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Preparation and characterization of injectable microspheres of contraceptive hormones

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Abstract

Present study describes the development of a new formulation of levonorgestrel and ethinylestradiol based on double emulsion-solvent evaporation technique using poly(ε-caprolactone) (PCL) as biodegradable polymer. The effect of polymer concentration on microspheres and entrapment of drug into microspheres were studied. PCL was selected because of its hydrophobicity and advantages over other biodegradable polymers. Characterization of biodegradable polymer used for controlled drug delivery is essential to ensure reproducibility of in vitro and in vivo performances. The selected characterisation techniques established for PCL microspheres include its loading and entrapment efficiencies, DSC to analyse thermal behaviour, SEM to observe surface morphology, drug content of microspheres and in vitro release of drugs from microspheres. The SEM reports showed that microspheres were with smooth surface and DSC thermograms revealed no interaction between drug and polymer. The entrapment was found to be 58 and 47% for 1:10 and 1:5 batches and in vitro release studies showed that about 69.7% of LNG and 66.7% of EE from 1:10 batch and about 80% of LNG and 75.5% of EE from 1:5 batch for 150 days.

Keywords: Levonorgestrel; Ethinylestradiol; Microspheres; Biodegradable polymers; Poly(ε-caprolactone)

1. Introduction

Modern drug carrier systems such as injectable implants play important role in controlled delivery of pharmacological agents. Parenteral controlled release systems are of considerable interest for drugs, which either require daily administration or have high toxicity or a very low oral bioavailability (Sandrap and Moes, 1993). In order to avoid daily administration of drugs, polymeric particulate carriers (microspheres and nanospheres) were developed as controlled release systems (Blanco-Prieto et al., 1997). Developing biodegradable injectable delivery systems to deliver drugs and to maintain the drug blood level in a desired therapeutic range for longer period of time, thereby improve patient compliance and convenience has been of considerable interest.

Microspheres, which consists of a drug dispersed in spherical polymer matrix, have already been extensively evaluated for administration of active compounds such as narcotic antagonists, local anaesthet-

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ics, steroid hormones, anti-tumour agents, and several peptides (Beck et al., 1979; Benita et al., 1984; Sanders et al., 1984; Ogawa et al., 1988). However, to avoid tissue overloading encountered with the administration of non-biodegradable polymers, special attention has been paid to biodegradable polymers. Among the various biodegradable polymers—poly(ε-caprolactone) (PCL), poly-glycolic acid (PGA), poly-lactic acid (PLA) and poly(lactide-co-glycolide) (PLG)—PCL is bioresorbable, biocompatible and more hydrophobic than the polymers mentioned above (Espuelas et al., 1997). It has been shown that the efficiency of particle absorption is correlated with hydrophobicity of wall material and the absorption increases with increase in hydrophobic nature of polymer. Unlike PLA and PGA, which generate extreme acid environments during their degradation, the delayed degradation characteristics of PCL do not generate any acid environment during drug release and therefore advantageous for developing a controlled delivery system (Vijaya Ramesh et al., 2002). These polymers lead to production of non-toxic degradation products that can be metabolised and excreted via normal physiological pathways.

Selection of microencapsulation technique is primarily determined by solubility of drugs (Blanco-Prieto et al., 1997). In this study, a technique based on water-in-oil-in-water (w/o/w) emulsion-solvent evaporation method was selected since it has been reported as the most appropriate method to encapsulate hydrophilic drugs within the microparticles (Alex and Bodmeier, 1989; Iwata and Mc Ginity, 1992). The drugs used in this study are contraceptive hormones as levonorgestrel (LNG) and ethinylestradiol (EE), which are hydrophobic in nature. A novel method has been developed to get these drugs into aqueous phase by using ethanol/water mixture. A combination of LNG and EE pills has to be taken daily to maintain their blood levels to achieve the desired effect of these steroidal hormones. Daily intake of these drugs requires compliance and bears the disadvantage like drug accumulation in the body, which may result in increased side effects. It would be effective if these drugs were made available in the blood at effective concentrations with reduced side effects for longer duration. Based on these considerations the present study investigates the feasibility of formulating LNG and EE into PCL microspheres using double emulsion-solvent evaporation technique, and to study the effect of polymer concentration on release rates, entrapment efficiencies and on size of microspheres.

2. Experimental

2.1. Materials

Poly(ε-caprolactone) has been obtained from Sigma-Aldrich, USA and levonorgestrel and ethinylestradiol were gifted by German Remedies, Mumbai, India. Polyvinyl alcohol (AR grade) from Sigma (St. Louis, MO, USA) and dichloromethane (AR grade) has been procured from Sisco Research laboratories Pvt. Ltd., Mumbai, India. Ethanol (AR grade) was bought from Hayman Ltd., UK. Acetonitrile, methanol and water (HPLC grade) were purchased from Qualigens Fine Chemicals, Mumbai, India. All other chemicals were of analytical grade.

2.2. Preparation of microspheres

Solution of drugs, prepared by dissolving both levonorgestrel and ethinylestradiol in a mixture of ethanol/water in the ratio 7:3, is emulsified in 10 ml of dichloromethane containing polymer. The emulsion formed is allowed to stirr at 4000 rpm for 10 min and then added to external phase containing polyvinyl alcohol (1%), which results in multiple, w/o/w, emulsion. Emulsion formed was mechanically stirred at 600 rpm for 4 h to evaporate dichloromethane. The microspheres formed were separated by centrifuging at 2000 rpm for 10 min and washed with phosphate buffer pH 7.4 for three times and dried under vacuum. The microspheres are prepared in two batches, i.e. Batch I and Batch II. The quantities of various ingredients used in batches are given in Table 1.

2.3. Determination of LNG and EE entraped in microspheres

Estimation of drug content of microspheres was carried out after dissolving 100 mg of microspheres in methanol, the sample was injected into SHIMADZU SCL 10A-VP of C18 column (250 mm × 4.6 mm) and flow rate of 2 ml/min. The sample was detected with UV at 215 nm. Mobile phase used was acetonitrile,

Table 1
Quantities of various ingredients used in preparation of microspheres

S. No.	*d*/*p* ratio[a]	Amount of drugs taken (mg)	Amount of polymer (mg)	Amount of PVA (mg)
1	1:5 (Batch I)	LNG (15) + EE (3)	90	33
2	1:10 (Batch II)	LNG (15) + EE (3)	180	33

[a] *d*/*p*—drug/polymer.

methanol and water in the ratio of 3.5:1.5:4.5 (Berzas et al., 1997). The percentage (w/w) of drug entrapped per dry weight of microspheres was determined. The percentage of entrapment efficiency was expressed by relating the actual drug entrapment to theoretical as described by Jeffery et al. (1993).

2.4. Determination of particle size of microspheres

Microspheres were analysed for their size and size distribution. Dried microspheres were dispersed in water, vortexed for 3 min and sonicated for 30 s before sampling. Particle size was measured using laser diffractometer (Shimadzu SALD 1100) and plotted for volume distribution using software supplied by manufacturer.

2.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) provides accurate and precise quantities data of the sample. Levonorgestrel, ethinylestradiol, poly(ε-caprolactone) and both batches (1:5 and 1:10 drug/polymer (*d*/*p*) ratios) were scanned for their melting temperatures in nitrogen atmosphere by PERKIN-ELMER DSC-7.

About 3 mg of sample was placed in hermetically sealed aluminum pans and was heated at a scan speed of 10 °C/min over a temperature range of 30–500 °C at a chart speed of 10 mm/min. The heat of fusion was calibrated with Indium.

2.6. Scanning electron microscopy

Scanning electron microscopy (SEM) is an excellent tool for physical observation of morphological features of microspheres both initially and during the degradation process. It is helpful to examine microspheres shape and surface characteristics in order to correlate other determined characteristics such as surface area and bulk density. The microspheres were sprinkled on to one side of adhesive stub. The stub was then coated with conductive gold with JOEL-JFC 1100E-SPUTTER COATER and were examined under JOEL-JFC 5300 scanning microscope for qualitative assessment of morphology of microspheres.

2.7. In vitro studies

Release studies of drug from both batches were determined by adding 15 mg of levonorgestrel equivalent microspheres to 50 ml of phosphate buffered saline pH 7.4 in a conical flask. These flasks were incubated at 37 ± 1 °C. A sample of 1 ml was collected at regular intervals and analysed for drug released by HPLC as described earlier.

3. Results and discussion

The choice of particular method of encapsulation is usually determined by the solubility characteristics of drug (Lamprecht et al., 1999). The method opted was double emulsion-solvent evaporation method as it is known to be superior to other incorporation methods (Tabata et al., 1993). The process of solvent evaporation method involves two major steps, the formation of stable droplets of primary emulsion and subsequent removal of solvent from droplets of secondary emulsion. LNG and EE encapsulation efficiency and particle size have been optimized by varying the amount of polymer.

3.1. Particle size and encapsulation efficiency

Microspheres prepared by using different polymer concentration resulted in diameters of length between 8 and 25 μm depending on the polymer concentration of each batch. The diameters and the percentage of entrapment were found to be 9.53 μm and 46.3% for Batch I and 17.85 μm and 57.7% for Batch II, respectively. These are given in Table 2.

Table 2
Percentage of loading efficiency and entrapment efficiencies of prepared microspheres

S. No.	*d*/*p* ratio	Mean size (μm)	Drug loading (%)	Encapsulation efficiency (%)[a]
1	1:5	9.5	7.7	46.3
2	1:10	17.28	5.25	57.7

[a] $E = Q_p/Q_t \times 100$, where E is the percentage encapsulation of microspheres, Q_p is the quantity of drug encapsulated in microspheres (g), Q_t is the quantity of drug added for encapsulation (g).

From the results it was evident that the increase in polymer concentration increases mean diameter of formulation as well as entrapment efficiency of microspheres. This is in agreement with the findings of Benoit et al. (1999). The entrapment efficiency of drugs was obtained as results described by Jameela et al. (1998) (57.7–60%).

The postulated reason for the decrease in entrapment by Batch I was that the quantity of polymer present was insufficient to cover the drugs completely (Benoit et al., 1999) and an increase in concentration of polymer in organic phase leads to increase of size of microspheres. These results were correlated with those of Lamprecht et al. (1999). They stated that when the *d*/*p* ratio increases it results into increase in viscosity of primary emulsion and thereby reduces partition of drugs into external phase and subsequently increases drug entrapment efficiency.

3.2. Differential scanning calorimetry

DSC thermograms of LNG, EE, microspheres of both batches and polymer were made in an attempt to define the physical state of drug in these carrier and possibility of interactions between the drug and polymer within the network of polymer in microspheres (Espuelas et al., 1997). The results are illustrated in Fig. 1.

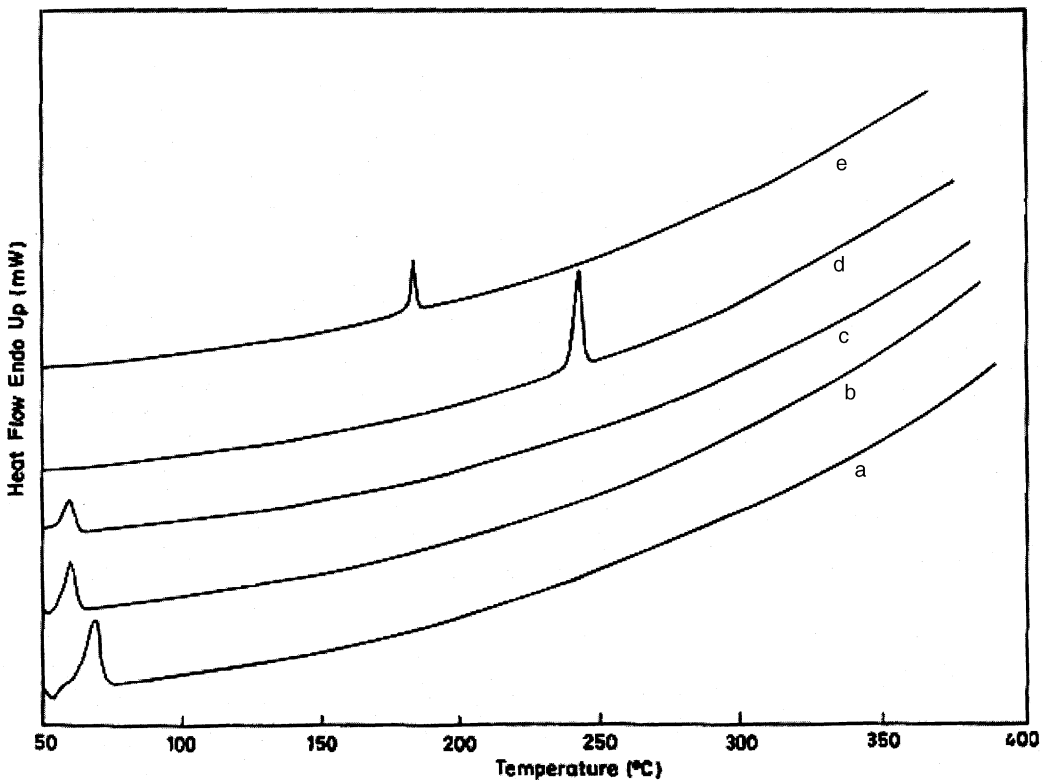

Fig. 1. DSC thermograms of (a) polymer (PCL); (b) Batch II (1:10); (c) Batch I (1:5); (d) levonorgestrel; (e) ethinylestradiol.

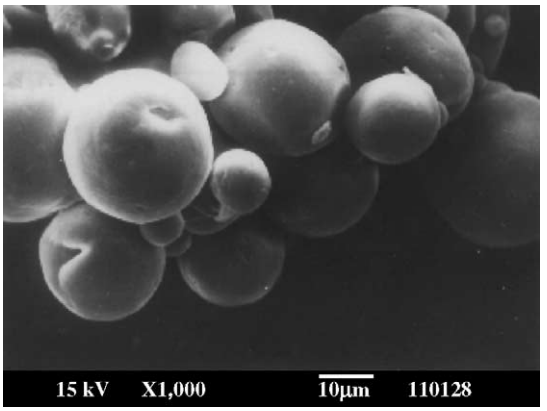

Fig. 2. SEM photographs of levonorgestrel and ethinylestradiol loaded microspheres. Batch II (1:10).

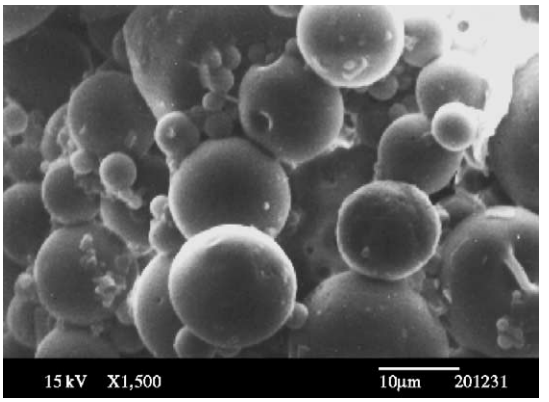

Fig. 3. SEM photographs of levonorgestrel and ethinylestradiol loaded microspheres. Batch I (1:5).

The thermograms of LNG and EE showed an endothermic around 243 and 183 °C, respectively. This corresponds to melting temperatures of drugs (LNG at 240 °C and EE at 180–186 °C). The thermograms of polymer and those of both batches were identical with an endometric peak at 60 °C corresponding to melting temperature of PCL. The result clearly indicates that there is no interaction between drugs and polymer. The thermograms of both batches do not show endothermic peaks of drugs, implying that the drugs are

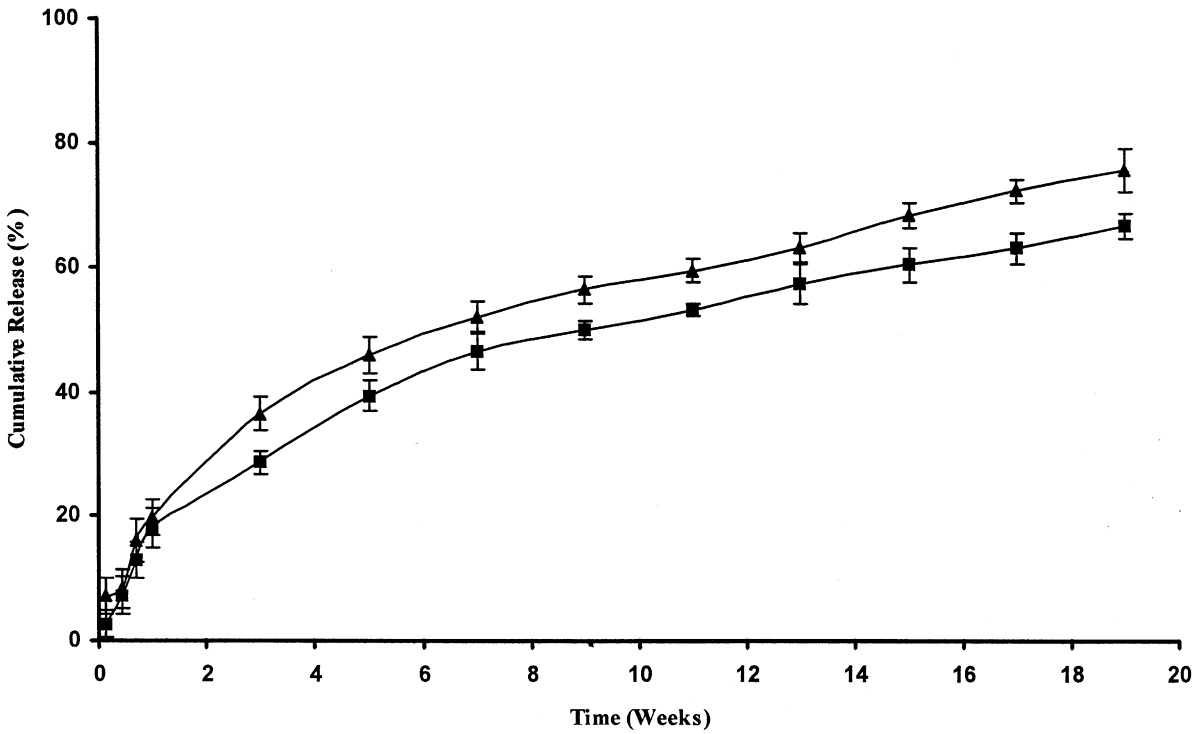

Fig. 4. Dissolution profiles of levonorgestrel from microspheres prepared by double emulsion-solvent evaporation method. (■) 1:10 and (▲) 1:5 batches. Data are shown as mean ± S.E. of three experiments.

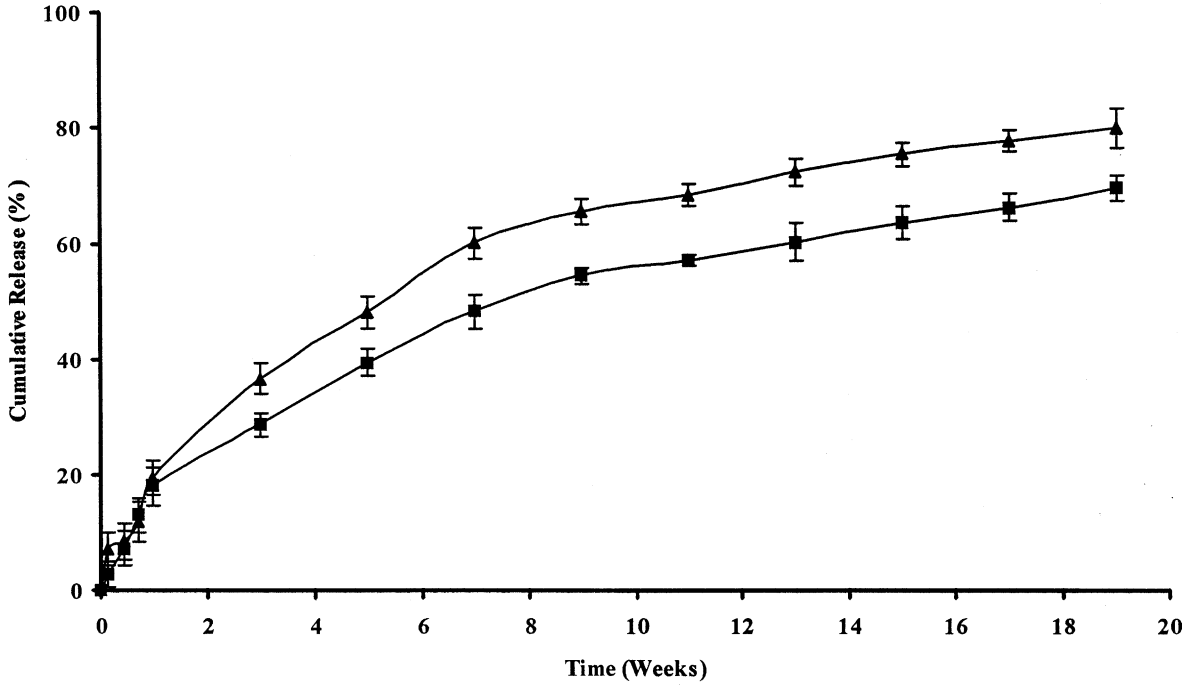

Fig. 5. Dissolution profiles of ethinylestradiol from microspheres prepared by double emulsion-solvent evaporation method (■) 1:10 and (▲) 1:5 batches. Data are shown as mean ± S.E. of three experiments.

present in batches in molecular level at polymer melting temperature, and it can be concluded that polymers maintained their characteristics in the microspheres prepared.

3.3. Scanning electron microscopy

The microspheres obtained by both batches were free flowing. The scanning electron micrographs in Figs. 2 and 3 show that the microspheres were spherical, smooth and individually homogeneously distributed without evidence of collapsed spheres. The micrographs do not show any pores on microspheres. Smooth surface reveals complete removal of dichloromethane from microspheres.

3.4. In vitro release studies

The release profiles of all batches of microspheres showed a trend of increasing release initially and thereafter the release is relatively slow. Batch I (1:5) with small particle size released more percentage of entrapped drugs (80% of LNG and 75.5% of EE). However, the burst release was about 19.4 and 19.8% of LNG and EE, respectively. In case of Batch II having larger particle size showed 69.7% of LNG and 66.7% of EE for 150 days with burst effect of 18.8 and 18.0% of LNG and EE, respectively.

From the results obtained it is clear that the initial burst release was followed by low constant rate of drugs. The burst release of drugs was normally considered to be due to surface located drug (Yan et al., 1994) and slow and constant rate of drugs release may be due to diffusion of drugs from polymer as well as due to erosion of polymer. The phenomenon seems to be more accentuated by high hydrophobicity of poly(ε-caprolactone). Figs. 4 and 5 show the in vitro graph of LNG and EE, respectively.

4. Conclusion

It is shown that PCL microspheres are capable of delivering sufficient quantity of drugs by diffusion for prolonged periods. The use of ethanol/water mixture

to attain solubility of highly water insoluble drugs in aqueous phase helped in using superior method, i.e. double emulsion-solvent evaporation method to encapsulate into microspheres. The biodegradable property of PCL and its hydrophobicity makes this delivery system a potential controlled drug delivery system.

The result of this work demonstrates the feasibility of formulating both levonorgestrel and ethinylestradiol (both are hydrophobic in nature) in the same biodegradable polymeric microspheres employing ethanol/water mixture and in conjection with w/o/w method. Because of less pronounced burst effect, microspheres prepared by such method may present a promising approach for achieving contraception for longer period after single administration.

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