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β-Estradiol biodegradable microspheres: Effect of formulation parameters on encapsulation efficiency and *in vitro* release

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The purpose of this work was to study the effect of organic solvent and surfactant type on the *in vitro* release behavior in general and on the burst release in particular of beta-estradiol from PLA/PLGA microspheres. Also the effect of these variables on the encapsulation efficiency was investigated. The microspheres were prepared by solvent evaporation technique using dichloromethane (DCM), ethyl acetate (EtAc), tetrahydrofuran (THF), chloroform ($CHCl_3$) or acetone (AC) as organic solvent and polyvinyl alcohol (PVA), Tween 80, sodium lauryl sulfate (SLS) or benzalkonium chloride (BKCl) as surfactant. The obtained microspheres were tested for encapsulation efficiency and *in vitro* drug release using 50% methanol/buffer pH 7.4 as dissolution medium. EtAC and PVA formulations showed the highest encapsulation efficiency and the lowest burst release. These microspheres were further characterized for particle size distribution, SEM and zeta potential. The results suggested that these materials could be starting materials to prepare a beta-estradiol biodegradable controlled delivery system.

1. Introduction

One major obstacle for development of injectable biodegradable microspheres for controlled peptide and protein delivery is the high initial burst of drug release occurring over the first day(s) of incubation. The conventional methods of preparing microspheres involve emulsification using organic solvent(s) and emulsifying agent(s). Successful entrapment of drugs, together with other properties of microspheres depend to a large extent on the selection of the organic solvent used to dissolve the polymer. Parameters considered when choosing an organic solvent include: the solvent's miscibility with water (if an oil in water emulsion is to be used), its ability to dissolve the polymer and drug, and its toxicity (McGinity and O'Donnell 1997; Soppimath et al. 2001). The ability of the solvent to dissolve large amounts of polymer makes it easier to control particle size distribution and drug encapsulation efficiency. However, the rate of solvent removal by the evaporation method strongly influences the characteristics of the final microspheres and depends on the temperature, pressure and solubility of the polymer in solvent and dispersion medium. Rapid solvent evaporation may cause local explosion inside the oil droplets and lead to formation of a porous structure on the microsphere surface (Arshady 1991). Dichloromethane is widely used because of its good solvent power and its high volatility which enables easy and fast removal by evaporation. The major problems with the use of dichloromethane in particular and other halogenated solvents in general are their toxicity and environmental hazardous problems. Toxicity is of special concern as there will always be trace amounts of solvent in the final microparticles and it may lead to dismissal of any proposed formulation regardless of potential benefits (Birnbaum et al. 2000). For this reason and for other reasons like the burst release, the encapsulation efficiency and the quality of the microparticles, researchers tried to find alternatives to avoid these problems. Soppimath and Aminabhavi (2002) reported the use of ethyl acetate to prepare PLGA microspheres by the solvent extraction process. Sah (1997, 2000) produced microspheres by a two-step extraction process using ethyl acetate as well as ethyl formate. Other solvents such as acetonitrile, dimethyl formamide, chloroform, acetone, dioxin, trifluoroethanol, ethanol and other solvents and cosolvents have been tried (Jain et al. 1998; Mandal 1999).

During the solvent evaporation/extraction process, there is a gradual decrease in the volume and subsequent increase in the viscosity of the dispersed oil droplets. This affects the droplet size equilibrium and the droplets tend to coalesce and produce agglomerates during the early stages of solvent removal. This problem could be rectified by adding a small quantity of an emulsifier in the continuous phase. The emulsifier provides a thin protective layer around the oil droplets, and hence reduces their coalescence and coagulation. As the solvent is removed, the emulsifier continues to maintain the spherical shape of the oil droplets and prevents their aggregation until the microspheres are hardened and isolated as discrete particles (Arshady 1991; Wu 1995). It has been reported that the encapsulation efficiency and the *in vitro* drug release are significantly influenced by the type of surfactant encountered. In addition, the physicochemical properties and the concentration of the emulsifier strongly influence the size, shape, and the quality of the resulted microspheres (Alpar et al. 1995;

Mu and Feng 2003). PVA is by far the most commonly used emulsifier in the O/W emulsion method. Others such as PVP, alginates, gelatin, methylcellulose, hydroxypropyl methylcellulose, Tweens, Spans, Brijs have been used (Jain et al. 1998).

The objectives of this study were: to investigate the effect of organic solvent and surfactant type on the encapsulation efficiency and *in vitro* release of beta-estradiol from biodegradable microspheres and to characterize the microspheres showing higher encapsulation efficiency and lower burst release via SEM, particle size distribution and surface charge analysis.

2. Investigations, results and discussion

2.1. Encapsulation efficiency and in vitro drug release

In a screening study, it was found that using a mixture of PLA, PLGA85/15 (1 : 1) in a drug polymer ratio of 1 : 7 is a good starting point to study the factors lessen the initial burst of release. Preparation of a lactide-rich formulation by mixing PLA with PLGA85/15 leads to a formulation of more hydrophobic characters, absorbs less water and subsequently degrades more slowly and hence sustains the release for long time. In addition, dl-PLA enables a more homogenous dispersion of the drug in the polymer matrix (Cohen et al. 1994). In order to evaluate the influence of formulation parameters on encapsulation efficiency and release pattern, one parameter was changed while the other was kept constant. In the first batch of the experiments, the surfactant was changed. The surfactants used were PVA, Tween 80, SLS, and BKCL. The results are showing in the Table. From the results it is obvious that PVA among all the surfactants showed the highest encapsulation efficiency; 99% compared to 89, 92 and 86% for Tween 80, SLS and BKCL, respectively. To study the *in vitro* release of these formulations, different amounts of microspheres containing equal amounts of beta-estradiol were used. This was based on the encapsulation efficiency of each formulation. The technique applied to perform the dissolution testing depends on withdrawing the entire release solution and substituting with a new one. This method prevented the pH change of the release medium on keeping in contact with polymer degradation products for a 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 a micropore filter on its tip was used. This method eliminated a) the undesired loss of microspheres during sample preparation and handling; b) shear stresses due to the centrifugation for sample recovery. 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 buffer pH 7.4. This cosolvent was found to provide sink conditions. The profiles of drug release are shown in Figs. 1–3. The percent of drug released after 120 h incubation were 47, 90, 77 and 63 for formulations containing PVA, Tween 80, SLS and BKCL respectively. From the results it is obvious that the PVA containing formulation showed the highest encapsulation efficiency and the lowest amount of burst drug release, therefore it was chosen as a stabilizer in the second patches of the experiments.

To study the effect of organic solvents on encapsulation efficiency and *in vitro* release, DCM, EtAC, THF, CHCL3 and AC were used. In each one of these, 1% PVA was added to the aqueous phase as a surfactant. The encapsulation efficiency results were 86, 97, 66, 93, and 52% and

Table: Encapsulation efficiency and in vitro drug release of different beta-estradiol biodegradable formulations

SAA/Solvent	Encapsulation efficiency (%)	*In vitro* drug release after 120 h (%)
PVA	99.2	47.3
Tween 80	89.1	90.7
SLS	92.0	77.1
BkCL	86.7	63.4
DCM	86.3	63.4
Et AC	97.6	52.1
THF	66.2	69.6
CHCL3	93.4	64.8
AC	52.5	72.2

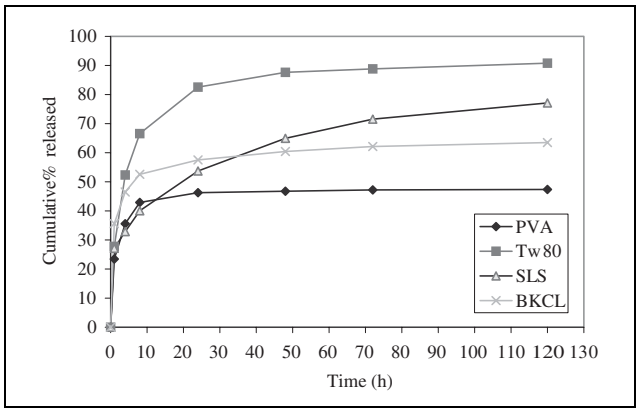

Fig. 1: Dissolution profiles of beta-estradiol biodegradable microspheres prepared using different surfactants in 50% MeOH/phosphate buffer pH 7.4

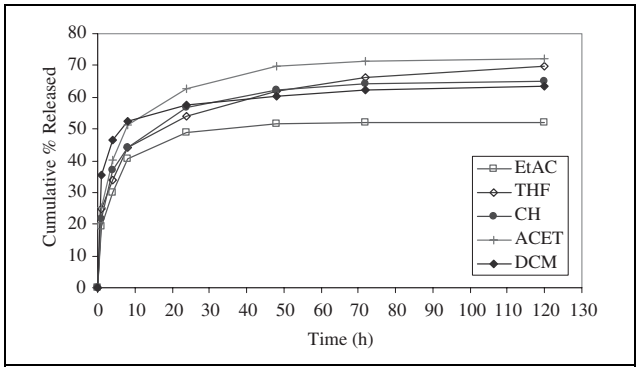

Fig. 2: Dissolution profiles of beta-estradiol biodegradable microspheres prepared from different organic solvents in 50% MeOH/phosphate buffer pH 7.4

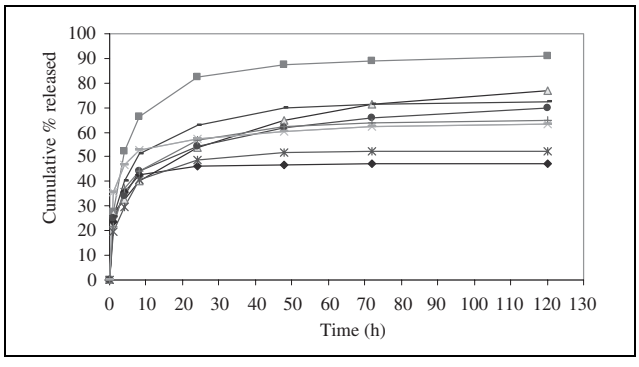

Fig. 3: PVA; Tween 80; SLS; BKCL; ETAC; THF; CH; ACET; DCM
Dissolution profiles of beta-estradiol microsphers prepared from different surfactants and organic solvent in 50% MeOH/phosphate buffer pH 7.4

the cumulative amounts of drug released after 120 h incubation were 71, 52, 69, 64, and 72% for DCM, EtAC, THF, CHCL3 and AC respectively. From these results, it was shown that using EtAC as an organic solvent and PVA as a dispersing agent showed the highest encapsulation efficiency and the lowest amount of drug released after 120 h. These findings were supported by Duan et al. (2000) who reported that formulations applying ethyl acetate are best suited for encapsulating the hormones. Bodmeier and McGinity (1988) found that the rate of polymer precipitation from the organic solvent phase was strongly affected by the rate of diffusion of the organic solvent into the aqueous phase. Organic solvents of low water solubility resulted in slow polymer precipitation which facilitated complete partitioning of the drug into the aqueous phase. The drug loading of the microspheres was found to be enhanced by the addition of water miscible organic solvents to the organic phase of the system. Luckily, EtAC as a solvent is preferred over DCM (the common solvent used in biodegradable formulation) because it is a good solvent for both drug and polymer which enable embedding the drug into the polymer matrix on precipitation. In addition it is less toxic and has less environmental hazards compared to DCM. However, because it is slightly soluble in water, care must be taken to ensure that high quality microparticles can be prepared. Problems encountered when using slightly water miscible organic solvents include irregular particle sizes and shapes, and high microparticle porosity. Thus, the next objective was to characterize these microparticles for particle size distribution, surface morphology and surface charge.

2.2. Particle size analysis

In general, the mean particle size is proportional to the volume and viscosity of inner and external phase which depend on the type and concentration of polymer and surfactant in addition to the type of the organic phase. In this study, the average particle size was about 18 μm and the cumulative results showed that 25% of distribution is less than 3 μm while 99% of distribution is less than 31 μm. The particle size distribution profile is shown in Fig. 4. These results may contribute to the initial burst of drug release. Microspheres with mean particle sizes of 12 and 18 μm have shown an initial release of about 10% of the drug load within the initial 5 h which was attributed to the large surface area (Sansdrap and Moes 1993). The broad distribution may be due to the viscosity induced by the high polymer/drug ratio (7 : 1) and the concentration of PVP in the aqueous phase. Particles fabricated from viscous solution harden much rapidly because the shearing forces induced by the stirrer have limited effects on them once the initial particles are formed (Jeffery et al. 1993).

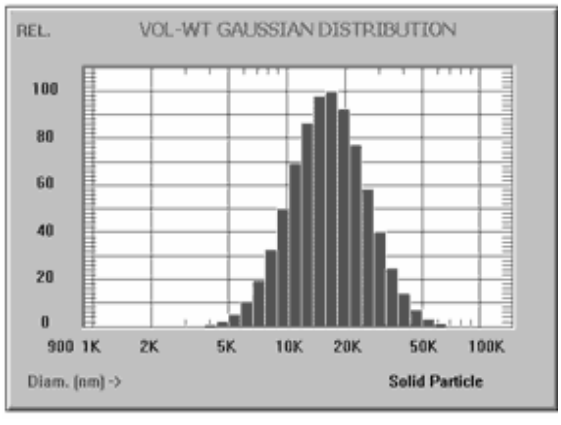

Fig. 4: Particle size distribution of beta-estradiol microspheres

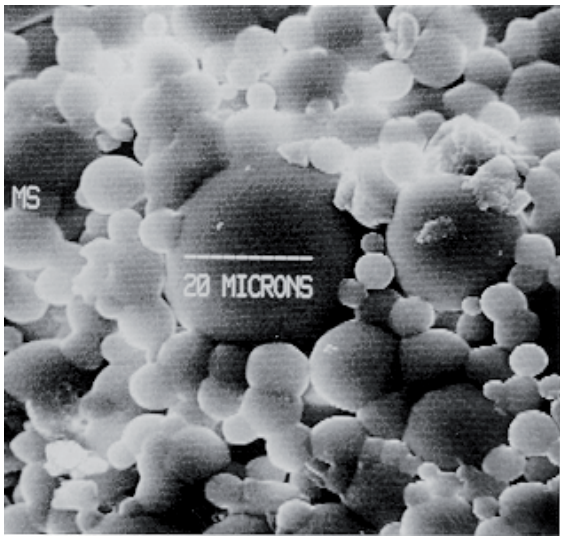

Fig. 5: SEM picture of beta-estradiol microspheres

2.3. Scanning Electron Microscopy (SEM)

The SEM picture (Fig. 5) showed smooth surface microspheres. Some of the microspheres were porous and some others were dense. The slow solvent evaporation rate of EtAC (BP about 77 °C) might led to a denser structure of the particles since they can have sufficient time to shrink in size and this contributed much to the higher bulk density of the microspheres obtained. The porous ones may be those close to the surface which are subjected to faster removal of the solvent compared to those in the bulk. These findings were supported by Duane et al. (2000) and Sah H. (1997) who found that PLGA microparticles prepared from EtAC are transparent to slightly translucent spheres but some of them are opaque and may contain hollow cores. Also, the picture showed some particle agglomerates which may be related to the slow removal of the solvent. Slow removal of solvent promotes the agglomeration of the particles when they come close to each other when they are in the rubbery state before hardening. However, these agglomerates are easy to disperse in buffer or saline (Sun et al. 2005).

2.4. Zeta potential

An aqueous dispersion of the microspheres was assessed for surface charge. When microcapsule particles are suspended in a polar medium, a surface electric double layer develops that governs the ion distribution from the particle surface into the solution. The magnitude and sign of the zeta potential provide valuable information regarding the electric charge present at the interface between the particles and the solution. In addition, surface charge associated processes are believed to be important in normal biological bone synthesis and remodeling. Fig. 6 shows zeta potential signals for the medicated microspheres. The zeta potential value for the medicated and non-medicated ones were almost the same (−6mv). This may be attributed to the negative charge created by PVA molecules (Ravi Kumar et al. 2004). Estradiol by being hydrophobic in nature is encapsulated in the oil core and did not show any influence on the surface charge. These findings were

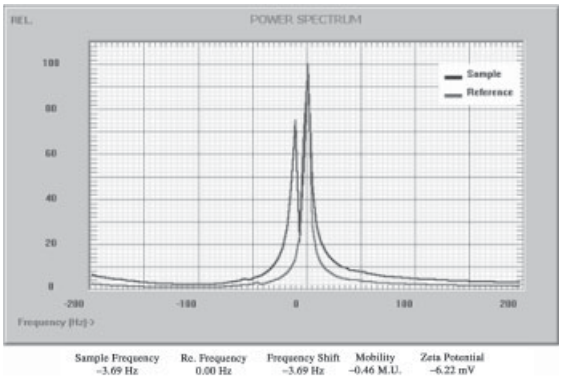

Fig. 6: Zeta potential measurement of beta-estradiol microspheres

supported by Berkland et al. (2004) who stated that the more hydrophilic compound is located toward the microsphere surface while the more hydrophobic one is distributed deep in the core.

In conclusion, it can be stated that different organic solvents and stabilizers showed magnificent effects on the encapsulation efficiency and *in vitro* drug release of beta-estradiol. The formulation containing ethyl acetate and PVA showed low burst drug release and high drug entrapment. The particle size analysis of this formulation showed an average particle size of 15 μm. The SEM showed spherical particles with smooth surface and the zeta potential was negative. The results emphasized the effect of formulation parameters on the characteristics of the resultant microspheres in general and on the entrapment efficiency and *in vitro* release in particular. EtAc and PVA may have a good potential in preparation of beta-estradiol biodegradable microspheres.

3. Experimental

3.1. Materials

Poly (DL-lactide); PLA (IV = 0.26, average MW 12,000–24,000), copolymer poly(DL-lactide-co-glycolide); PLGA 85 : 15 (IV = 0.61, average MW 80,000) were obtained from Birmingham Polymers Inc, (Birmingham, AL), Tween 80, sodium lauryl sulfate, benzalkonium chloride, poly-vinyl alcohol; PVA (average MW 30,000–70,000), and beta-estradiol were obtained from Sigma Chemical Co (St Louis, MO), dichloromethane, ethyl acetate, tetrahydrofuran, chloroform, acetone and methyl alcohol were obtained from Fisher Scientific Co. (Norcross, GA), mono-and dibasic potassium phosphate, calcium chloride and sodium hydroxide were obtained from EM Science (Gibbstown, NJ). Water used was distilled and deionized. Other solvents and excipients were of analytical grade and used without further purification.

3.2. Preparation of beta-estradiol microspheres

Microspheres were prepared by means of the emulsion solvent evaporation technique using PLA and PLGA 85/15 polymer mixture (1 : 1) at drug polymer ratio 1 : 7. The polymer solutions were prepared by dissolving 1400 mg of polymer in 20 ml of the organic solvent using a magnetic stirrer (Fisher Co., Fair Lawn, NJ). After complete dissolving, 200 mg of beta-estradiol were 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% of PVA or Tween 80 or 0.5% of sodium lauryl sulfate or benzalkonium chloride while homogenizing at 10,000 rpm for 5 min using PRO 250 homogenizer (Monroe, CT). The organic phase was allowed to evaporate overnight at a continuous stirring speed of 1400 rpm. The resultant microspheres were filtered using a 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 to remove the residuals of surfactants 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 obtained microspheres were stored in tightly closed glass scintillation vials at temperature and humidity controlled conditions for further investigation.

3.3. Drug encapsulation efficiency

An accurately weighed amount of prepared microspheres was dissolved into 50 ml of 60/40 dichloromethane/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 281 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:

$$\text{Encapsulation efficiency}\,\% = \frac{\text{experimental drug loading}}{\text{theoretical drug loading}} \times \text{X100} \qquad (1)$$

3.4. In vitro drug release

Dissolution experiments were carried out according to Parikh et al. (1993) with slight modification. Briefly, amounts of microspheres 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 a micropore 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 281 nm and the corresponding drug concentration was calculated. The formulation showed high encapsulation efficiency and low burst release was subjected to particle size analysis, SEM and zeta potential characterization.

3.5. Particle size

The particle size and its distribution were measured by laser diffractometry using Nicomp® 380 Particle Sizing Systems (Santa Barbara, CA). Dry microspheres were suspended in de-ionized distilled water and sonicated for a few seconds to segregate the agglomerated particles. The average particle size in μm and particle size distribution were obtained.

3.6. Scanning Electron Microscopy (SEM)

The shape and surface morphology of the particles 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.

3.7. Zeta potential

The surface charge was identified and measured using Nicomp 380/ZLS (Santa Barbara, CA). The process was carried out by suspending few milligrams of medicated and non medicated microspheres in distilled de-ionized water and placed in the electrophoresis cell. After doing the necessary adjustment, the zeta potential signals were recorded.

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