CHAPTER - 3

SUMMARY

&

CONCLUSIONS

In recent years there has been a growing interest in the subject of design and development of **controlled release drug delivery systems** for bioactive agents. These systems have been found particularly useful for long-term medications that require continuous administration of drugs at therapeutically effective doses. Fertility regulation is one of the situations where prolonged administration of contraceptive agents is desirable. The present study was therefore aimed at the development of controlled release injectable and implantable dosage form(s) for levonorgestrel (LNG), an antifertility agent.

Extensive literature survey justified the selection of the antifertility agent and the delivery systems. It also provided useful information which assisted in the logical development and evaluation of controlled release injectable and implantable dosage forms of LNG.

The investigations undertaken are briefly summarized as :
A) preformulation studies viz., standardization of LNG, characterization of PCL & PLGA, solid state stability of drug polymer mixtures & feasibility of sterilizing LNG, PCL & PLGA by gamma radiation and B) studies pertaining to development of injectable and implantable dosage forms such as, 1) optimization of process parameters for the preparation of microspheres; 2) preparation of drug loaded microspheres and implants; 3) *in-vitro* release studies of the microspheres and implants; 4) selection of suitable vehicle for dispensing microspheres and formulation of injectable preparation; 5) radiation sterilization of selected injectable and implantable

formulations; 6) accelerated stability testing of radiation sterilized formulations and 7) *in-vivo* evaluation of selected injectable and implantable formulations.

Levonorgestrel samples which complied with the British pharmacopoeial and United States pharmacopoeial specifications were used in the study. The biodegradable polymers viz., poly-ϵ-caprolactone (PCL) and poly (dl-lactide-co-glycolide) [PLGA] were characterized with respect to their molecular weight distributions, glass transition/ melting temperature and moisture content. PCL utilized in the study was found to have a weight average molecular weight (M_w) of 155,000 dalton, polydispersity (PD) of 3.95 and melting temperature 63.38°C. Whereas, the M_w, PD and glass transition temperature of PLGA employed in the investigation was observed to be 89,000 dalton, 2.56 and 57.95°C, respectively. Both the polymers were found to be free from moisture.

Preliminary investigations were performed to probe the stability of drug-polymer mixtures at 0-4°C. DSC thermograms of drug-polymer mixtures were registered before and after storage at 0-4°C for one month. The drug content of these mixtures was determined by a previously standardised UV spectrophotometric method. The DSC scans performed on the drug-polymer mixtures, before and after storage, were found to be superimposable, indicating absence of any significant changes in the polymer properties at the studied temperature. Further, the amount of LNG present in these mixtures was found to remain identical.

The feasibility of sterilizing LNG by gamma radiation, without adversely effecting its properties, was ascertained at 1.0, 1.5, 2.0 and 2.5 Mrad doses. The irradiated samples were characterized by pharmacopoeial methods. The samples exposed to radiation dose of 1.5, 2.0 and 2.5 Mrad were found to be sterile, while others were non-sterile. The physicochemical properties of the sterilized samples of LNG were observed to comply with the pharmacopoeial specifications.

Based on these results, two radiation doses viz., 1.5 & 2.5 Mrad were selected to sterilize the polymers and study their impact on the polymer properties. The irradiated polymer(s) samples were characterized by the gel permeation chromatography (GPC) and DSC. A significant decrease in M_W and T_g of PLGA was observed at the studied doses. Whereas, the changes in M_W and T_m of PCL were found to be less than 5% and 1°C respectively.

The dosage form development studies were initiated with the optimization of process parameters for the preparation of plain microspheres. Eleven batches of PCL microspheres, without drug, were prepared by an emulsion solvent evaporation technique. The influence of process variables viz., stirring rate (SR), type of emulsifier (ES), emulsifier concentration in aqueous phase (EC), polymer concentration in organic phase (PC) and organic to aqueous phase ratio (OAR), on the particle size distribution of microspheres was examined. Each variable was studied at three different levels while keeping other variables constant. The levels at which these variables were investigated include:

SR - 4000, 2000 & 1000 rpm;
ES - 0.5% w/v PVA, 0.5% w/v HPMC & 0.5% w/v MC;

EC - 0.5% w/v, 0.2% w/v & 0.1% w/v;
PC - 10% w/v, 5% w/v & 2.5% w/v and
OAR - 1:40, 1:20 & 1:10.

The particle size distribution of the microspheres, thus prepared, was determined by light microscopy. One way ANOVA was performed for each variable to evaluate statistical difference between the mean diameter of the microspheres prepared at different levels of a variable. All the variables studied were found to have significant ($p < 0.05$) influence on the mean particle diameter of the microspheres, at the scrutinized levels. The impact of these variables was found to decrease in the order of PC > ES > EC > SR > OAR as judged by their calculated F values.

Consequently, LNG loaded microspheres were prepared under conditions optimized to produce microspheres with particle size ranging between 10 µm to 250 µm i.e., SR - 4000 rpm; ES - PVA; EC - 0.5% w/v; PC - 5% w/v and OAR 1:10. Total six batches of drug loaded microspheres were prepared. PCL microspheres were prepared with three drug loadings viz., 5%, 10% & 20% w/w. In addition, three batches of microspheres were prepared with the combination of two polymers viz., PCL & PLGA, at 10% w/w drug loading. The PCL to PLGA ratios of these batches were 80:20, 60:40 and 40:60. The drug content and moisture content of all these batches was determined by the UV spectrophotometric method and Karl Fischer method respectively. A laser diffraction particle size analyzer was used to find the particle size distribution. Whereas, the surface morphology was observed under a scanning electron microscope.

The yields of the process were found to be about 83%, with approximately 90% of the microspheres in the desired particle size range. The drug content ranged between 97.32 and 99.28% of theoretical drug loading. The microspheres appeared to be spherical in shape with rough, porous surface, as revealed by scanning electron microscopy.

The *in-vitro* release studies of the microspheres were performed in two different dissolution media viz., 10% v/v ethanolic phosphate buffered saline pH 7.4 (EPBS) and 50% v/v EPBS, by a reported method. The UV spectrophotometric methods for estimation of LNG in these dissolution media, were standardized. The fractional release of drug was examined at regular time intervals for a period of 60 days or 7 days in 10% v/v EPBS or 50% v/v EPBS respectively. The fractional drug release profiles were expressed in terms of fractional drug release as a function of time as well as square root of time. An apparent linear relationship ($0.97<r<0.999$) was found to exist between the amount released and the square root of time data, indicating that the release of drug from the microspheres followed the Higuchi's diffusion model.

The microspheres with LNG:PCL:PLGA ratios of 10:90:00 (DLM-2) and 10:36:54 (DLM-6) were selected for further investigation owing to their favourable release profiles, surface characteristics and particle size distribution. These batches were further characterized for their polymer properties by GPC & DSC. The vehicle for dispensing these microspheres was also selected.

Three suspending agents viz., Sod.CMC, HPMC & MC were evaluated, at 0.5% w/v concentration in normal saline, for their suitability as a vehicle to dispense the microspheres in an injectable dosage form. 10% w/v suspensions of microspheres were prepared in these vehicles. Suspendibility, sedimentation volume, sedimentation rate, redispersibility and syringeability of these suspensions were studied. Normal saline containing 0.5% w/v HPMC was selected as a dispensing medium based on the results of this study.

Implants (circular discs) containing LNG were prepared from the microspheres by a method reported for the preparation of progesterone implants. Eight batches of implants were prepared with different weights, drug loading and polymer compositions. The drug content, thickness and friability of these implants were determined. The *in-vitro* release studies were performed in 50% v/v EPBS. Implant with LNG:PCL:PLGA ratio of 10:90:00 & thickness 1.1 mm (IMP-2) was selected for further investigations based on the results of *in-vitro* release studies.

The selected formulations, i.e. DLM-2, DLM-6 & IMP-2, were sterilized by gamma radiation at a dose of 2.5 Mrad. Important physicochemical properties and *in-vitro* release profiles of the radiation sterilized formulations were characterized. A favourable change in the *in-vitro* release profile of LNG was observed from the DLM-6 microspheres after the sterilization. Whereas, the *in-vitro* release profiles of drug from DLM-2 and IMP-2 were unaltered and closely matched the desired release pattern. Other properties of these formulations were not changed during sterilization.

Accelerated stability testing of the radiation sterilized formulations, packed and sealed in USP type I vials, was carried out at different temperature and humidity conditions viz., 0-4°C, 25°C, 37°C, 45°C and 75% RH at 37°C for a period of 3 months. A significant change in *in-vitro* release pattern was noticed for DLM-6 and IMP-2, when stored at 45°C and 37°C (or higher temperature) respectively. Further, these changes were observed to be associated with change in M_w (in case of DLM-6) and deformity of shape (in case of IMP-2). The *in-vitro* release profile of DLM-2 microspheres stored at different conditions remained same at the end of the study.

Safety of these formulations was assessed in adult Wistar rats, in terms of their biocompatibility with subcutaneous tissue, over a period of 3 months. Both the batches of microspheres were found to be safe as revealed by the histology of the subcutaneous tissue at the site of administration. However, mild to moderate inflammatory response was observed, for the first 30 days, in animals injected with DLM-6 microspheres. Whereas, no signs of gross inflammatory response were noticed in animals treated with IMP-2, in the early or late phase of the investigation. The implantable formulation was also found to be safe.

Based on the results of these studies two formulations viz., DLM-2 & IMP-2, were evaluated for their effectiveness in adult female Wistar rats. The efficacy of these formulations was judged by their ability to block the estrous cycle in the diestrous phase for prolonged period. Both the formulations were found to be effective for a period of 60-65 days. However, a delay in onset of action was observed in animals treated with the implant.

Following conclusions can be drawn from this study :

1) LNG & PCL can be radiation sterilized, at 2.5 Mrad dose, without any significant changes in their important properties. Whereas, a significant decrease in M_w and T_g of PLGA occurs at a gamma radiation dose of 1.5 & 2.5 Mrad.
2) The biodegradable microspheres containing levonorgestrel can be obtained in good yields with the desired particle size distribution by the emulsion solvent evaporation technique.
3) The biodegradable implants can be easily prepared by the method used in the present study. However, improvization in the fabrication method is required for industrial scale production.
4) The LNG containing dosage forms (microspheres as well as implants) prepared using PCL alone can be sterilized by gamma radiation at 2.5 Mrad dose, without any significant changes in their important properties. Whereas, a careful selection of radiation sterilization dose is required for the dosage forms prepared using PLGA alone or in combination with other polymers.
5) The LNG containing dosage forms prepared using PCL & PLGA can be stored at 0-4°C without any significant changes in their important properties.
6) The results of the *in-vivo* studies demonstrates the

potential viability of levonorgestrel loaded poly-ϵ-caprolactone microspheres and implants to deliver levonorgestrel for prolonged period of time.