Influence of Manufacturing Parameters on Development of Contraceptive Steroid Loaded Injectable Microspheres

Magharla Dasaratha DHANARAJU,*[a,b] Rajadas JAYAKUMAR,[c] and Chandrasekar VAMSADHARA[b]

[a] *Department of Pharmaceutics, Vel's College of Pharmacy, Old Pallavaram; Chennai 600 117, India:* [b] *Institute of Pharmacology, Madras Medical College; Chennai 600 003, India: and* [c] *Bioorganic and Neurochemistry Laboratory, Central Leather Research Institute; Adyar, Chennai 600020, India.* Received January 23, 2004; accepted May 18, 2004

The main objective of this work was to develop a system consisting of polymeric microspheres loaded with steroid drugs. The drugs were encapsulated using biodegradable poly(lactide-co-glycolide) (PLG) and poly(ε-caprolactone) (PCL) by double emulsion solvent evaporation method. The lipophilic drugs, levonorgestrel and ethinylestradiol were made soluble by adding ethanol/water mixture. The effects of parameters like polymer concentration and stabilizer concentration were studied on the size, size distribution, surface properties and loading efficiencies of microspheres. The formulated microspheres were smooth, spherical and uniform in shape and size. Fourier transformed infrared spectroscopy and differential scanning calorimetry studies seemed to confirm the absence of chemical interaction between the drugs and the polymers, while the drugs were dispersed in the polymer. The increase in polymer concentrations increased the size as well as the loading efficiency of microspheres. Data obtained in this study demonstrated that the PLG/PCL microspheres may be a suitable polymeric carrier for long acting injectable drug delivery.

Key words microsphere; poly(lactide-co-glycolide); poly(ε-caprolactone); steroid drug; levonorgestrel; ethinylestradiol

The use of specific polymers and design of new drug delivery dosage form are currently perhaps one of the most exiting areas in pharmaceutical formulation. The major aim is to improve the efficiency of treatment and to decrease its side effects.[1] Modern drug carrier system plays an important role in controlled delivery of pharmacological agents to its target at therapeutically optimum rate and dose. Formulation of microspheres is one such way to achieve the desired effect.

Effectiveness of microspheres lies mainly on the polymers used to prepare microspheres. Among various types of polymers, biodegradable polymers are of interest in pharmaceutical field for developing a controlled release formulation. These polymers are biocompatable and slowly disappear from the site of administration. These are non-toxic, non-immunogenic and degrade within the body to natural metabolic products. Poly(ε-caprolactone) (PCL), poly lactic acid (PLA), poly glycolic acid (PGA) and poly(lactide-co-glycolide) (PLG) are some of such polymers.[2] In this study PCL and PLG are used to prepare microspheres. Many authors[3—7] has previously shown that hydrophilic drugs and proteins can be encapsulated into microspheres.

Selection of microencapsulation technique is primarily determined by solubility of drugs.[6] In this study the lipophilic drugs were made aqueous soluble by dissolving in a mixture containing ethanol and water then further proceeded with multiple emulsion solvent evaporation method, the method is most successful with drugs which are insoluble or poorly soluble in aqueous medium.[8,9] Many types of drugs with different physical and chemical properties have been formulated into polymeric systems, including anti cancer drugs,[10,11] narcotic agents,[12,13] local anaesthetics,[14] steroids[15,16] and fertility control agents[17,18] using solvent evaporation method of microencapsulation.

The combination of levonorgestrel and ethinylestradiol[19] has been proved for their effectiveness as contraceptives and has been used from decades. These drugs have been selected for entraping into microspheres because the disadvantages associated with oral route is requirement of daily intake and subsequent daily variations in blood concentrations[20] as well as accumulation in body. Therefore, developing a biodegradable polymeric delivery system of antifertility steroids would improve patient compliance and reduce risk of adverse effects.

The purpose of this study was to investigate the feasibility of formulating levonorgestrel (LNG) and ethinylestradiol (EE) into controlled drug delivery system using microsphere technology with poly(ε-caprolactone) and poly(lactide-co-glycolide) as polymers using double emulsion solvent evaporation method and to investigate the influence of concentration of polymer and concentration of stabilizer (PVA) on microsphere size and loading efficiencies.

Experimental

Poly(ε-caprolactone) (PCL) (MW 40000) was purchased from Sigma Aldrich-USA; Poly(lactide-co-glycolide) (PLG) (MW 70000) was bought from Bringham Polymers, Inc., U.S.A.; Levonorgestrel and Ethinylestradiol were obtained as gift samples from German remedies, Mumbai, India; Polyvinylalcohol from Sigma (St.Louis, MO, U.S.A.); Dichloromethane (AR Grade) from Sisco research lab Pvt Ltd, Mumbai, India; Ethanol (AR Grade) from Hayman Ltd., England; Acetonitrile, Methanol and Water (HPLC Grade) from Qualigens fine chemicals, Mumbai, India. All other ingredients used were of analytical grade.

Preparation of Microspheres The microspheres were prepared by w/o/w emulsification-solvent evaporation method as described earlier by us[9] as per quantities mentioned in Table 1. Briefly, a saturated solution of LNG (15 mg) and EE (3 mg) were taken in the ratio of 1 : 5 in ethanol water mixture (7 : 3) and was emulsified at 4000 rpm for 10 min using Remi propeller mixer into 10 ml dichloromethane containing polymer. The resulting w/o emulsion was further emulsified with PVA (0.5, 1, 2%) solution to produce w/o/w emulsion same as that of w/o emulsion. The formed multiple emulsion was kept under constant stirring for 4 h with 600 rpm by a magnetic spin bar assembly. Microspheres were separated by centrifugation at 2000 rpm for 10 min and washed with phosphate buffer pH 7.4 for three times and dried in nitrogen atmosphere. The solvent used for microspheres preparation retained during the encapsulation process were analyzed by GLC and it was well below the limit of detection.

Scanning Electron Microscopy (SEM) When working with microspheres, it is often helpful to visualize particle shape and surface characteristics in order to correlate other determined characteristics such as surface area and bulk density. The size and shape texture of microspheres was determined by JOEL-JFC-5300 scanning electron microscope. The microspheres

* To whom correspondence should be addressed. e-mail: mddhanaraju@yahoo.com

were sprinkled on to one side of adhesive stub and coated with gold using JOEL-JFC 1100E sputter coater and microphotographed.

Particle Size Distribution Particle size is one of the important factors, which decides the release rate of drugs from microspheres and the free flow of microspheres through syringe needle. Microspheres were dispersed in water, vortexed for 3 min and sonicated for 30 s before sampling. The particle size of microspheres was determined by laser diffraction method using Shimadzu SALD 1100, Japan.

Drug Content of Microspheres The drug content of microspheres was determined by dissolving 100 mg of microspheres in 5 ml dichloromethane. To this, 5 ml of methanol was added and the solution was evaporated under vacuum to eliminate dichloromethane and the polymer was allowed to precipitate. The drugs dissolved in the solvent methanol were filtered by using 0.1 μ millipore filter assembly and suitably diluted, subsequently injected into Hypersil C 18, 250×4.6 mm column. The mobile phase used was a combination of acetonitrile/methanol/water in the ratio of 3.5 : 1.5 : 4.5 at flow rate of 2 ml/min, the eluted sample was detected at 215 nm[21] using Shimadzu HPLC LC 10AT-vp.

Fourier Transformed Infrared Spectroscopy (FTIR) FTIR spectra of the formulated microspheres, polymers and drugs were recorded on a Nicolet spectrometer (Avatar Model 320) using the conventional KBr pellet method. For each sample 50 scans were recorded with a nominal resolution of 4 cm^{-1} at 25 °C.

Differential Scanning Calorimetry (DSC) The thermal behaviour of LNG, EE, polymers and drug loaded microspheres *i.e.* glass tranisition temperature and melting temperature were determined by using Perkin–Elmer DSC-7 (calibrated with cadmium) at a chart speed of 10 mm/min. the samples were heated from 30—500 °C in nitrogen atmosphere.

Results and Discussion

Morphology of Microspheres The microspheres on visualized in SEM were spherical and uniform with smooth surface indicating the complete evaporation of solvent. Fig. 1 and Fig. 2 show the morphological characteristics of PCL and PLG microspheres respectively. It was clear that the microspheres were not porous in nature. The particles appeared to be homogeneously distributed without evidence of collapsed particles.

Effect of PVA in External Aqueous Phase Since exchanges between the internal and external aqueous phases should be kept to a minimum during the second emulsification step, the stability of second emulsion is critical. Further during the solvent evaporation process, there is a gradual decrease in volume and subsequent increase in viscosity of dispersed droplets. This affects the droplets size equilibrium, involving the coalescense and agglomeration of droplets during the early steps of solvent removal.[22] This problem can overcome by adding a surfactant into the continuous phase, which provides a thin protective layer around the droplets and hence reduce their coalescence. The different concentrated solutions of PVA (0.5%, 1%, 2%) were used for emulsification.

Aggregation of microspheres was noticed when less amount of PVA was used (0.5%) and there was no such aggregation when 1% and 2% solutions were used. So, it seems 1% PVA to be optimum and therefore all batches were prepared using 1% PVA. The PVA may have prevented coalsecence by forming a film on globules. However at all concentrations of PVA the microspheres obtained were seemed to be stable enough to harden after solvent evaporation. The results were shown in Fig. 3.

Concentration of Polymer LNG and EE microspheres were prepared using PCL and PLG by varying concentration as given in Table 1. By varying the weight of the polymer dissolved in dichloromethane (DCM) to investigate the corresponding modification of particle size, drug loading and encapsulation efficiency.

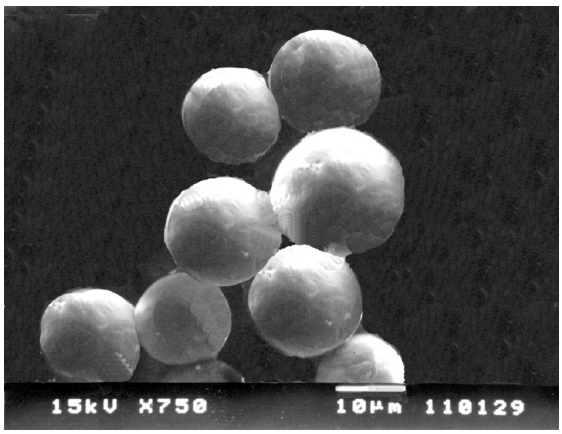

Fig. 1. SEM Photograph of Contraceptive Steroid Loaded PCL Microspheres

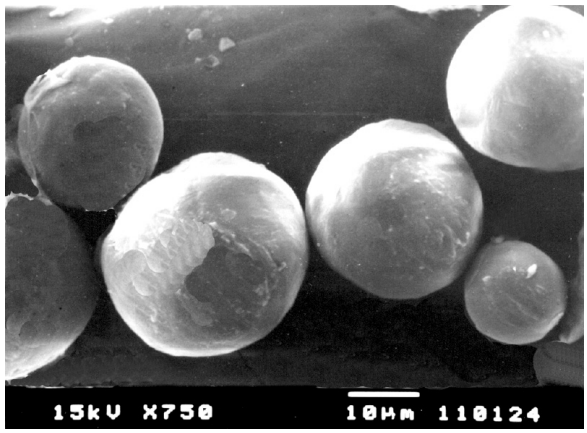

Fig. 2. SEM Photograph of Contraceptive Steroid Loaded PLG Microspheres

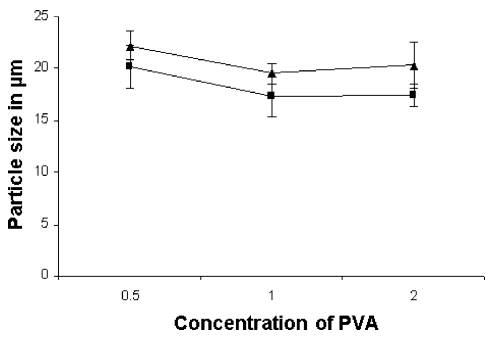

Fig. 3. Effect of PVA Concentration in the External Aqueous Phase on the Particle Size (PCL —■—), (PLG —▲—)

Data are shown as mean±S.E. obtained from three formulations.

Increase in concentration of polymer resulted in increase in mean particle size and an improvement in drug entrapment efficiency. This effect was also observed by Benoit.[23] It stands true with both the polymers. The phenomenon suggests that higher concentration of polymer may lead to an increased frequency of collisions, resulting in fusion of semiparticles and finally producing bigger particles thereby increasing the size of microspheres. Moreover the high concentration of polymer in emulsion droplets led to an enhance-

ment because of high viscosity of organic phase tends to restrict migration of the inner aqueous/drug phase to the external water phase. The increase in polymer concentration increased mean particle size and encapsulation on microspheres. Figures 4 and 5 show the effect of polymer concentration on mean size and on encapsulation efficiency, respectively.

Fourier Transformed Infrared Spectroscopy FTIR spectroscopy was used to ensure that no chemical interaction between the drugs and polymer had occurred. From the inspection of FTIR spectra (Fig. 6, curve a) LNG showed an ester C=O band near 1652 cm^{-1}, and a shoulder band at 1617 cm^{-1}. The band at 3348 cm^{-1} is characterized of presence of terminal ···C≡CH bond. Further, EE (Fig. 6, curve b) showed a broad band between 3400—3470 cm^{-1} which is characteristic of phenolic OH group. Figure 6, curve c and e showed an intense band at 1721 cm^{-1} is due to presence of ester carbonyl group in PCL and PLG polymers.

On the other hand, FTIR spectra corresponding to microspheres (Fig. 6, curve d) were identical to polymer spectra. This seems to indicate the absence of chemical interaction between polymer and the drugs in microspheres preparation. These spectra did not display the intense bands characteristic of drugs because there were of low intensity and were hidden by the bands produced by the polymer. Similar results were observed for PLG microspheres (Fig. 6, curve f).

Differential Scanning Calorimetry DSC thermograms of free drug, polymers and drugs loaded microsphere formulations, were made in an attempt to define the physical state of the drug presented in the carriers and the possibility of interactions between the drug and polymer within the network of the polymer in the microspheres. DSC studies indicated no interaction between drugs and polymer (Fig. 7). PCL showed a melting temperature at 68 °C and glass transition temperature of PLG at 49 °C and similar results were obtained when microspheres of 1 : 10 and 1 : 5 were subjected to thermal analysis (about 60 °C for PCL and 42 °C for PLG formulations). This data suggested that LNG and EE were not dissolved whereas they were dispersed in polymers.

Table 1. Different Formulations of Microspheres

S. No.	Polymer	Drug : Polymer	Amount of drugs (LNG+EE)[a)] in mg	Amount of polymer in mg
1	PCL	1 : 1	15+3	18
2	PCL	1 : 5	15+3	90
3	PCL	1 : 10	15+3	180
4	PCL	1 : 20	15+3	360
5	PLGA	1 : 1	15+3	18
6	PLGA	1 : 5	15+3	90
7	PLGA	1 : 10	15+3	180
8	PLGA	1 : 20	15+3	360

a) LNG, levonorgestrel; EE, ethinylestradiol.

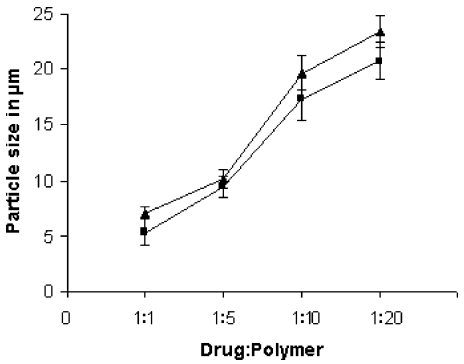

Fig. 4. Effect of Various Drug/Polymer Ratios on Particle Size of PCL (—■—) and PLG (—▲—) Microspheres Prepared by w/o/w Technique

Data are shown as mean±S.E. obtained from three formulations.

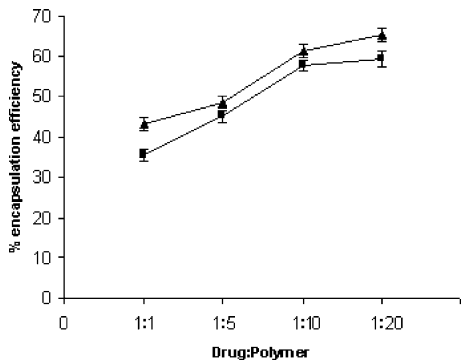

Fig. 5. Encapsulation Efficiency of Steroid Loaded Microspheres *versus* Drug/Polymer Ratio PCL (—■—) and PLG (—▲—)

Data are shown as mean±S.E. obtained from five formulations.

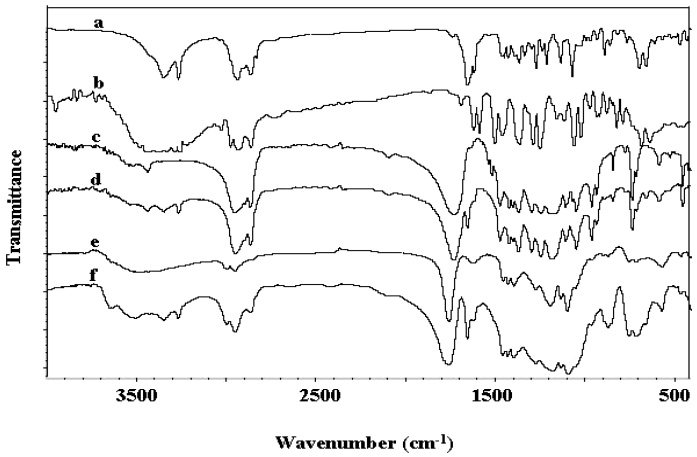

Fig. 6. FTIR of Levonorgestrel (a), Ethinylestradiol (b), PCL Polymer (c), Steroids Loaded PCL Microspheres (d), PLG Polymer (e) and Steroids Loaded PLG Microspheres (f)

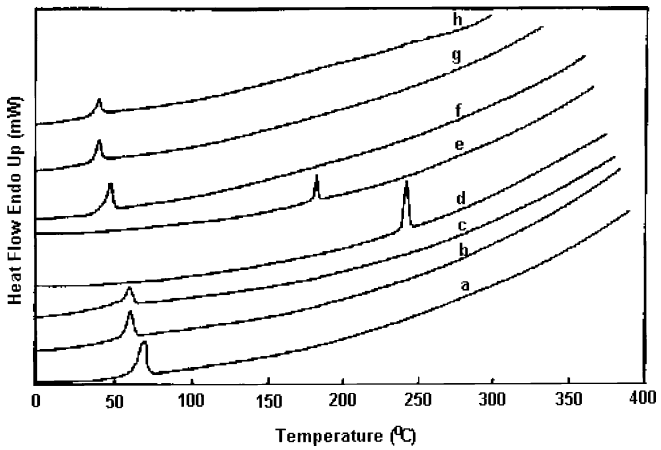

Fig. 7. DSC Thermograms of PCL Polymer (a), 1 : 10 PCL Microspheres (b), 1 : 5 PCL Microspheres (c), Levonorgestrel Alone (d), Ethinylestradiol Alone (e), PLG Polymer (f), 1 : 10 PLG Microspheres (g) and 1 : 5 PLG Microspheres (h)

DSC thermograph of LNG and EE shows sharp endothermic peaks at 243 °C and 184 °C respectively which corresponds to their melting temperatures (LNG 240 °C and EE 180—186 °C). The drug-loaded microspheres did not show any such peaks. This suggests that drugs were at molecular level at polymer melting temperature, and it can be concluded that polymers maintained their characteristics in the microsphere formulations.

Conclusion

The study has demonstrated that microspheres of poly(ε-caprolactone) and poly(lactide-co-glycolide) loaded with levonorgestrel and ethinylestradiol expected to be used as carrier of lipophillic compounds prepared by a w/o/w emulsion solvent evaporation technique. Selection of appropriate conditions had enabled the preparation of smooth, spherical and uniform polymeric microspheres. The parameters (concentration of polymer and concentration of stabilizer) were selected to check their effect on particle size and loading efficiency of microspheres. Further more, the present microspheres are attractive for parenteral application because of their optimum micron size structure and their biodegradability. The biodegradable property of the polymers makes this delivery system a potential carrier for long acting controlled drug delivery for of contraceptive steroids.

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