CHAPTER — 1

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1.1 CONTROLLED RELEASE DRUG DELIVERY SYSTEMS – A BRIEF OVERVIEW

The limited efficacy of conventional dosage forms characterized by the first order release kinetics and the associated limitations such as of duration of action and patients' compliance led to the concept of CONTROLLED RELEASE DRUG DELIVERY SYSTEMS (CRDDS). Teorell established pharmacokinetic basis of these systems almost 60 years ago.[1] He demonstrated that when the rate of drug input was controlled, the duration of action was prolonged. Subsequently, attempts were made to slow and delay absorption of drugs administered by an oral or parenteral route and few sustained release products appeared in the market in 1950's.[2-5] However, initial commercial enthusiasm for slow release products was not matched by corresponding evidence of clinical advantage and thus the growth of such systems was slow.[6] Nevertheless, the reliability of CRDDS has improved greatly in the last two and a half decades and their popularity is again increasing, as judged by their current sales value.

The world market of CRDDS has increased from US $ 6 billion in 1990 to US $ 15 billion in 1995 and will probably reach over US $ 50 billion, by the year 2005.[7] This rapid growth in the field of CRDDS is attributed to several factors, the most prominent are; (i) a marked decline in the rate of appearance of new drug entities, due to increase in time and cost of development; (ii) the growth of pharmacokinetic discipline due to acquisition of capabilities in precise analytical techniques; (iii) a better understanding of mechanisms of drug transport across different biological membranes and methods to enhance the drug transport; (iv) the availability of a wide range of polymers and carriers for drug delivery; (v) well designed *in vitro* release tests and clinical trials; and (vi) medical and economical benefits offered by these systems. These benefits include new therapeutic uses of the same drug, therapeutic applications of drugs difficult to administer orally, decreased costs of therapy, improved patient compliance, reduction in medication side-effects and increased product life through patents.[8,9]

A variety of CRDDS intended for oral, parenteral, ocular, transdermal, buccal, nasal, rectal, pulmonary, vaginal or intrauterinal administration are being manufactured or are under development.[10] However, oral controlled release dosage forms led the market in 1995, with roughly US $ 6 billion in revenue and site specific pulmonary delivery systems at US $ 5.1 billion. Transdermal therapeutic systems came in third place with US $ 1.9 billion, followed by nasal site specific and controlled release parenteral formulations at US $ 0.85 billion and US $ 0.8 billion, respectively.[7]

In the developed part of the world, such as United States, Europe and Japan, there is a tremendous level of interest in the development, utilization and promotion of the CRDDS. The incorporation of controlled release societies, regular organization of national and international symposia and publication of many journals, books and reports reflect the importance gained by CRDDS.

On the other side, the situation in India, with respect to CRDDS has not been so bright and promising. There are two main reasons for this; firstly, majority of Indian population (about 75%) is still dependant on the traditional systems of medicine, where as most of the CRDDS are developed for modern drugs. Secondly, the priorities of Indian pharmaceutical industry have been to become self reliant with respect to the production of bulk drugs as well as conventional formulations. As a result, the predominant research activity in India was confined to manufacturing processes, rather than technology development. However, with the signing of GATT, the Indian pharmaceutical industry is in a transition period. The Indian pharmaceutical companies have now become interested in the development of CRDDS for existing drugs. This offers an excellent business opportunity to them, as the cost of research and development in India is minuscule, in comparison to product development cost in USA and Europe. Also, regulatory requirements are fewer for generic drugs than new drug molecules.

At the present time, about 90 oral CRDDS are manufactured and available in the Indian market and only 2 transdermal CRDDS are marketed in India. But the potential of advanced drug delivery systems has not been exploited as yet. The major categories for therapeutics marketed as CRDDS in India are NSAIDs, Ca-channel blockers, antihistaminics, bronchodilators, antiepileptics, haematinics, nitrates, diuretics, peripheral vasodilators and vitamins, in the descending order of their market share. Thus majority of CRDDS available today are for the treatment of chronic diseases or conditions, such as rheumatoid arthritis, cardiovascular disorders, and asthma, which require a prolonged treatment.[11]

However, CRDDS for treating diseases, like leprosy, malaria, tuberculosis, which are more prevalent in this country, are not available as yet. Equally important is the need for CRDDS for contraception. The rapid increase in human population in India has been a source of concern to the managers and policy planners of the National Family Planning Programme. Therefore, there is an urgent need to develop an efficacious and reliable fertility regulating method, for both male and female partners. The studies conducted throughout the world, clearly indicate that the hormonal contraception for men is still years away, as the present approaches are not adequately safe, effective and reversible.[12] On the other hand, hormonal contraception for women (oral contraceptive pills [OCPs]) has proved to be a preferred and an effective method of contraception. However, the use of OCPs is associated with an increased risk of side-effects and poor patient compliance.[13-16] Other methods of contraception devised for women also have many limitations.[17]

CRDDS for use in human contraception offer several advantages such as improved therapeutic ratio by decreasing the total drug dose, localised delivery of drug to the target tissue, improved patient compliance and reduced incidence of side effects as compared to conventional dosage forms.[18] The present study is therefore directed to the design and development of a long acting drug delivery system for a female antifertility agent.

1.2 LONG ACTING DRUG DELIVERY SYSTEMS FOR CONTRACEPTION – A REVIEW

The reported fertility regulating methods could be classified into five broad categories; (i) Natural methods, which do not involve the use of any device or drug, e.g. coitus interruptus, rhythm method; (ii) Physical barrier methods, which involve the use of barriers such as condoms and diaphragms; (iii) Use of spermicidal formulations, which contain a spermicidal agent such as nonoxynol; (iv) Surgical methods such as tubectomy and vasectomy and (v) Drug Delivery Systems (including devices), which deliver contraceptive agent(s) e.g. estrogens, progestogens, either systemically or locally to the target tissues. The first three methods are reversible but have high failure rates. Though the surgical methods are reported to have low failure rates, these are not popular because of their irreversibility. The use of drug delivery system (DDS) would be a preferred method for family planning.[17]

Although the explosion of population has been a problem of developing countries, major contribution in the area of developing contraceptive drug delivery systems, based on modern medicine, comes from the developed countries. This is evident by the fact that the first OCP and intrauterine devices were developed by the western countries.[19,20] Further, technologically advanced and more sophisticated drug delivery systems for contraceptive agents (e.g. Progestasert^{R}, Norplant^{R} and LNg-IUD^{R}) have been developed and commercially made available in the western world. Many other delivery systems are also in the pipeline in advanced countries. It is interesting to note that, many of these research projects are sponsored by the World Health Organization and the work is carried out in government laboratories or universities. Very few industrial houses, like Organon (The Netherlands), are actively involved in this field.[21,22]

Amongst developing countries India, China and few Latin American countries are actively engaged in this field for the last one and a half decade but, they have not been able to commercialise any product till date. In India, research in the development of contraceptive drug delivery systems is carried out by the government organisations and laboratories. These include Indian Council of Medical Research (ICMR), IIT's, Department of Biotechnology (DBT), National Institute of Immunology (NII), Institute for Research in Reproduction (IRR), National Chemical Laboratory (NCL) and Bhabha Atomic Research Centre (BARC).

The current long acting contraceptive drug delivery systems and devices are briefly reviewed. These can be classified into; (i) devices which releases drug into target tissue e.g. medicated intrauterine devices, intravaginal devices, intracervical devices, transdermal drug delivery systems and (ii) devices which releases drug into systemic circulation, e.g.long acting injectables, biodegradable and non-biodegradable subcutaneous implants.

First medicated intrauterine device Progestasert^{R} was marketed by ALZA Corp., USA, in 1976. Progestasert^{R} contains progesterone and is effective for a period of one year.[23-25] Recently, another medicated intrauterine device, Lng-IUD^{R}, is marketed by Leiras, Finland. This device is effective for a period of 3 to 5 years.[26-28] Many intravaginal and intracervical devices releasing either a progestogen or combination of progestogen and estrogen have been reported in the literature.[29-35] However, in very few cases the clinical studies are completed and none of these are available commercially. The major problems with these devices are high expulsion rate, high discontinuation rate due to increased discomfort & pain and high failure rates as compared to injectable or implantable systems.[36-41] Transdermal delivery of contraceptive agents has also been studied, but no such system is available commercially as yet. Major problems in the development of transdermal system are; (i) poor permeability of contraceptive drugs through skin; (ii) metabolism of drugs in the skin and (iii) chances of skin rashes after prolonged use of such systems.[42,43]

The devices releasing contraceptive drug into systemic circulation are more effective than those releasing into target tissues.[44] The first report on the use of subdermal implant of a steroid to achieve contraception came in 1969.[45] Since then, many implants of different size, composition and containing a variety of progestins have been tested at various subcutaneous sites in women and the contraceptive effectiveness of the implants have been documented. They are either hollow cylinders filled with drug suspended in a suitable medium and sealed at both ends or solid rods, in which drug is blended with a polymer and co-extruded to form a rod. Solid rods may or may not be covered with a rate limiting membrane. Release of steroids from these devices, is primarily diffusion controlled and release rate is dependent on surface area of the implant and wall thickness. Polymers used for the fabrication of implantable systems can be non-biodegradable e.g., silicone elastomers and ethylene-vinyl acetate or biodegradable eg., poly-ϵ-caprolactone, poly (lactide) and poly (glycolide).[46-53]

One such system, Norplant[R], developed by the Population Council, USA, is commercially available. Norplant[R] is designed to release levonorgestrel, a progestogen, over a period of 3 to 5 years. Another implantable system, Implanon[(R)] containing 3-Ketodesogestrel is under development at Organon, The Netherlands. A biodegradable implant, Capronor[(R)], developed by Research Triangle Institute, USA containing levonorgestrel has successfully completed phase-II clinical studies. All these implantable systems require a minor surgical procedure for insertion and removal, whereas the injectable systems can be administered without any surgical procedure.[21]

The very first approach for developing a long acting contraceptive was in the form of an injection.[44] The first injectable system contained micro crystals of a progestogen such as medroxyprogesterone acetate (Depoprovera[R]) and norethisterone enanthate (Noristearate[R]) suspended in a suitable vehicle. Other such systems containing fatty acid esters of levonorgestrel are under investigation.[54-56]

When administered intramuscularly, these systems form a drug depot from which the drug is released over a period of time into the muscle and is eventually absorbed into systemic circulation, from where it is transported to the site of action. These preparations are effective for a period of three months. However, they produce very high initial systemic drug concentrations which is the cause of side-effects. To overcome this limitation, injectable microspheres and microcapsules are being developed.

Microspheres are extremely small solid particles containing drug homogeneously dispersed in a polymer matrix, where as, microcapsules are polymeric spherical particles containing drug concentrated in the central core. A variety of synthetic and natural polymers are being used for the preparation of microcapsules and microspheres, such as poly-ϵ-caprolactone, poly (lactide), poly glycolide and copolymers thereof. These polymers degrade in the muscle and release the hormone over a period of time. One such system containing progesterone is under development in Japan.[57] Injectable formulations are effective for a period ranging from 3 months to 6 months and treatment cannot be reversed or terminated abruptly.

Majority of injectable and implantable contraceptive systems contain only a progestogen e.g progesterone or levonorgestrel. However, recently attempts have been made to incorporate biotechnology based molecules in these systems. Such systems are called as antifertility vaccines or contraceptive vaccines.[58] Various molecules that have been identified for the development of contraceptive vaccines include human chorionic gonadotrophin (hCG), gonadotrophin releasing hormone (GnRH) and follicle stimulating hormone (FSH). Antifertility vaccines developed using FSH and GnRH are still at the experimental stage, whereas vaccines containing hCG are being evaluated in clinical trials.[59-62]

At the present time, only injectable, implantable and intrauterine devices are commercially available. The literature review also indicates that currently investigations are mostly directed on development of long acting injectable preparations or subcutaneous implants containing steroid or hormones.

1.3 SELECTION OF AN ANTIFERTILITY AGENT

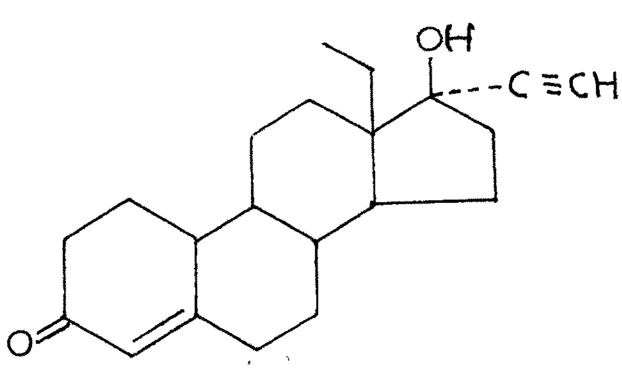
Hormonal contraception for women involves two classes of sex hormones, the estrogens and the progestins.[14] The progestogens are most commonly used in the long acting contraceptive devices because the use of estrogens is associated with an increased incidence of side-effects.[16,63] Further progesterone, which is a naturally occurring progestogen, is not preferred due to its low potency as compared to synthetic progestogens like levonorgestrel (LNG). Progestogen component of a contraceptive dosage form is selected on the basis of its antiestrogenic (progestogenic) and androgenic activities. The older progestogens, norethindrone and norethisterone, have lower antiestrogenic and higher androgenic activity as compared to LNG.[64-66] The newer progestogens, desogestrel, norgestimate and gestodene have very weak or no androgenic effects. However, desogestrel and norgestimate are prodrugs, which get transformed to 3 - keto desogestrel and levonorgestrel respectively, after an oral administration. These transformations take place in the intestinal mucosa and liver. Thus, these drugs are not suitable for developing a long acting parenteral drug delivery system (PDDS). On the other hand, gestodene have low affinity for progesterone receptor.[67] Thus levonorgestrel, with balanced antiestrogenic and androgenic activities, was found suitable antifertility agent for the present study.

The successful development of a safe, stable and effective CRDDS involves careful selection of excipients, analytical methods, fabrication methods, critical parameters for evaluation of the dosage form, suitable dissolution method for *in-vitro* release study and suitable animal model for the *in-vivo* evaluation.

1.31 LEVONORGESTREL – A DRUG PROFILE

The DRUG PROFILE, which is a compilation of literature information about physicochemical, pharmacological and pharmacokinetic properties of the drug, not only provide important guidelines for selection of excipients & methods and preparation of an experimental design, but also give useful leads about the experimental data that need to be generated and possible problems that may be encountered during the dosage form development. The drug profile of LNG is presented in following few pages.

Levonorgestrel, is a 19-nor testosterone analogue, developed by Wyeth Lab. Inc., USA, in 1960's.[68] It is a highly potent progestogen.[69] The d-enantiomer have twice the potency of racemic form (dl-norgestrel), where as the l-enantiomer is reported to be inactive.[70] Chemically, LNG is (-) 13β - ethyl 17β - hydroxy - 18,19 - dinor - 17α pregn - 4 en - 20yn -3 one. Empirical formula is $C_{21}H_{28}O_2$ and structural formula is



Molecular weight of drug is 312.5; elemental composition is : C-80.73%, H-9.03%, O-11.24%. Melting point is in the range of 232-239° C. The drug occurs as white, crystalline powder and is odourless. It is practically insoluble in water (0.001 mg/ml), slightly soluble in 95% ethanol (13 mg/ml), acetone (15 mg/ml), ether (2 mg/ml) and benzene (7 mg/ml) and soluble in chloroform (111 mg/ml). Levonorgestrel is optically active and optical rotation of 2 % w/v solution in chloroform ranges between -30° to -35°. The wavelength of maximum absorption of ethanolic solution of drug is 240 nm.[71,72]

DL-norgestrel is stable, for 5 years, at room temperature. However, under severe hydrolytic and accelerated thermal and photolytic conditions it is reported to undergo decomposition.[73] British Pharmacopoeia and United States Pharmacopoeia recommend to store LNG in a light resistant container, below 15° C.

In laboratory animals, dl-norgestrel is reported to be 10 times and 80 times more progestogenic, than progesterone and norethisterone respectively, after subcutaneous administration. It is reported to be 30 times more potent than progesterone and norethisterone, after intravenous administration.[64-66] It has 10 times the antiestrogenic activity and 18 times the progestational : androgenic ratio of norethisterone. LNG is reported to block ovulation by suppressing the hypothalamic-pituitary gonadal mechanism. Subcutaneous administration of LNG, at low doses, has been reported to selectively decrease the secretion of luteinizing hormone (LH) in rats. However, at high doses, it is reported to produce a significant reduction in serum FSH and LH. Minimum effective dose, to prevent ovulation in rat, is reported to be 20 μg/100 gm body weight.[74,75]

In women, LNG is reported to cause a variable suppression of FSH, LH and hence ovulation. Therefore, the OCPs containing only LNG has higher failure rates than the combined contraceptive pills.[76-79] The antifertility effect of LNG at low doses is attributed to the alterations in the structure of endometrium and cervical mucus.[80] LNG, when administered by other routes such as subcutaneous implant, intrauterine device, intravaginal device or intracervical device, exerts its antifertility effects by the same mechanism.[13,81] However, subcutaneous implants of LNG is reported to block release of LH.[82]

The minimum effective dose of LNG to prevent ovulation in women is 60 μg/day. The daily dose of LNG ranges between 50-250 μg for combined OCPs therefore, these preparations effectively suppress ovulation. Whereas, the only "progestogen" pills, which contain 75 μg of dl-norgestrel (equivalent to 37.5 μg of LNG) do not suppress ovulation.[81]

Pharmacokinetics of LNG in animal models have been studied by several workers.[83-89] Peak plasma concentration (conc.) of LNG, in women, have been reported to range between 2.2 to 3.7 ng/ml, after an intravenous dose of 30 μg. The time course of LNG conc. in plasma is reported to follow three compartment open model, with an average half lives of 10 min., 60-90 min. and 18-20 hrs. for the α, β and τ phases.[90]

LNG has been reported to bind with sex hormone binding globulin (SHBG). Use of ethinyl estradiol (EE) is reported to increase the plasma concentration of SHBG in women. Therefore, simultaneous administration of EE and LNG is reported to result in large intra and inter individual variations and cause gradual increase in plasma concentration of LNG.[91]

LNG is completely metabolized by liver and metabolites are excreted in urine and faeces. Metabolic clearance rate is reported to range between 50-180 ml/min. About 70% of the administered dose is excreted in urine and 15 % in faeces after 8 days as sulphate and glucoronide conjugates, after hydroxylation and reduction of LNG.[90,92] Ruelius et al, observed selective biotransformation of norgestrel and its enantiomers in laboratory animals.[93,94] Metabolism and excretion of norgestrel, in women, is documented to be stereoselective. Plasma concentrations of LNG (d-enantiomer) are lower than those of l-enantiomer and racemic form, after administration of a tablet containing 1 mg of drug. The half lives of ß and τ phase are significantly lower for d-norgestrel (2 hrs and 7 hrs) than those of dl- (3.2 hrs and 10.3 hrs) and l- (5.5 hrs and 10.4 hrs). The average percentage of dose recovered in urine and faeces was significantly different for dl-norgestrel and its enantiomers.[95-97] About 15% of the plasma concentration of the drug is reported to be excreted in the milk of nurturing mothers.[98]

LNG is completely absorbed after oral administration and peak plasma concentration is reached within 30-60 min. The fractional bioavailability of LNG after oral administration have been reported as 0.87 ± 0.9, 1.00 ± 0.16 and 1.04 ± 0.22 in three different studies. The bioavailability of LNG from capsules is reported to be same as that from tablets.[90,99-101] The peak plasma concentration is reported to range between 0.8-2.8 ng/ml, 2.9-4.1 ng/ml, 3.3-5.1 ng/ml and 14.0-23.0 ng/ml after an oral dose of 30, 150, 250 and 1000 µg respectively. The average half lives of 1.6 ± 0.3 hrs. and 1.1 ± 0.2 day are reported for β and τ phases respectively. The mean volume of distribution of LNG is 1.5 ± 0.19 L/Kg.[90,102]

LNG is also used in long acting contraceptive devices such as Norplant^R and LNg-IUD^R. *In vivo* release of LNG, from Norplant^R is 85 µg/day at the beginning which declines to 50 µg/day and 35 µg/day by the 9th and 18th month respectively. Thereafter daily 30-35 µg of drug is released for the next 3 years. Plasma concentrations of 1600 ± 1100 pg/ml are reported following administration of a subdermal implant (Norplant^R) of LNG. It has been reported that the plasma concentrations decline to around 400 pg/ml, 327 pg/ml and 258 pg/ml at the 3rd, 12th and 60th month respectively. The rate of decline in the plasma concentration is 1.4 pg/ml/month.[25]

On the other hand, initial release of LNG from LNg-IUD^R is 24 µg/day, during the first month, decreasing to an average of 16 µg/day during the following period of 23 months.[103]

Intravaginal and intracervical devices, releasing 20 µg of LNG per day have been reported to be effective in clinical trials.[104,105] It has been reported that, after intracervical administration of LNG the maximum plasma concentrations reaches within 24 hrs. The mean plasma concentrations after administration of a 20 µg/day releasing intracervical device are reported to be 128 ± 47, 107 ± 35 and 106 ± 40 pg/ml for the first, second and third month respectively, which decline to 81 ± 22 pg/ml in the 48th month of use.[105]

The mean plasma concentration of LNG after administration of a vaginal ring releasing 20 µg/day is reported to range between 0.5 to 1.4 nmol/L. The plasma concentrations are reported to decline to 72% and 52% of the initial value after 6 months and 12 months use. The LNG level has been reported to decrease daily by 1.1 pmol/L (0.13%) on the average.[106] The fractional bioavailability of LNG after vaginal administration is 0.88 ± 0.16. It has been reported that T_{max} is significantly longer and C_{max} is significantly reduced after vaginal administration.[99]

In conventional laboratory studies of acute and sub-acute toxicity, there is no indication of organic damages or haematopoietic disturbance.[79] The incidence of side-effects of norgestrel is the lowest (1%) among all the progestogenic and estrogenic compounds studied. No abnormal findings have been reported in any of the patients treated with either LNG or combination of LNG with EE.[64,78]

Norgestrel, alone or in combination with therapeutic levels of an estrogen, has been reported to show absence of tumorogenicity in mice and rats, in a long term study. Norgestrel is reported to be non-mutagenic in dogs and monkeys.[107] The use of LNG in combination with estrogens, during early pregnancy, is reported to cause tracheo-oesophageal fistula and hepatoblastoma in infants.[108,109]

The use of subdermal implants of LNG, in singaporean women, have been reported to increase tendency of thrombosis and potential for hypercoagulation, during first 12 months of use.[110] However, it has been reported that some of the haematological changes returned to normal values in the 2nd year of the study.[111]

The continuation rate of Norplant at 1st , 2nd, 3rd, 4th, and 5th year is reported to be 96, 92, 88, 82 and 78% respectively. Bleeding problems have been reported as the most common cause for removal.[112] However, Norplant is reported to provide safe, effective and reversible method of fertility regulation.[113]

Several analytical methods for LNG have been reported. The simplest method is UV spectrophotometric method. This method involves recording the absorbance of ethanolic solution of drug at about 241 nm.[72] Colorimetric methods based on isoniazid reagent[114-116] and tetrazolium blue[117,118] are reported for the assay of drug, in tablets, in presence of an estrogen and excipients. Fluorometric methods have been reported for the determination of norgestrel in tablets and biological fluids.[119,120] Titrimetric method based on the reaction of ethinyl group with silver nitrate in tetrahydrofuran have been reported in British Pharmacopoeia. The end point is determined potentiometrically.[71]

Thin layer chromatographic method for identification of drug and related substances is official in British Pharmacopoeia and United States Pharmacopoeia. This method makes use of a solvent system comprising of 96 volumes of chloroform and 4 volumes of alcohol and the spots are visualized with phosphomolybdic acid reagent.[71,72] HPLC and GC methods for quantitative determination of LNG from different dosage forms, have been reported.[73,121,122]

Several radioimmunoassay methods have been reported for determination of LNG, in biological samples.[123,124]

Further, LNG is commercially available, in various dosage forms such as combined OCPs, progestogen only mini pills, subdermal implant and intrauterine device. Other contraceptive dosage forms such as intravaginal and intracervical device containing LNG are under development.

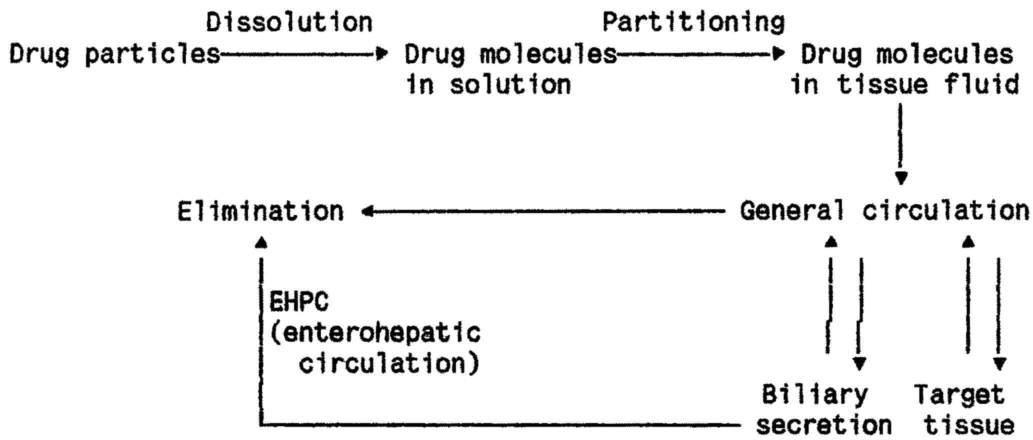
1.4 DESIGN CONSIDERATIONS FOR A LONG ACTING PDDS

1.41 General Considerations :

The long acting PDDS are depot type systems administered via a suitable parenteral route. Although the intravenous, subcutaneous, intramuscular, intraperitoneal, and intrathecal routes are the parenteral routes of drug administration, not all of these are useful as routes for controlled drug delivery. The subcutaneous and intramuscular routes are the major routes of administration for the majority of long acting PDDS. The intravenous route is used occasionally for liposomes, nanoparticles, erythrocytes, mainly in drug targeting. The intraperitoneal route is sometimes used where macromolecules are used as carriers to target antineoplastic agents into the lymphatic system.[9] Therefore, we shall restrict our discussion to the systems administered via subcutaneous and intramuscular routes.

1.42 Biopharmaceutical Considerations :

When a sustained release drug formulation is administered subcutaneously or intramuscularly, it forms a depot at the site of injection. This depot acts as a drug reservoir and releases the drug molecules continuously at a rate which is largely determined by the physicochemical characteristics of the drug, the design of formulation and physiological factors. A possible sequence of events is as follows



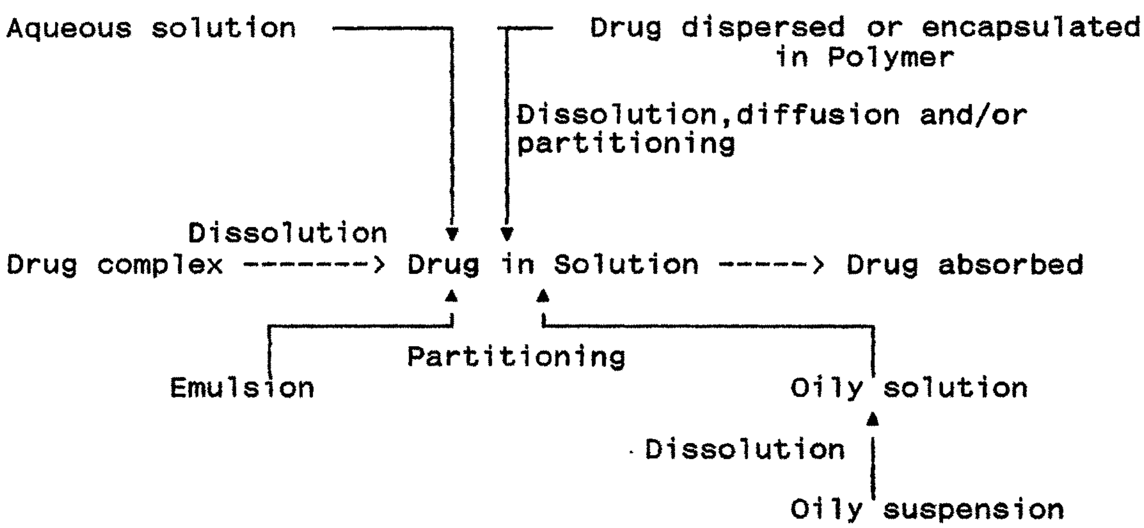
The rate limiting step in most cases is dissolution of drug particles from the formulation and/or partitioning of drug molecules from the vehicle into the surrounding tissue fluids. Some physicochemical characteristics which affect dissolution and partitioning are particle size, crystal form, polymorphism and partition coefficient of the drug. Unionized form of drug partitions into the tissue rapidly and hence is preferred for enhanced absorption. Volume of injection is also an important factor both from the standpoint of tissue injury and absorption rate. It is recommended that volume injected subcutaneously should not exceed 1.5 ml and by intramuscular route should not exceed 5 ml.

Physiological factors which affect drug release and absorption include site of administration, body movement, tissue injury, increased muscular activity and increased blood flow to muscles.

The onset and magnitude of drug response is affected by blood flow through the sites of injection. Coadministration of agents that affect blood flow through the injection site influence absorption rate.[9]

1.43 Formulation Approaches :

At the present time, there are a number of injectable depot formulations in the market e.g., Penicillin G procaine suspension, medroxy progesterone acetate suspension, Insulin-Zinc suspension etc. Majority of these are either aqueous/oily solutions or suspensions. The prolonged action in these preparations is achieved by retarding drug diffusion/dissolution by various approaches such as increasing viscosity, use of less soluble drug derivative or salt, use of a release retarding polymer, selecting an oily vehicle so that partitioning of drug into surrounding tissue is modified.[125-130] Following scheme illustrates the rate limiting step in drug release with several of these approaches.



A recent approach in developing a long acting PDDS is the use of a suspension of microspheres or microcapsules. Decapeptyl CR[R] containing triptorelin, Enantone LP[R] containing leuprorelin and Parlodel LAR[R] containing bromocriptin are the examples of commercially available long acting PDDS based on this approach.[57] Some of the other approaches include the use of emulsions, liposomes, macromolecules, erythrocytes and implants to provide prolonged drug release by the parenteral route.[131-142] The use of release retarding polymers is essential in microsphere and implantable systems and therefore the selection of polymer is equally important.

1.44 Selection of Polymers :

The pioneering studies in the field of PDDS are reported with the use of biostable commercial polymers such as polyethylene and silicone rubber.[143-145] The rate of release of the drug from the polymeric matrix or reservoir device was determined solely by diffusion; biodegradation of the polymer was considered as a poorly defined and unnecessary experimental variable. Subsequently, interest in biodegradable polymers developed for two reasons. Firstly, as the field expanded from research to application, it was recognized that surgical removal of a drug depleted delivery system was difficult, yet leaving non-biodegradable foreign materials in the body for an indefinite time period constituted an undesirable toxicological hazard.[146] Secondly, while diffusion controlled release is an excellent means of achieving predefined rates of drug delivery, it is limited by the polymer permeability and the characteristics of the drug.[147-149] Drugs which have either a high molecular weight (>500) or poor solubility in the polymers are not amenable to diffusion controlled release.

As a result, attention turned to biodegradable polymers and three basic approaches to drug delivery have evolved i) erosion of the polymer surface with concomitant release of physically entrapped drug, ii) Cleavage of covalent bonds between the polymer and drug, occurring in the polymer bulk or at the surface followed by diffusional drug loss, and iii) Diffusion controlled release of the physically entrapped drug, with bioabsorption of the polymer delayed, until after drug depletion. The latter approach avoids any irreproducibility of the bioerosion rate and the difficulty of trying to synchronize the diffusion and bioerosion process to achieve a specified delivery rate.[150-156]

Biodegradable polymers investigated for PDDS can be divided into natural and synthetic polymers. Natural biodegradable polymers includes i) Proteins and polypeptides (e.g. albumin, fibrinogen, gelatin, collagen, and casein) ii) Polysaccharides (e.g. starch, dextran, dextrin, hyaluronic acid, alginic acid, chitin and chitosan) and iii) Viral envelopes and living cells (e.g. erythrocytes, fibroblasts and myoblasts)[157]. Natural polymers usually vary in purity because they come from natural sources and often require some crosslinking methods (chemical agents), which can lead to denaturalization of the polymer and the embedded drug. These changes in the compound result in variation in the quality of the DDS and immunogenic adverse reactions. Therefore, synthetic polymers are preferred for the development of commercial products.

Synthetic biodegradable polymers have certain properties which makes them more useful in controlled release applications.[158] These properties include i) ability to be formed into desired shapes; ii) release of contained drugs by a suitable mechanism; iii) adequate dimensional stability together with strength loss characteristics; iv) little toxic response to either polymer, drug system or degradation products; v) complete biodegradation of the device and disappearance of material and vi) sterilizability. Synthetic biodegradable polymers investigated for drug delivery systems are polyorthoesters,[159-161] polyanhydrides,[162-164] polyamides[165] and poly amino acids,[166] poly alkylcyano acrylates,[167,168] polyphosphazenes,[169] and polyesters.[170]

The polyesters are by far the most widely studied synthetic polymers for PDDS. They are easily prepared as homo and co-polymers in a wide range of molecular weights. They undergo simple hydrolysis forming naturally occurring nontoxic metabolites. In addition, their relative strength and the long standing safe use and acceptance of these polymers in surgical procedures has encouraged the application of the polyesters as drug carriers. The degradation pathway is by homogeneous, bulk degradation. The stages involve a) hydration which disrupts hydrogen bonds and other hydrophobic forces; b) initial cleavage of the covalent bonds randomly through out the polymer; c) mass loss from continued cleavage and solubilization of the low molecular weight fractions and d) complete absorption.[170]

Various polyesters that have been studied extensively include poly(lactide), poly(glycolide), poly-ϵ-caprolactone, and their copolymers.

Polylactide/glycolide :

One of the earliest application of these polymers was for synthetic resorbable sutures.[171,172] During seventies, a large number of publications on these polymers became available from American Cyanamid, Johnson & Johnson and the U.S.Army institute for Dental Research. A drug delivery system fabricated using this polymer, has originally been described by Dupont.[173] Since then, these polymers have been used in the preparation of controlled release drug delivery systems.[174-177]

The homo and copolymers of lactic acid and glycolic acid have been synthesized by two methods a) the direct polycondensation of the monomers i.e. lactic acid or glycolic acid or b) the ring opening melt condensation of the cyclic diesters i.e. lactide or glycolide, in presence of suitable catalysts. The polymers prepared by the polycondensation methods have low molecular weight than those prepared by the ring opening melt condensation method. Stereo regularity of the lactic acid is preserved during polymerization process, therefore optically pure polymers i.e. poly (D-lactide) & poly (L-lactide) or racemic polymers i.e. poly(DL-lactide) can be synthesized. The properties of these polymers are quite different. Primarily poly(L-lactide) and poly(DL-lactide) are used for controlled release delivery system, but, poly (DL-lactide) is preferred because it is amorphous polymer and forms better coatings than the crystalline poly(L-lactide). The other polymer, poly glycolide, is high melting (M.P 230°C), crystalline and more hydrophillic than poly lactides.[178-180]

Solubility of these polymers in common organic solvents is an important factor in regard to fabrication of drug delivery system. The homopolymers DL, D and L lactide have good solubility in halogenated hydrocarbons, ethyl acetate, tetrahydrofuran, dioxane and a few other solvents. At glycolide content less than 50% lactide-glycolide copolymers have solubility characteristics similar to those of lactide homopolymers. Polyglycolic acid and the glycolide rich copolymers have solubility only in solvents such as hexafluoro isopropanol.[181]

A well proven advantage of lactide-glycolide polymers is their versatility in terms of polymer properties and performance characteristics. A wide range of degradation rate and duration of drug release (1 month to 18 months) have been demonstrated by these polymers depending upon monomer stereochemistry, co-monomer ratio, polymer chain linearity and molecular weight of the polymer.

Biodegradation of these polymers occurs by bulk erosion, the polymer chains are cleaved by hydrolysis to monomeric acids and are eliminated from the body through the Krebs cycle, primarily as carbon dioxide in urine. Crystallinity and water uptake are the key factors in determining the rates of *in vivo* degradation. Very little difference is observed in rate of degradation at different body sites.[182-184]

Development of various long acting contraceptive systems, such as microspheres, implants and fibres, based on these polymers have been reported.[185,186]

Poly-ϵ-caprolactone :

The successful use of polymers of lactic acid and glycolic acid in biodegradable drug delivery systems and biodegradable sutures led to an evaluation of other aliphatic polyesters. In 1977, Pitt C.G. et al demonstrated the *in-vivo* biodegradability of poly-ϵ-caprolactone, for the first time.[156] However, scientists working at Union Carbide had suggested, in 1973, that poly-ϵ-caprolactone may be used as a biodegradable packaging material.[187]

Poly-ϵ-caprolactone (PCL) is a semi crystalline polymer, melting in the range of 59-65°C depending on the crystallinity. The heat of fusion of 100% crystalline PCL is reported to be 139.5 J/gm.[186] PCL is soluble in number of solvents at room temperature, these include; tetrahydrofuran, chloroform, methylene chloride, carbon tetrachloride, benzene, toluene; it is poorly soluble in acetone, ethyl acetate, acetonitrile and dimethyl formamide and insoluble in alcohols, petroleum ether and diethyl ether.

The studies have established that the permeability of PCL and its copolymers, to low molecular weight drugs (< 400D) are comparable to that of silicone rubber. The permeabilities of copolymers of ϵ-caprolactone and dl-lactic acid have been reported to be relatively same for compositions up to 50% lactic acid.[189] The permeability of polymer could be altered by changing crystallinity and molecular weight of the polymer. The homopolymer, itself degrades very slowly when compared with poly lactic acid, poly glycolic acid and their copolymers.

The high permeability and longer biodegradation time of the PCL makes it an ideal polymer for the development of diffusion-controlled drug delivery devices. The degradation rate of the polymer has been reported to be same; even after ten fold increase in the surface to volume ratio of the polymer, indicating bulk hydrolysis as a major mechanism of degradation.[190,191] *In-vivo* degradation rate is reported to be comparable to *in-vitro* degradation rate at

40°C in water.[192] The degradation rate and hence the rate of drug release is reported to be dependant on crystallinity, molecular weight, polymer composition, end group capping and addition of acidic or basic component.[192-196]

Poly-ϵ-caprolactone has been reported to get completely metabolized to ϵ-hydroxy caproic acid and water, in the body, PCL and ϵ-caprolactone tested negatively in the Ames mutagenicity assay; the same result has been reported after their exposure to mammalian liver homogenates under conditions designed to effect metabolism.[197] Poly-ϵ-caprolactone based implants have been reported to show minimal tissue reaction, when tested for their effects on cell ultra structure, surface characters and population doubling times, in organ culture.[198]

The use of poly-ϵ-caprolactone and its copolymers have been reported in the preparation of drug containing microspheres, implants and fibres. Levonorgestrel containing implant (Capronor(R)), has successfully completed phase-II clinical trials.[199]

High permeability, lack of toxicity, compatibility with other polymers and long biodegradation time has made PCL and its copolymers an ideal choice for development of diffusion controlled, biodegradable drug delivery system.[181]

1.45 Sterilization of Long Acting PDDS :

It is essential that all parenteral controlled delivery systems are sterile. The effects of the sterilization method on the drug and polymers used must be considered in detail to ensure that there are no changes in physicochemical properties, stability and safety of the delivery system. Some of the changes are time dependent and become apparent only on storage and aging. Hence, suitable stability study guidelines should be developed in the study of the effects of various sterilization methods.[200,201]

Of the various sterilization methods, thermal methods (dry heat and moist heat) are unsuitable for most plastics and polymers. Hence these methods cannot be used for polymers utilized in drug delivery systems. However, it can be used for oily solutions and suspensions if the candidate drugs are thermostable. Two cold sterilization methods which may prove useful are ethylene oxide (EtO) sterilization and radiation sterilization. EtO sterilization involves exposure to EtO gas at 55°C and 40% RH. This method is reliable but there is a risk of harmful residues like EtO, ethylene glycol (EG) and ethylene chlorohydrine (ECH) in the treated material. When this method is used it is essential to assess the levels of these toxic residues.[202]

Radiation sterilization involves exposure to ionizing radiation obtained from high energy electrons or gamma rays from cobalt-60 or Caesium-137. The accepted sterilization dose is 25 KGy or 2.5 Mrad. However, before adopting this technique, detailed investigations are necessary to ensure that the treated material does not show adverse changes.[203]

Sterilization of Polyesters :

Aseptic processing and radiation sterilization are the two major routes of preparing sterile dosage forms using these polymers. Because of the good solubility of these polymers in organic solvents, it is feasible to filter sterilize drug-polymer solutions in a clean room environment under GMP conditions. Aseptic processing is particularly useful with microencapsulated products, which almost always involve solutions of the polymer in organic solvents.

Gamma radiation has proven useful for many lactide/ glycolide formulations. Various studies have been made on the effects of radiation on lactide/glycolide polymers. In most cases doses of 1.5 to 3.0 Mrad range can be utilized without serious degradation of the polymers.[178,204,205]

1.46 *In-vitro* Evaluation of Long Acting PDDS :

Assessment of *in-vitro* release profiles of prolonged action parenteral delivery systems is a formidable task. Variables like analytical sensitivity of drug concentrations, maintenance of sink conditions, hydrodynamic characteristics of solution diffusion, reproducibility of sampling and compartment volumes and system temperature must be precisely controlled. Continuous flow systems, constant rotation apparatus, static method and USP paddle apparatus have been reported for determining release profile.[206-208]

1.47 *In-vivo* Evaluation of Long Acting PDDS :

Since, these drug delivery systems remain or reside at a body location for extended periods of time ranging from few hours to months or years, the biocompatibility of the polymers and the DDS is extremely important. Biocompatibility of a polymeric material with tissue is described in terms of acute and chronic inflammatory responses and the subsequent formation of fibrous capsule around it and it can be measured in terms of sensitivity reactions and infections.[209,210] Pain on injection is a subjective phenomenon and a little difficult to assess. The form in which a polymeric device is administered and its duration of residence in the body play an important role in deciding the degree of tissue injury.

Injury in the muscular tissue are of different types such as focal necrosis, adoptive lymphatic response and granulomatous response.[211] Subcutaneous injury has been characterized into four types. In these processes the injured connective tissue is replaced by a more resistant layer of granulation tissue, which chiefly consists of proliferating fibroblasts. Subcutaneous sarcomas have been reported on administration of CMC, PVP and Tween-60.[212,213] In case of i.v.route, tissue irritation is rare. When it occurs, it is characterized by pain, cellular inflammation and granulation tissue. Thrombus formation may occur in some cases, which will prove hazardous.[214]

The best method to evaluate biocompatibility of polymeric material and drug delivery system is to carry out actual implantation trial in animals like rabbits, dogs, rats, mice or pigs and assess the degree of tissue injury periodically by histopathological studies. USP specifies various Biological reactivity Tests for plastics and polymers.[215] *In-vitro* tests are based on agar diffusion methods and make use of tissue cultures. *In-vivo* studies include :

* Systemic injection test - in mice
* Intracutaneous test - in rabbits
* Implantation test - in rabbits

Another important aspect in *in-vivo* evaluation of the these systems is the efficacy of the formulation. The efficacy of the formulations needs to be evaluated in a suitable animal model.

Various attempts to formulate particulate delivery systems and implants of LNG, based on biodegradable polymers, have been reported. However, such systems are not commercially available as yet. Hence, formulations were designed as biodegradable microspheres and implants of LNG for subcutaneous administration. The experiments conducted are briefly discussed in the experimental design.

1.5 EXPERIMENTAL DESIGN

Levonorgestrel was chosen as the drug candidate for the study since its therapeutic use, pharmacokinetic and physicochemical properties prompt its administration as controlled release dosage form.

A methodical approach was designed in consonance with the characteristics of the drug and an integrated experimental plan was adopted. The study was phased into approaches dealing with the development of an injectable dosage form (biodegradable miccrospheres) and feasibility study for the development of an implantable dosage form of the drug. Experiments were designed to cover areas of characterization of drug and polymers, compatibility of drug with polymers, feasibility of sterilizing drug, polymers and dosage forms by gamma radiation, development of injectable and implantable dosage forms with suitable release characteristics, study of influence of formulation variables on drug release pattern, assessment of stability of the dosage forms and evaluation of *in-vivo* performance, of the systems developed, in terms of biocompatibility and pharmacodynamic activity in animals.

The samples of LNG were standardised to ensure its compliance with pharmacopoeial specifications. The polymers, PCL and PLGA were characterized with respect to their molecular weight, glass transition temperature (Tg), and moisture content. Infrared spectrums of the polymers were also recorded. Compatibility of LNG with the polymers was evaluated. Feasibility of radiation sterilization of drug and polymer samples was evaluated.

Microspheres and implants of LNG were prepared using different combinations of the two polymers - PCL and PLGA. Microspheres were prepared by emulsion solvent evaporation technique using methylene chloride as the solvent. Process variables, for the preparations of microspheres, were optimized. Implants were prepared by compressing the microspheres in a IR press. Varying drug : polymer ratios were tried for both, microspheres and implants.

The microspheres were characterized to assess various physicochemical properties like particle size distribution, moisture content,drug content and surface morphology. The implants were evaluated for thickness, friability and drug content.

Ethanolic phosphate buffered saline pH 7.4 [EPBS] was used to evaluate *in-vitro* release profile of both formulations. *In-vitro* release studies of microspheres were carried out in 10% v/v, and 50% v/v EPBS, at 37°C, for 60 days and 7 days respectively. Where as the *in-vitro* release studies of implants were carried out only in 50% v/v EPBS, at 37°C for 15 days.

The influence of three suspending agents, namely HPMC, MC and Sod. CMC, on the suspendibility of the microspheres was investigated in normal saline. The suspensions were characterized for suspendibility, sedimentation, ease of redispersion and syringeability.

Freedom from microbial contaminants (sterility) is an important criterion for all parenterals. Therefore, the next experiments were designed to assess the ability of the selected formulations to retain their physicochemical characteristics and *in vitro* drug release pattern, when sterilized by gamma radiation at 2.5 Mrad dose. All the sterilized formulations were evaluated to detect any change in physicochemical properties. These samples were also subjected to a test for sterility as per IP.

The selected formulations were then subjected to stability testing at aggravated temperature and humidity conditions. The dosage forms were stored at 0-4°C, 25 ± 0.5°C, 37 ± 1°C, 45 ± 1°C and 75% RH at 37 ± 0.5°C and evaluated at suitable intervals for appearance, drug content, moisture content, suspendibility and *in-vitro* release profile.

A biocompatibility study was undertaken to determine safety of selected formulations in adult female wistar rats in comparison with a negative and a positive control.

The selected formulations were then assessed for pharmacodynamic activity in adult female wistar rats by observing the ability of the formulation to block estrous cycle for a prolonged period in comparison with suitable controls.