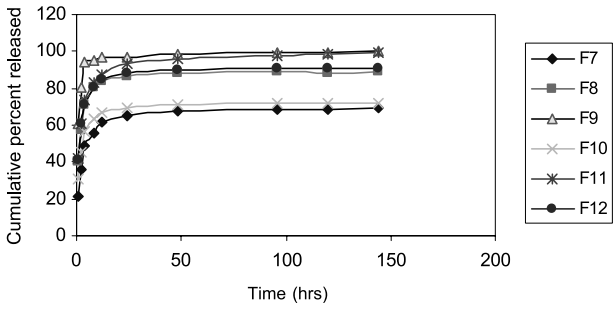

FIGURE 2 **Release Profiles of Beta-Estradiol Microparticles in 50% Me OH/Phosphate Buffer pH 7.4 (F7:F12).**

buffer pH 7.4. This cosolvent was chosen because of the low solubility of beta-estradiol in pH 7.4 buffered solution alone. It is important that the solubility of beta-estradiol in the release solution is enough to create sink condition and the drug released would be due to polymer degradation and not the rate of drug solubility (Higuchi et al., 1987).

The initial drug loading in each of these formulations ranged from 12.5 to 25%. All the formulations showed high initial rate of drug release followed by slow sustained release. Drug released in the first hour of experiment ranged from 25% (F4) to 66% (F3). It has been stated that the initial burst release is probably due to poorly entrapped drug and/or the drug is loosely attached to the internal and the outer surface (Mehta et al., 1996). The experiment was run for 6 days. Some formulations showed complete drug release after this time period (F3, F5, F6, F9, and F11). These formulations were fabricated using high polymer concentration (D/P ratio is 1:5 or 1:7). This may be due to high percentages of the drug that remained unencapsulated or loosely bound to the polymer. This explains the high initial burst release of these formulations. By the same token, the lowest cumulative drug release was shown in F1, F4, F7, and F10. These formulations were fabricated using low polymer concentration and showed low amounts of pulsatile drug release. This may be attributed to the intact and strong coating of the drug within the microparticles.

Since PLGA degrades by bulk hydrolysis in water (Gopferich, 1996), the rate of degradation would control the release of any encapsulated pharmaceutical agent. This may be the reason for the high cumulative drug released in case of F9 (100%) which contained a high percentage of the hydrophilic polymer PGA. It has been stated that the higher the glycolide content and the lower the molecular weight of the polymer, the higher the degradation rate (Witschi & Doelker, 1998).

Interestingly, all the microparticles produced showed similar release profile after initial burst. It may come from the similar surface and internal morphology of these particles as presented above. From the release profiles, it appears that F4 shows low initial burst and a characteristic steady release of drug. For these reasons, F4 was chosen for particle size, SEM, XRD, and FT-IR characterization.

Particle Size Analysis

Samples of F4 microparticles were suspended in deionized water and size distribution was obtained. Particle size ranged from 3 to 31 μm with an average diameter of 7 μm. The cumulative results showed that 25% of distribution is less than 3 μm while 99% of distribution is less than 31 μm. Results of particle size analysis based on intensity, volume, and number are seen in Fig. 3. Controlling the size of the particles is

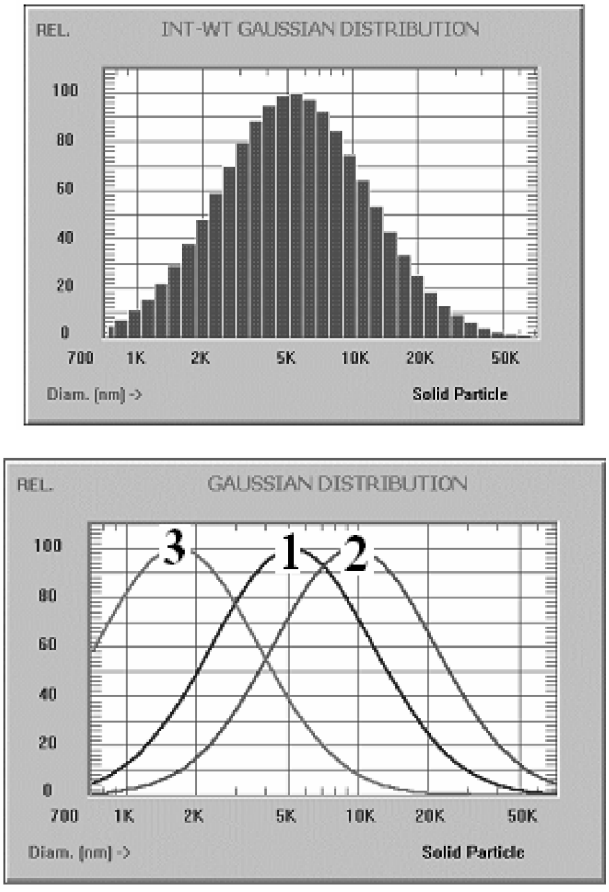

FIGURE 3 **Size Distribution of Beta-Estradiol Microparticles with PLGA 85/15 Biodegradable Polymer, 1-Intensity wt. Mean Diameter, 2-Volume wt. Mean Diameter and 3-Number wt. Mean Diameter.**

important since the size affects the degradation rate (Mehta et al., 1994), loading and initial burst release (Deng et al., 1999), and finally the utility of the particles (Jeffery et al., 1993; O'Hagan, 1998). The size of the microparticles can be easily affected by a variety of processing factors such as volume and viscosity of inner and external aqueous phase (Jeffery et al., 1993) and molecular mass of the polymer (Mehta et al., 1996). Particles fabricated from viscous solution harden much rapidly. This probably causes them to have broader size distribution because the shearing forces induced by the stirrer have limited effect on them once the initial particles are formed. The broad distribution that was shown here may be attributed to particle agglomeration during the analysis or the presence of broken spheres and polymer bits. The reduction of particle size to micrometer size range may be due to the strong and long time homogenization during emulsion process. Keeping the prepared microparticles within the micrometer size range might have

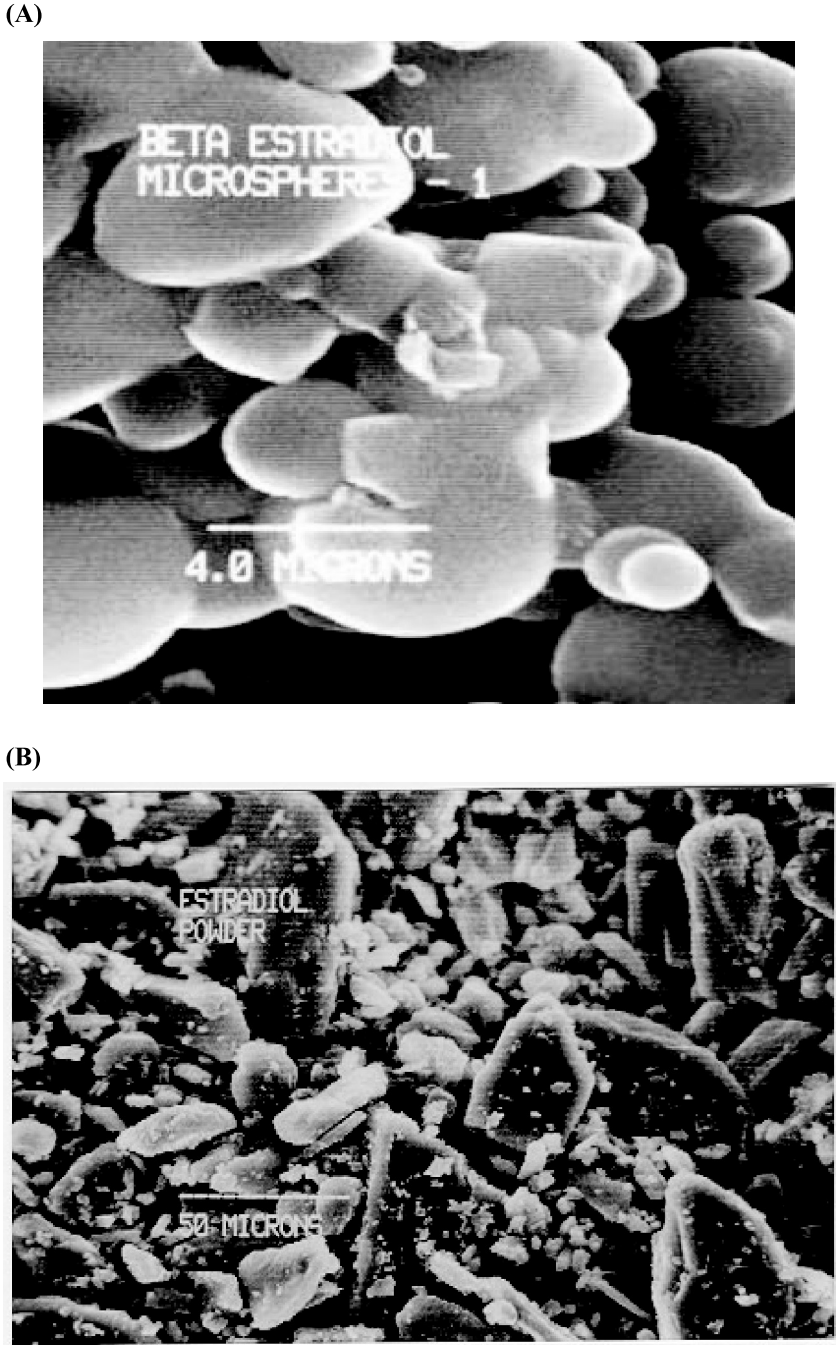

FIGURE 4 SEM Pictures of Beta-Estradiol Microparticles with PLGA 85/15 Biodegradable Polymer (A) and Beta-Estradiol Powder (B).

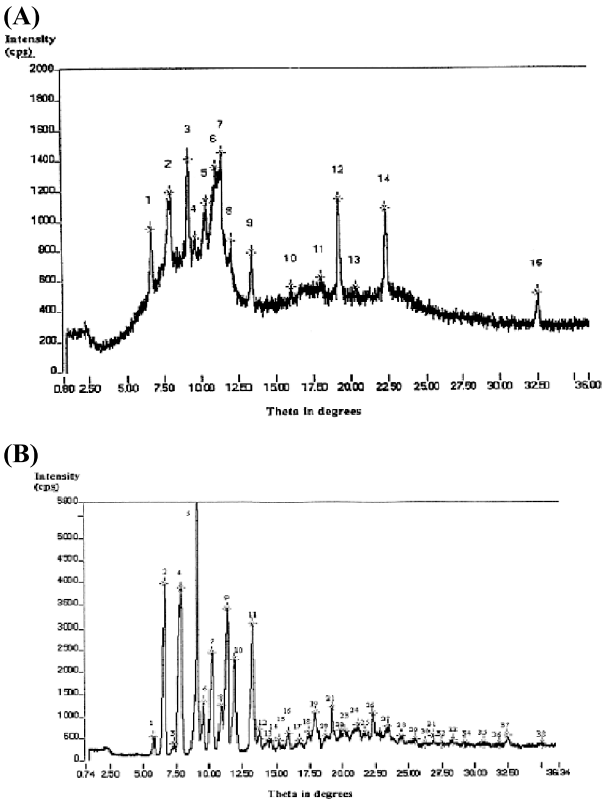

FIGURE 5 **XRD Diffractograms of Beta-Estradiol Microparticles with PLGA 85/15 Biodegradable Polymer (A) and Beta-Estradiol Powder (B).**

its impact on the behavior of drug release generally and on pulsatile release particularly as we have seen above.

SEM

SEM photographs of the microparticles are shown in Fig. 4. The surface of the particles looked irregular in appearance. Some particles appeared to be fractured and hollow inside, some others contain a hollow core or are highly porous in nature. Porosity of microspheres is known to vary with numerous factors such as polymer molecular mass, co-solvent concentration, dispersed phase to continuous phase ratio, drug concentration, rate and method of solvent removal, as well as method of fabrication (Jeyanthi et al., 1996, 1997). In our case, the internal porosity is generally high because the polymer concentration is quite low. Low polymer concentration would result in high distribution of solvent and internal water in the polymer. During precipitation, more water from the continuous phase could diffuse in to create more water pockets (Li et al., 1995). Once the microparticles are dried, the water pockets will become holes. The absence of core formation in some of the microspheres could be due to the presence of emulsifier in the inner aqueous phase, which prevents coalescence of the internal water droplets.

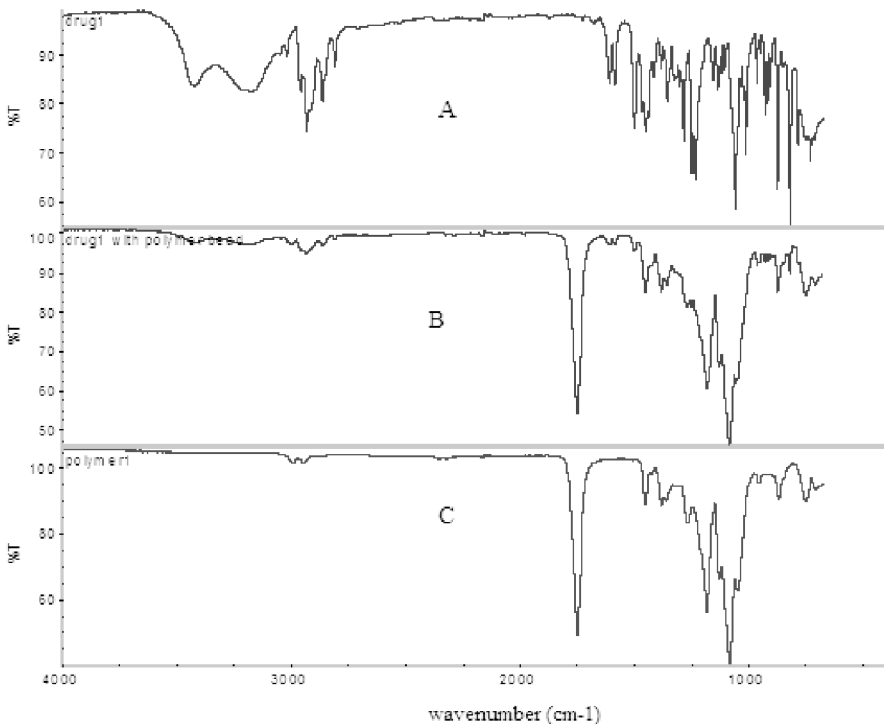

FIGURE 6 **FT-IR Spectra of Beta-Estradiol Microparticles with PLGA 85/15 Biodegradable Polymer (B), Beta-Estradiol Powder (A), and PLGA Polymer (C).**

XRD

The X-ray diffraction scans of F4 and beta-estradiol powders are shown in Fig. 5. Comparing the two diffractograms, it is obvious that the crystallinity of drug was reduced. It is known that the copolymers of lactide and glycolide are less crystalline than the two homopolymers. Since the drug is soluble neither in the organic nor in the aqueous solvent, it is not expected that it will precipitate in complete amorphous form. Reducing the crystallinity of beta-estradiol and/or the polymer might be because of the fabrication technique. In our case, the solvent evaporation takes place slowly allowing the drug to be coated with strong polymer sheath. The changing of the drug from crystalline to semi-amorphous was reflected on the release behavior of the microparticles which was fast at the beginning but slow and sustained after that.

FT-IR

It is important to determine whether or not the beta-estradiol-PLGA microparticles may promote a chemical interaction between drug and polymer. To investigate this possibility, the microparticles were analyzed side by side with drug alone and polymer alone using FT-IR. Figure 6 displays the IR spectra over the range 1000–4000 cm^{-1}. FT-IR spectra corresponding to beta-estradiol loaded microparticles displayed all the characteristic bands of both, drug and polymer, without any spectral shifts in either band. This indicates that there is no chemical interaction between the components of the microparticles.

CONCLUSIONS

The obtained results demonstrated the feasibility of efficiently encapsulating beta-estradiol into biodegradable microparticles for controlled release by solvent evaporation method. Different polymers at different drug-polymer ratios showed different encapsulation efficiencies and different release behavior. PLGA 85/15 showed the best drug release behavior and the lowest burst. Characterization of this formulation showed irregular spherical shape of micrometer size range. X-ray scans showed decrease in the crystalline structure of the drug, and FT-IR spectra showed the characteristic peaks of both polymer and drug. From the results, beta-estradiol biodegradable microparticles with PLGA 85/15 (1:3) may present a promising approach for long term therapy for osteoporosis. The burst release might be useful as a starting dose and the gradual drug release as the maintenance dose.

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