CHAPTER 2

LITERATURE REIVEIW

2.1 CONTRACEPTION

Throughout history, mankind has tried to limit family size Until the last century, this was largely achieved by behavioral modifications, including abstinence, infrequent coitus, the avoidance of intercourse during the fertile period of the cycle and coitus interruptus (the withdrawal method) Modern barrier methods emerged during the 1800s, while intrauterine devices (IUDs) and hormonal contraception are 20th century developments Political and legal developments over the past century are closely tied to advances in contraception Despite evidence of need and market demand, little contraceptive development has taken place in the US for several decades. Concern over product liability and dwindling research support has stifled development. In spite of reduced contraceptive research and development activity, several new contraceptives may reach the US market in the next several years These include new implants and injectables. a transdermal contraceptive patch, a new IUD, new cervical caps, and a vaginal contraceptive ring New male methods of fertility control are still many years away

2.1.1 INTRAUTERINE DEVICES

The intrauterine device is one of the most commonly used methods of fertility regulation, especially in developing country programs It is a safe and effective method for women who are in a monogamous sexual relationship and not at risk of sexually transmitted infections (PATH/Outlook, 1992; FHI, 2000, IPPF, 2003) WHO estimates that more than 150 million women use IUDs, with more than 74 million users in China alone A review of studies confirmed that (1) IUDs are not abortifacients. (2) newer comprehensive IUDs are highly effective and long-lasting; (3) IUDs can be safely used by lactating women; and (4) IUD use is not associated with an increased risk of pelvic inflammatory disease (PID), of ectopic pregnancy, or of subsequent infertility (Chi, 1993)

Intrauterine devices (IUDs) are small flexible devices made of metal and/or plastic that prevents pregnancy when inserted into a woman's uterus through her vagina. The most widely used IUDs are copper-bearing IUDs. Inert (unmedicated) and progestin-releasing IUDs (levonorgestrel or progesterone) are less widely available. Drawbacks of IUDs are their tendency to cause heavy menstrual bleeding and Pelvic Inflammatory Disease

New Generation of IUDs

The newest generations of copper IUDs combine high continuation rates with very low pregnancy rates (JHU/CCP, 1995). Since little can be done to increase the efficacy of these devices, recent research has focused on developing devices to address side effects, particularly bleeding and pain, which account for a significant number of removals. The levonorgestrel-releasing IUD, a device with high effectiveness and acceptability, reduces menstrual blood loss compared to pre-insertion levels (Luukkainen, 1995). The levonorgestrel-releasing IUD, Mirena®, has been available in Europe for 10 years and has been used by 2 million women. It was approved for sale in the United States in December 2000. Frameless IUDs, such as the Gynefix (Kishen, 1998, Van Os, 1998; Wildermeersh, 1999) have been specifically designed to reduce cramping and pain. This device consists of a surgical nylon thread that holds copper sleeves and is anchored to the uterine fundus during insertion. It recently became available in Europe, and is licensed for five-year use. Studies suggest that the Gynefix is as effective as the Copper T380A, and expulsion rates are less than 1 per 100 women years.

2.1.2 ORAL CONTRACEPTIVES

- Combined oral contraceptives
- Progestin-only oral contraceptives pills

2.1.21 Combined Oral Contraceptives

Modern combined oral contraceptives are made from very low doses of synthetic estrogen and progestin. These combined oral contraceptives (COCs)—often called combined pills, the Pill, and birth control pills—are very effective in preventing pregnancy when taken consistently and correctly (at the same time every day). Their use does not interfere with intercourse. COCs are safe for most women; only some cardiovascular conditions, severe chronic conditions, and heavy smoking in women age 35 and over preclude use of the method. Most conditions that restricted use of high-dose COCs do not apply to low-dose formulations. Some characteristics of COCs are highlighted in the table below.

Combined oral contraceptives (COCs) are among the most intensely investigated family planning products in history. A growing body of research confirms that, in addition to being safe for most women, COCs provide significant noncontraceptive

health benefits (Blackburn, 2000, IPPF, 1998) By reducing menstrual bleeding, COCs help prevent iron deficiency anemia Recent studies have confirmed that long-term COC use protects against ovarian cancer and endometrial cancer Results suggest that protection is long-lasting, and may persist for 15 years or more after stopping COC use Although many previous studies in developed countries indicated a greater risk of liver cancer in women who used combined OCs for a long period of time, new data from several studies suggest the effect of OCs on liver cancer is negligible (MILTS, 1997; Blackburn, 2000)

Although OCs have been proven safe for most women, some health issues remain unresolved—primarily the relationship between OC use and breast or cervical cancer OC use does not increase lifetime risk of developing breast cancer (Blackburn, 2000). For most women, especially those in developing countries, the benefits of effective pregnancy prevention outweigh the very slight, increased risk of breast cancer associated with COC use (FHI, 1996; IPPF, 1998, PATH/Outlook, 1997) After more than 50 studies, most experts believe that pills have little, if any, effect on the risk of developing breast cancer (Hatcher, 2002) Most studies in the past decade have found that long-term OC use is associated with a slight increase in the risk of cervical cancer. However, many researchers believe that this observed association may be part of a larger behavior pattern that increases risk of cervical cancer rather than a causal relationship (PATH/Outlook, 1997; IPPF, 1998; Blackburn, 2000)

Another concern has been the risk of certain diseases of the cardiovascular system Although COC use does carry the risk of cardiovascular disease, the risk is very small, except in older women (over age 35) who smoke or women with high blood pressure

Table 2.1 Various methods of Contraception

Method	Failure rate during First year use	Age limitations	Mode of action	STI risk	Drug interaction	Duration of use	Return to fertility
IUDs	0 4% to 2 5% for copper IUDs and 0 1% for the levonorgestrel-IUD	No restrictions on use for women age 20 and over	Inhibiting sperm migration in the upper female genital tract, inhibiting ovum transport, and stimulating endometrial changes	NP	None	Copper T device remains effective for up to 10 years; the Multi-load copper IUD remains effective for up to five years, the levonorgestrel-releasing IUD is effective for at least five years Can be used safely throughout their reproductive years	Immediately upon removal
COCs	0 1% to 8%	No restrictions on use from menarche to age 40	Inhibiting ovulation, thickening of the cervical mucus, changing endometrium, and reducing sperm transport	NP	Certain antiseizure medications and may reduce the contraceptive effect of COCs	Throughout reproductive years, no need for periodic discontinuation	Immediately or after slight delay (average 2–3 months)
POPs	0 5% to 10%	No restrictions on use for women age 16 and over	Thickening cervical mucus, inhibiting ovulation	NP	Certain antiseizure medications and may reduce the contraceptive effect of COCs	Throughout reproductive years, no need for periodic discontinuation	Immediately or after slight delay

Method	Failure rate during First year use	Age limitations	Mode of action	STI risk	Drug interaction	Duration of use	Return to fertility
Injectable	Progestin-only injectables 0 1% to 0 6% Combined injectables 0 2% to 0 4%	No restrictions on use for women age 16 and over	Thickening cervical mucus, inhibiting ovulation	NP	Certain antiseizure medications and may reduce the contraceptive effect of COCs	Throughout reproductive years, no need for periodic discontinuation	3 to 6 months delay for progestin-only injectables, within three months for combined injectables
Male Condoms	3% to 12%	No restrictions	By preventing sperm from reaching the female reproductive tract	P		Throughout reproductive years, no need for periodic discontinuation	Immediately upon removal
Female Sterilization	0 2% to 0 5%	No restrictions	By blocking the fallopian tubes	NP	Certain antiseizure medications and antibiotics may affect the effectiveness of anesthetics	Permanent	Never
Male Sterilization	0 1% to 0 15%	No restrictions	By blocking the vas deferens	NP	Certain antiseizure medications and antibiotics may affect the effectiveness of anesthetics	Permanent	Never

2.1.22 Progestin-only Oral Contraceptive Pills

Progestin-only oral contraceptive pills, often called progestin-only pills (POPs) and minipills, are estrogen-free oral contraceptives made from very low doses of synthetic progestin POPs are appropriate for older women, especially smokers who want to use an oral hormonal contraceptive method, women for whom estrogen-containing formulations are not recommended because of side effects and postpartum or breastfeeding women POPs are effective in preventing pregnancy when taken consistently and daily, at the same time Their effectiveness is slightly less than that of COCs, especially in younger women, but effectiveness is high in women over 35 years of age and when use compliance is good POPs are safe for most women, only a few conditions—pregnancy, unexplained vaginal bleeding, and breast cancer—preclude use of the method POPs protect against endometrial cancer, decrease pelvic pain during menstruation, and may be protective against pelvic inflammatory disease (Blackburn, 2000, IPPF 1998; Blumenthal and McIntosh, 1996).

2.1.3 INJECTABLES

Injectable contraceptives contain synthetic hormones that are administered by deep intramuscular injection Injectables are safe and effective method of reversible contraception for most women (IFFP, 1999). Two types of injectable contraceptives are available progestin-only injectable contraceptives and combined injectable contraceptives that contain both a progestin and an estrogen hormone. Available progestin-only injectables include DMPA (depot medroxyprogesterone acetate) and NET-EN (norethindrone enanthate) Available combined injectables are Cyclofem™ (also called Lunelle) and Mesigyna ®.

DMPA (known widely under the brand name Depo Provera) has been the most widely studied injectable contraceptive. According to a nine-year WHO study, DMPA did not increase women's overall risk of breast cancer, invasive cervical cancer, liver cancer, or ovarian cancer, and it decreased the risk of endometrial cancer. Women may face a slightly increased risk of breast cancer in the first five years after they start DMPA, perhaps due to accelerated growth of existing tumors (PATH/Outlook, 1992, Lande, 1995)

An interesting review of hormonal contraceptives and bone mass is presented in the IPPF Medical Bulletin ((Meirik, 2000). In this article, Dr Olav Meirik concludes that

the long-term effects of hormonal contraceptives on bone mass are dependent on age and the life cycle. For women in the middle years of their reproductive lives, bone-mass changes resulting from hormonal contraceptives are small and transient. In adolescents, however, DMPA in particular does seem to slow the accumulation of bone mass. It is not yet known whether this is a transient effect

New injectables

Two new, once-a-month, combined injectable contraceptives have been developed by the Special Programme of Research in Human Reproduction (HRP) of the World Health Organization Both Cyclofem® (also called CycloProvera or Lunelle) and Mesigyna® have been tested in large multicenter clinical trials, and have been proven effective with relatively low incidence of side effects Recent studies on the efficacy, causes of discontinuation, and side effects of these two injectable contraceptives in Egypt found they could be a positive addition to contraceptive choice (Hassan, 1999)

2.1.4 IMPLANTS

The first contraceptive implant system developed was the Norplant system, which consists of six thin, flexible capsules made of silicone contains 36 mg of the progestin levonorgestrel. Norplant-2 (also known as Jadelle) is a two-rod system that has been approved in various countries for 5 years (Sivin, 2002). Several other implant systems are currently being tested. Implants are a safe and effective method of reversible, long-term contraception for most women They do not interfere with intercourse and are effective within 24 hours after insertion (IPPF, 2000)

Data from a recent five-year, international, post-marketing surveillance study of nearly 8,000 Norplant users confirmed the safety and efficacy of this method (Meirik et al., 2001).

New Implants

Norplant®, introduced in the 1980s, was the first contraceptive implant that became available to family planning programs. Despite the changes in menstrual bleeding patterns common to all progestin-only methods, Norplant proved highly acceptable to many women Progestin implants for female contraception are now growing into a family of options. So far, four different progestins and two polymers have been used to design six different implants (IPPF, 2000):

Norplant (Population Council)	• A system of six Silastic capsules that release levonorgestrel • Duration of action 5 years • Registered in 60 countries • Used by nearly 6 million women
Jadelle (Population Council)	• A system of two Silastic rods that release levonorgestrel • Duration of action 5 years • Ongoing registration in Europe
Implanon (NV Organon)	• A single implant system that releases etonogestrel • Duration of action 3 years • Registered in Australia, Indonesia, and 11 European countries • Introduced in the Netherlands and the United Kingdom in September 1999
Uniplant	• A single-rod, one-year implant that delivers nomegestrol acetate • No commercialization plans at this time

2.1.5 BARRIER METHODS

2.1.51 Male barrier methods

Condoms can be very effective in preventing pregnancy when used correctly and consistently with every act of intercourse (perfect use), however, they are less effective with typical use A meta-analysis of the male condom in preventing HIV suggests that their effectiveness at preventing HIV is 87 percent (with a range from 60 to 95 percent depending on the incidence among nonusers) (Davis, 1999) Condoms do not affect breastfeeding or have hormonal side effects, no medical condition restricts a client's eligibility for use of the method except allergy to latex. Reviews of literature confirm that condoms can prevent both pregnancy and STIs, including HIV (Lisken, 1990; FHI, 1998; PATH/Outlook, 1994; Gardner, 1999).. Male condoms may be less effective in protecting against those STIs that are transmitted by skin-to-skin contact, since the infected areas may not be covered by the condom (WHO, 2001; NIAID/NIH/DHHS, 2001)

Condoms and nonoxynol-9

Results of several recent studies on the effects of the spermicide nonoxynol-9 have led to a rethinking of the policy and recommendations about spermicidally-coated condoms in family planning and HIV prevention programs (AGI, 2002) In a recent revision to the WHO List of Essential Medicines, WHO stated that nonoxynol-9 coated condoms are no longer recommended (unless there is no alternative condom available) because nonoxynol-9 does not provide additional protection against pregnancy or STIs, and could perhaps be harmful by causing epithelial disruption on frequent exposure, resulting in an increased risk of STI and HIV infection In particular, recent findings suggest that products containing N-9 should not be used during rectal intercourse, as they cause rectal epithelial cells to slough off and therefore increase susceptibility to infection with HIV (Phillips, 2000)

2.1.52 Female Barrier Methods

Research has continued to develop several new female barrier methods that are modified versions of diaphragms, cervical caps, and sponges These devices have been designed to be easier to insert and remove, and more difficult to dislodge during intercourse.

The Lea's Contraceptive™ is a modified diaphragm-like device in one size It is available as an over-the-counter product in Germany, and was approved by the United States Food and Drug Administration (U.S FDA) in March 2002 It should remain in place at least 8 hours after intercourse, but be worn no longer than 48 hours before removing to wash A study carried out by CONRAD indicated that the 12-month pregnancy rate of the Lea Contraceptive™ compared favorably with other barrier methods. Pregnancy rates associated with the Lea Contraceptive were 15 percent, compared to the 10 to 21 percent for the standard diaphragm with spermicide (FHI, 2000, CONRAD, 2000)

The FemCap™ is a modified cervical cap with a strap to aid in removal of the device. It is available in some European countries and was approved for use in the United States in March 2003. In a study by CONRAD and Family Health International, the FemCap™ used with spermicide was found to be somewhat less effective as a contraceptive than a conventional diaphragm with spermicide. CONRAD estimates a

12-month pregnancy rate (based on 6-month pregnancy rates) of about 23 percent for the FemCap™ (FHI, 2000, CONRAD, 2000)

Two contraceptive sponges currently are available, primarily in Canada and Europe The Pharmatex sponge—available in Europe—contains the spermicide benzalkonium chloride The Protectaid™ Sponge contains a combination of spermicides

2.1.6 STERILIZATION

- Female sterilization
- Male sterilization

2.1.61 Female sterilization

Female sterilization, also called tubal occlusion or ligation, is a permanent contraceptive method for women. The two most common female sterilization approaches are minilaparotomy, which is usually performed under local anesthesia with light sedation, and laparoscopy, which requires general anesthesia. Female sterilization does not affect breastfeeding or interfere with intercourse and it is free from the side effects associated with some temporary methods.

Quinacrine sterilization

Intrauterine application of quinacrine hydrocholoride is a method of nonsurgical female sterilization that has received considerable attention during the last decade and has generated significant controversy Although recorded failure rates and persistent side effects related to quinacrine sterilization have been low, controversy has developed around quinacrine's long-term safety, efficacy, and link to upper genital tract infections As a result, several countries and regulatory agencies, including the U.S. FDA, have taken steps to ban both the manufacture and use of quinacrine for sterilization (PATH/Outlook, 1999)

2.1.62 Male sterilization

Male sterilization, also called vasectomy, is a permanent contraceptive method for men. The method requires a simple surgical procedure and is performed under local anesthesia. Male sterilization is not castration; it does not affect the testes. The method does not interfere with intercourse or affect a man's sexual ability. Male sterilization is generally safer and less expensive than female sterilization and it is a good way for men to share in the responsibility of family planning.

2.1.7 MALE CONTRACEPTION: RECENT DEVELOPMENTS

Research to develop safe, effective, reversible and acceptable methods of fertility regulation for men has been supported by several International Agencies, many National Research Councils and some Pharmaceutical Companies Recently clinical and biomedical investigations have grown out of the basic physiological studies performed during the preceding decades Through increased public awareness, research on male methods and the greater involvement of men in reproductive health have received support from several quarters, including International Women's Organisations Together with strong support from the governments of some developing countries with major population growth e g the People's Republic of China, Indonesia, and India, these trends are encouraging. The clinical and scientific bases for the research have been well reviewed in recent years (Wu,1988, Bardin et al , 1991, Nieschlag et al ,1992, Springer et al , 1991, Waites. 1993).

2.1.71 Hormonal methods

The suppression of sperm production by hormonal means has been a general research strategy for all agencies interested in male contraception (Wu, 1988, Swerdloff et al, 1989, Nieschlag et al , 1992). There are three main aspects to this strategy. the suppression of the secretion of gonadotrophins, either of both LH and FSH or of FSH alone; the recovery of circulating androgen to physiological levels without re-stimulation of spermatogenesis, and the assessment of the functional capacity of residual sperm, should the treatment fail to achieve azoospermia in all cases. To date, suppression of spermatogenesis by hormonal means has been shown to be fully reversible in all clinical and non-human primate studies (Wu, 1988, Swerdloff et al, 1989, Nieschlag et al., 1992)

Testosterone enanthate

The first ever multicentre contraceptive efficacy study of normal men receiving a prototype hormonal regimen, which was conducted during 1986-1990, provided convincing evidence that, once the laboratory diagnosis of azoospermia had been achieved, normal men were rendered infertile and able to sustain safe, effective and reversible contraception for at least 12 months (WHO, 1990). There were variations in the rate of achievement of azoospermia among men of the same genetic background.

Long-acting androgen preparations

Studies on androgen suppression of spermatogenesis to date have been conducted with relatively short-acting preparations (Swerdloff et al., 1989; Wu, 1988, Nieschlag et al, 1992) More physiological means of androgen replacement with prolonged duration are now becoming available, not only for the treatment of male hypogonadism but also in the development of all types of hormonal methods for men. These are biodegradable testosterone microcapsules (Bhasin et al, 1992), testosterone pellets (Handelsman et al , and testosterone buciclate, a testosterone ester

Progestogen-androgen combinations

Studies in the 1970s established that progestogen-androgen combinations were safe and relatively effective in suppressing sperm production in Caucasian men but rarely achieved more than 50% incidence of azoospermia (Swerdloff et al , 1989). Recently, it was demonstrated that three injections at monthly intervals of depot-medroxyprogesterone acetate (DMPA, 200 mg or 100 mg) and testosterone enanthate (250 mg or 100 mg) caused suppression of spermatogenesis to azoospermia in 19 out of 20 Indonesian men (Pangkahila, 1991). One advantage of such a regimen would be that the dose of exogenous androgen required would be much less than in an androgen-alone approach Long-acting formulations of progestogens developed for female applications, are being evaluated for their potential for fertility suppression in men. All would require long-acting androgen supplementation

GnRH analogue-androgen combinations

Clinical studies (Pavlou et al., 1991; 1989) and studies in non-human primates (Weinbauer et al. 1989 have shown that GnRH antagonists are more potent in the suppression of gonadotrophin secretion and of sperm production than are GnRH agonists When combined with androgens, a depot-release form of GnRH agonist even had a blunting effect on the suppression of pituitary and testicular function (Behre, 1992). Research on these compounds is well justified for their application for the treatment of cancers but at present the cost of synthesis of peptide hormones such as the GnRH antagonists is likely to remain too high for contraceptive use in developing countries

2.1.72 Non-hormonal agents acting directly on spermatogenesis

A large number of chemical agents have been described (Behre, 1992) but all tend to lead to total spermatogenic arrest and, ultimately, to irreversible sterility. Gossypol was one of the more attractive drugs in this category. It was identified as an antifertility agent by Chinese scientists and clinical studies on more than 8,000 men were conducted (Swerdloff et al., 1989). Because of the high incidence of irreversibility (Liu et al., 1987) and potentially serious side effects such as hypokalaemia (Liu et al, 1987, Tang et al., 1988), gossypol has not been widely used outside China.

Physical agents such as irradiation, ultrasound and high temperature also lead to spermatogenic arrest when applied at appropriate dose levels. Their limitations lie in the equipment needed and the careful monitoring of the dosage required to avoid irreversible damage. One exception is the local application of heat (Rock and Robinson, 1965), Kandeel and Swerdloff, 1988. Recent clinical studies have shown that long-term mild elevation (1-2^{0}C) of temperature by the simple expedient of close apposition of the testes to the abdominal cavity during waking hours can lead to azoospermia or severe oligozoospermia (Mieusset et al., 1987, 1991). The safe reversibility, contraceptive efficacy and potential acceptability of this simple and inexpensive procedure need to be established.

2.1.73 Drugs and plant products for inhibition of sperm maturation

A reversible, post-testicular drug action on the normal function of sperm stored in the epididymis would be rapid in onset and, on withdrawal of the drug, normal sperm would return quickly in the ejaculate. There would be no disruption of normal endocrine function and the long latent period required to suppress spermatogenesis would be avoided. Since sperm spend only a relatively short time in the epididymis (3-10 days in the human), any interference with their competence at this stage would be more likely to involve their motility, capacitation and/or the acrosome reaction, events specific to sperm.

Many chemical compounds with reversible effects on sperm stored in the epididymis have been described but all have been discarded for their toxicity (Ray et al., 1991). Alpha-chlorohydrin and the 6-chloro-6-deoxy sugars were amongst the best explored (Ford and Waites, 1986). They at least established that the principle was attainable.

and, at antifertility doses, demonstrated the ideal characteristics of a post-testicular drug. Other compounds and their analogues are currently under investigation by various agencies e g sulphasalazines, imidazoles, and pyrimethamine.

Chinese investigators showed that a multiglycoside extract of the plant Tripterygium Wilfordii, long used in Chinese traditional medicine for the treatment of psoriasis, caused reductions in sperm motility and concentration in male patients (Qian, 1987) A collaborative programme has been established to isolate, identify and screen pure compounds extracted from the plant for their antifertility action (Waites, 1993)

2.1.74 Contraceptive vaccines

Passive or active immunisation against FSH has resulted in significant decreases in sperm counts in macaque monkeys but inconsistent effects on fertility (Moudgal et al., 1988, Nieschlag, 1986). Classical immunologists would prefer to avoid immunisation against a molecule so central in endocrine control, for fear of creating autoimmune reactions This dogma may be changing and the Population Council has developed a vaccine strategy based on GnRH Several agencies are supporting studies to establish if sperm surface proteins, crucial for sperm-egg interactions offer hope as immunogens for the development of a vaccine (Wu, 1988, Primakoff and Myles, 1990)

2.1.8 SCREENING OF ANTIFERTILITY AGENTS (Ghosh, 1984)

A screening programme should clearly demonstrate the potentiality of a compound to produce temporary and fully reversible sterility in laboratory animals The initial routine fertility tests may be carried out in both sexes followed by special tests to determine the mechanism of action. Rats are commonly used although mice rabbits and monkeys are also employed for the purpose.

2.1.81 Test in the Female Rat

Antifertility action in the female may result from the following:

(i) Inhibition of ovulation,

(ii) Prevention of fertilization;

(iii) Interference with the transport and/or implantation of the fertilized ovum; and

(iv) Destruction and resorption of the early implanted embryos.

Oestrous Cycle in Rats

The cycle makes its appearance at puberty at the age of two or three months, and the whole cycle lasts for about four to five days being divided into four stages according to the cell types found in the vaginal smear These are as follows (Hafez, 1970)

i. Oestrus (9 to 15 h) –is characterized by sexual receptivity when the female will allow copulation. During this period there are increased running activity quivering of the ears and lordosis in the presence of another rat The vaginal smear shows 100 percent cornified epithelial cells

ii. Metestrus (about 29 hr) - follows estrous and occurs shortly after ovulation Vaginal smear is characterized by many leucocytes with a few cornified cells

iii. Diestrous (60-70 hr) - is the longest of the phases and the vaginal smears consist mainly of leucocytes

iv Proestrus (about 12 hr) - which follows diestrous is a preparatory phase prelinary to the next oestrus phase The vaginal smears are characterized by nucleated epithelial cells either singly or in sheets

Vaginal smears may be taken in the following way to confirm the actual stage

Cotton swab made with tooth picks is moisten with the saline and gently inserted and slightly rotated with in the vagina The swab is pressed in a drop of saline on a microscope slide and examined under low power An alternative and better method is to take the vaginal wash with the few drops of the saline with the help of a capillary pipette fitted with a rubber teat The following tests are usually carried out for screening of antifertility drugs

Cohabitation test:

Female rats of established fertility (having produced two consecutive litters) are examined on seven consecutive days by vaginal smear for the presence of normal oestrous cycle. After administration of the test compound or solvent, the females are paired with males of proven fertility The females are examined for the following (a) sign of mating as indicated by the presence of sperm in vagina; prolongation of the average time from pairing to the first insemination is indicative of antifertility effect; (b) oestrous cycle changes by vaginal smear examinations, (c) inspection of uterus for

number of implants, (d) number of litters at birth, and (e) resorption of fetuses revealed by the differences between (c) and (d)

Ovarian weight in unilateral ovaiectomized rat: The ovarian weight increases in control animal 7-14 days after removal of the other ovary A decrease in the ovarian weight in the treated animals compared to the control will indicate an inhibition of ovulation through suppression of follicular stimulating hormone Ovarian histology may show corpus luteum, etc

Changes in the uterine weight: Any change in uterine weight will indicate antioestrogenic action

Deciduomata: The development of deciduoma in the endometrium will indicate progestational action.

2.1.82 Tests in the Male Rat

In the male, there are two possible mechanisms by which an antifertility agent may act (i) suppression of spermatogenesis at any of the stages resulting in sterility associated with oligospermia or aspermia, and (ii) a qualitative change in spermatozoa rendering them nonfunctional.

Routine testing of male fertility is most conveniently carried out in rats, since tests extending over 12 weeks after treatment cover possible effects on any stage of spermatogenesis normally of 9 weeks duration

Sperm count and motility:

Sperm count is carried out to evaluate the effect of the anti-fertility agent on spermatogenesis.

Cohabitation test:

Treated males are mated each with two females of known fertility and examined for the following. (a) sign of mating as indicated by the presence of sperm in vagina of females, calculation of the date of insemination from the date od birth (gestation period approximately 21 days) permits an estimate of the duration of sterility; pseudopregnancy (presence of leucocytes in vaginal smears for 10 to 14 days) is indicative of aspermic copulation, (b) normal estrous cycle is an indication of failure to mate due to an effect on libido, and (c) motility test of sperm from base of epididymis in males.

2.2 NASAL DRUG DELIVERY

The intranasal administration provides a useful way of taking a range of systemic drugs The rate of absorption, plasma concentration and pharmacokinetics often compares well to that obtained by intravenous medication because of rich vasculature and high permeability of nasal mucosa The large number of fenestrated capillaries just below the surface epithelium may well contribute to absorption (Fisher, 1990) The compliance of patients who require long-term medication has been shown to be better due to the simplicity and ease of administration when compared to the parenteral route (Pontiroli et al , 1985; Hirai et al , 1981)

Table 2.2 Advantages and Limitations of Nasal Drug Delivery

Advantages	Limitations
Avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation	Volume that can be delivered into nasal cavity is restricted to 25-200 µl
Avoids degradation of drug resulting from hepatic first pass metabolism	High molecular weight com pounds cannot be delivered through this route (mass cut off~1 kDa)
Results in rapid absorption and onset of effect	Adversely affected by pathological conditions
Results in higher bioavailability thus uses lower doses of drug	Large interspecies variability is observed in this route
Easily accessible, non-invasive route	Normal defence mechanisms like mucociliary clearance and ciliary beating affects the permeability of drug
Self-medication is possible through this route	Enzymatic barrier to permeability of drugs Irritation of nasal mucosa by drugs
Direct transport into systemic circulation and CNS is possible Offers lower risk of overdose	Limited understanding of mechanisms and less developed models at this stage
Does not have any complex formulation requirement	

2.2.1 FACTORS INFLUENCING NASAL DRUG ABSORPTION

2.2.11 Physiological Factors

In designing the nasal products, it is worth considering some basic nasal physiology. First, when a simple nasal formulation is placed in the nasal cavity weather as a solution or a powder it will normally be cleared quite rapidly to the throat by a process of mucociliary clearance, the average half time for the clearance in man is approximately 15 minutes as measured by scintigraphic methods (Soane, 1999) In order to achieve high absorption with only minimal effects on the mucociliary mechanism, the absorption process must be rapid, preferably within the first 15 minutes after administration (Gizurarson, 1991) Moreover, it should be remembered that at any time we predominantly use only one side of the nose for breathing, essentially one nostril is open (patent) while the other side is obstructed A nasal cycle mechanism operates in switching the nostril from patency to obstructed, over a period of 8 hours or so (Lund, 1996)

Table 2.3 Physicochemical, anatomical, physiological and formulation factors affecting the nasal absorption of drugs

Physicochemical factors	Anatomical and physiological factors	Formulation factors
Charge	Membrane transport	PH
Mol Wt	Deposition	Osmolarity
Lipophilicity	Enzymatic degradation	Viscosity
	Mucociliary clearance	Concentration
		Volume
		Dosage form

Irritation

As discussed above, some drugs can be well absorbed from the nasal cavity without the need for specific delivery system. The main question with these drugs is often one

of the possibilities of local irritation If drugs need to be given at large dose, such as 50mg or more, the nasal route will probability is unsuitable Some drugs by their very nature or the concentration used (hyper osmotic solution) can cause irritation in the nasal cavity. The measurements of irritation itself in non-human tests can present experimental challenges However, some compounds may be totally non-damaging but have irritant effect. Tolerance may be an important factor. Nicotine is a good example of this problem, where irritation occurs but the effect is transient and tolerance is soon obtained The measurement of non-damaging irritation can be achieved in animal models using techniques like measurement of evoked potential or the measurement of immunocytochemical markers such as C-FOS protein (Anton, 1991) Such studies can be performed as prelude to the best test for irritation, dose escalation studies in panels of human subjects

2.2.12 Disease factors

Mucociliary clearance is the most significant physiological factor that affects nasal drug absorption, because it determines the time a drug remains at the absorption site Some pathophysiological conditions, such as rhinitis, the common cold, hay fever, sinusitis, asthma, and nasal polyposis, may affect the retention of drugs in the nasal cavity Environmental factors such as humidity, temperature, air borne toxins and chemicals, and many pharmaceutical excipients may also affect the clearance (Hermens, 1987). The common cold or any pathological conditions involving mucociliary dysfunction can greatly affect the rate of nasal clearance and subsequently the therapeutic efficacy of drug administered intranasally Nasal obstruction as a result of extensive nasal polyposis would reduce the capacity of nasal absorption (Proctor, 1985) In addition, atrophic rhinitis or severe vasomotor rhinitis could also reduce the usefulness of the nose to absorb the drug. In some people, an extensive response of the secretary system to some irritants could drain away whatever is introduced prior to absorption. Such tendency may exist in persons with severe nasal allergies. Nevertheless, it has been shown that the common cold and rhinitis do not decrease the bioavailability of buserelin (Larsen, 1987) and desmopressin (Olanoff et al, 1987).

2.2.13 Formulation factors

The formulation plays an important role in the nasal absorption of drugs as it does in case of other route of administration.

Dosage forms

Various dosage forms available for nasal delivery are solution, suspension, emulsion and dry powders The liquid formulations are usually water based but may also contain alcohol, oils or other organic solvents Liquid spray and drops are the most widely used preparations for intranasal delivery The nasal spray deposits anteriorly in the nasal atrium while the drops are dispersed throughout the length of the nasal cavity Nasal sprays deposit more anteriorly, resulting in slower clearance of sprays than of drops (Hardy 1985) The solution deposited from nasal drops cleared more rapidly than from the nasal spray. Also, the particles with an aerodynamic size above 10-20 μm are all deposited in the nasal cavity, whereas particles smaller than 1 μm pass with inspired air into the lungs The nasal bioavailability of desmopressin was significantly increased following spray administration as compared to nasal drops (Harris, 1986) Powder Vs liquid dosage forms

Liquid preparations are common dosage forms for nasal delivery Because the nasal epithelium is essentially a lipophilic transport barrier, trans-nasal transport is related to the nasal mucosa tissue-water partition coefficient, suggesting also an important role of the stereo-chemical conformation during membrane transport (Corbo 1990). Therefore, aspects such as formula pH, ionic strength, surface active agents, viscosity, and drug concentration have to be considered in order to facilitate the transport (Hussain 1998). However, from a technological point of view, the liquid preparations present problems linked to formula stability, low drug concentration at the absorption site, and short residence time in nasal cavity.

These drawbacks accelerated the development of nasal powders as alternative nasal dosage forms with improved chemico-physical and microbiological stability. Further more, drug dissolution on nasal mucosa provides elevated drug concentration at the deposition site, giving rise to a high flux of active ingredient In many cases the superiority of nasal powder compared with nasal liquid was demonstrated, in particular with peptide drugs. For instance, the bioavailability of elcatonin (ECT) via the nasal route was investigated (Ishikawa 2001) with a powder dosage form utilizing

water-insoluble calcium carbonate ($CaCO_3$) in comparison with the liquid dosage form Total radioactivity and the radioactivity of intact [3H] ECT were measured to evaluate the nasal absorption in vivo and the nasal mucosal transport in vitro The systemic bioavailability of both total radioactivity and intact [3H] ECT following intranasal administration of the powder formulation in rats was significantly greater than in the case of the liquid formulation In contrast, similar permeability of ECT across excised rabbit nasal mucosa was seen for both formulations, and was close to that of [14C] inulin, suggesting that the ECT transport is predominantly para-cellular in each case However, the powder formulation significantly prolonged the residence time of [3H] ECT in the rat nasal cavity, compared with the liquid formulation In another study, Insulin powder formulations with Dimethyl β-cyclodextrin as an absorption enhancer was found to be much more effective than liquid formulations (Schipper 1993) A clinical study compared the administration of powders and solutions of glucagons and human Calcitonin with dihydrofusidate as enhancer (Pontiroli 1989). It showed that the powder formulations were as effective as the spray solutions

2.2.14 Physical and Chemical Factors

Molecular size

The absorption rate of particles has been shown to fall off sharply when molecular weight exceeds 1000 daltons (Chien et al., 1989) Absorption can be enhanced by surfactants, which modify the permeability of the nasal mucosa at a molecular level to alter its physiochemical properties or else by conversation of the drug into salt or esters.

Nasal pH

Nasal absorption is also pH dependent. The absorption rate of peptide based drugs such as insulin has been shown to be affected by pH (Hirai et al., 1978) When the rate was assessed by the intranasal administration of insulin in dogs, low pH resulted in a bigger reduction in the glucose levels.

Drug Lipophilicity

The trasnasal permeation behaviour cannot in general be predicted by lipophilicity. A fourfold change in nasal absorption was noted between pentobarbital (which has a

sulphur link which enhances lipophilicity) and a barbital in a study of the effect of lipophilicity on nasal absorption

Nasal Enzymes

Several enzymes are present in nasal secretions which may affect the metabolism of drugs Cytochrome P-450 dependent mono-oxygenase has been reported to metabolize drug slike cocaine, decongestants, nicotine and progesterone

2.2.2 MECHANISM FOR ABSORPTION

It was discovered empirically that drugs such as lignocaine (Jones et al , 1986), proparanol (Hussain et al , 1979), progesterone (David et al , 1981, Hussain et al., 1981), enkephalins (Su KSE, 1985) and nicardipine (Visor et al . 1986) were readily absorbed by the nasal mucosa with bioavailability similar to intravenous administration Several mechanisms have been described In one study in rats, a fast lipophilic-dependent mechanism, and a slower one influenced by molecular size were distinguished Absorption of water-soluble compounds is dependent upon diffusion through aqueous chanels Insulin, Mannitol and proparanolol has transport mechanisms which are dependent on passive diffusion but phospholipids have been shown to act as an absorption enhancer (Drejer et al . 1992) as have non-ionic surfactants (Paquot et al., 1988). Aminoacids like tyrosine and phenylalanine were absorbed by an active transport process in rat mucosa and this transport appears to be an active Na+-dependent process. Higher molecular weight polypeptides are poorly absorbed without an enhancer. The olfactory area may be the site of absorption for certain compounds Hormones such as progesterone and oestradiol are absorbed via olfactory neurons into the CSF. Herpes viruses enter the CNS via cranial nerves in the olfactory mucosa (Chien et al., 1989)

2.2.3 STRATEGIES TO IMPROVE NASAL ABSORPTION

Though the nasal absorption of small non-peptide drugs is considerably good, the nasal bioavailability of peptides and protein drugs is low To improve the bioavailability of peptides and protein drugs several strategies have been tried.

1. Synthesis of more lipophilic analogues
2. Peptidase and protease inhibitors
3. Absorption enhancers

4 Colloidal or carrier based drug delivery systems

5 Bioadhesive drug delivery systems

2.2.31 Synthesis of more lipophilic analogues:

Prodrugs have been used to overcome poor solubility, insufficient stability, incomplete absorption across biological membranes and premature metabolism to active species This review examines the importance of various physicochemical factors affecting nasal absorption of drugs Novel trends in nasal prodrug development in the areas of targeted delivery to the CNS and selective targeting of the nutrient transporter system of the nasal mucosa have received considerable attention

Many potent peptide analogues have been synthesized which possess high lipophilicity and increased stability to enzyme degradation This approach has lead to the development of many nasally active peptides for example, metkephamid (Su et al , 1985), antidiuretic drug desmopressin (Harris et al , 1986; Christolini, 1991), LHRH agonist buserelin (Sandow and Petri, 1985), leuprolide (Yamazaki, 1984; Adjei et al , 1992) and nafarelin (Anik et al , 1984, Vickery et al , 1985) The effect of these nasally administered LHRH analogues on the induction of ovulation is increase to 50-200 times in comparison to the parent compound LHRH, but their nasal bioavailability remained very low (2-3%) (Sandow and Petri, 1985)). The discrepancy found between nasal bioavailability and induced biological activities can probably be attributed to high affinity of the pituitary receptors for these LHRH agonists

2.2.32 Peptidase and protease inhibitors:

The nasal epithelial tissue contains substantial amounts of peptidases and proteases. These enzymes are able to degrade peptides and proteins like enkephalins, insulin and pro-insulin. The predominant enzyme present is amino-peptidase

The amino-peptidase inhibitors such as bacitracin, bestatin and amastatin have been found to promote the nasal absorption of LHRH peptides (Raehs et al , 1988), salmon Calcitonin (Hanson et al . 1986), leucine enkephalin (Hussain et al., 1989) and human growth hormone (O'Hagan et al., 1990) in rats. A new amino-peptidase inhibitor, boroleucine and phosphinic acid dipeptide analogue Appeared to be highly potent amino-peptidase inhibitors, resulting in remarkably enhanced nasal absorption of leucine-enkephalin (Hussain et al., 1989, Hussain et al , 1990; Hussain et al., 1992)

These compounds were found to fealty inhibit the degradation leucine enkephalin in the nasal perfusate Enzyme inhibition was greater with boroalanine derivatives The boroleucine derivatives were more than 1000 times more effective than puromycin

2.2.33 Absorption Enhancers:

Absorption enhancers have most frequently been used to improve the bioavailability of intranasally administered peptides and proteins (Lee et al , 1991) For example, bile salts and STDHF can largely enhance the nasal absorption of insulin and growth hormone in rats, rabbits, and sheep (Moses et al . 1983. Gordon et al , 1985, Deurloo et al , 1989) The absorption promoting effect of the enhancers is due to their ability to achieve any of the following, like increase in membrane fluidity by extracting proteins from nasal membrane and creating transient hydrophilic pores, altering the properties (e g. Decreasing the viscosity) of the mucus layer, facilitating the leaking of lipids, and opening of tight junctions between epithelial cells For instance, bile salts and STDHF have been assumed to increase nasal insulin transport by interference of different mechanisms, such as decreasing the viscosity of mucus layer, solubilization of the insulin molecules in mixed micelles with these enhancers and subsequent formation of reversed micelles in the nasal epithelium. and by inhibiting enzymatic degradation (Moses et al., 1983; Gordon et al , 1985, Longenecker et al., 1987) EDTA and fatty acid salts (e g. Sodium caprate and sodium laurate) increase the para-cellular transport by removal of lumenal calcium with a subsequent increase in permeability of tight junctions Non-ionic detergents like Laureth-9 change membrane structure and permeability.

Studies in rat showed that DM- β-CD in concentrations of 2-5% is a very potent enhancer for the nasal absorption of insulin resulting in absolute bioavailability of approximately 100% (Merkus et al., 1991) Microcrystalline cellulose, which is commonly used in commercial preparations for allergic rhinitis was used as an absorption enhancer Nasal glucagon delivery using microcrystalline cellulose in healthy volunteers were studied (Teshima et al., 2002). The intranasal powder form with some spray solutions of glucagon were compared with regard to glucagon absorption, concentration of blood glucose, stability and nasal irritation. The absorption of glucagon from the spray solution including 1.5% sodium glycol-cholate or 1% sodium caprate was 1.3 and 2.6 fold higher than that from the powder form mixed with microcrystalline cellulose at a ratio of 1.69, respectively. The C_{max} values

of plasma glucose were 2 18, 3 39 and 1 56 mmol/l in the spray solutions including sodium glycol-cholate and sodium caprate and in the powder form, respectively For drugs with molecular weights below 10,000 Da, the use of Chitosan can lead to an improvement in bioavailability of from 5-10 folds Medium molecular weight Chitosan are able to enhance the nasal absorption in animals and human volunteers of polypeptides and other polar drugs, such as insulin, salmon Calcitonin (Illum et al , 1994) The absorption-enhancing effect of aminated gelatin was investigated (Wang et al , 2002) in rats using insulin and fluorescein isothiocyanate-dextran with a molecular weight of 4 4 K Da (FD-4) as model drugs The absorption of insulin was estimated by measuring the changes in plasma glucose levels following intranasal administration, and that of FD-4 was determined by measuring its plasma concentration after dosing The hypoglycaemic effect after intranasal administration of insulin with aminated gelatin significantly increased compared with that after intranasal administration of insulin in phosphate buffered saline, indicating that aminated gelatin effectively enhanced the nasal absorption of insulin In contrast, neither kind of native gelatin (isoelectric point = 5.0 and 9 0) showed any absorption-enhancing effect The pH of the formulations and the concentration of aminated gelatin were found to affect the hypoglycaemic effect In addition, aminated gelatin at a concentration of 0 2% significantly enhanced the absorption and the efflux of FD-4 through the rat nasal mucosa The possible perturbation of aminated gelatin to nasal mucosa was evaluated by measuring the leaching of lactate dehydrogenase (LDH) using an in-situ perfusion rat model Aminated gelatin presented a concentration-dependent (0 1-0 4%) but relatively small effect on the LDH leaching from the rat nasal epithelial membrane

2.2.34 Bioadhesive and colloidal drug delivery systems

Prolonging the contact of the drug with the absorptive surfaces by means of an appropriate delivery system can increase the bioavailability of intranasally administered drug This system utilizes bioadhesive gels or microspheres.

Chitosan delivery systems investigated (Soane et al., 2001) in solution and microsphere system had significantly reduced rates of clearance from the sheep nasal cavity, as compared to the control The data show that the control was cleared rapidly from the sheep nasal cavity with a half time of clearance (time taken for 50% clearance; t (50%) of about 15 min. The bioadhesive chitosan delivery systems were

cleared at a slower rate, with half times of clearance of 43 min and 115 min, for solution and microsphere formulations respectively

The effects of starch microspheres on the absorption enhancing efficiency of various enhancer systems in formulations with insulin after application in the nasal cavity of sheep were evaluated (Illum et al., 1996) The enhancers studied were lysophosphatidylcholine, glycol-deoxycholate and sodium tauro-dihydroxyfusidate, a bile salt derivative The bioadhesive starch microspheres were shown to increase synergistically the effect of the absorption enhancers on the transport of the insulin across the nasal membrane Dependent on the potency of the enhancer system the increment in absorption enhancement was shown to be from 1 4 times to 5 times that obtained for the absorption enhancer in solution. Starch microspheres were also shown to increase the nasal absorption of human growth hormone and salmon calcitonin considerably in sheep (Illum, 1992) Enhancement of insulin absorption was observed when insulin was incorporated into the continuous aqueous phase of an o/w emulsion (Mitra et al, 2000). The presence of a small fraction of oil droplets along with insulin in the aqueous phase appeared to favour insulin absorption When the oil phase constitutes the external phase, as in w/o emulsion, no insulin absorption was noted Inhibition of insulin absorption might arise from a rate limiting barrier effect of the membrane completely covered by a stagnant oil layer

The suitability of gelatin microspheres for nasal delivery of salmon calcitonin (sCT) was examined (Morimoto et al, 2001) Negatively and positively charged gelatin microspheres were prepared both types of gelatin microspheres were capable of adhering to the nasal mucosa. The mucoadhesion of positively charged gelatin microspheres was significantly higher than that of their negatively charged counterparts. The absorption of sCT after intranasal administration was evaluated by calculating the area above the hypocalcemic time curve (AAC) in rats The AAC values after nasal administration of sCT in positively and negatively charged gelatin microspheres were significantly greater than that in pH 7 0 PBS.

In another study (Chen and Mao, 2000) after intranasal administration in rabbits, the bioavailability of melatonin-gelatin microspheres (MT-GMS) was evaluated MT-GMS were 87 47% as compared to vein injection, while the bioavailability of MT solution was 69.72% showed increase the bioavailability of MT in microspheres.

2.2.4 ANIMAL MODELS FOR NASAL ABSORPTION STUDIES

The animal models employed for nasal absorption studies can be of two types, viz., whole animal or in vivo model and an isolated organ perfusion or ex vivo model These models are discussed in detail below

2.2.41 *In vivo* Nasal Absorption Models

Rat Model

The surgical preparation of rat for in vivo nasal absorption study carried out as follows: The rat is anaesthetized by intraperitoneal injection of sodium Phenobarbital An incision is made in the neck and the trachea is canulated with a polyethylene tube Another tube is inserted through the oesophagous towards the posterior region of nasal cavity. The passage of the nasopalatine tract is sealed so that the drug solution is not drained from the nasal cavity through the mouth The drug solution is delivered to the nasal cavity through the nostril or through the canulation tubing. The blood samples are collected from the femoral vein (Chien et al, 1989). As all the probable outlets of drainage are blocked, the drug can be only absorbed and transported into the systemic circulation by penetration and/or diffusion through nasal mucosa.

Using the rat model, the nasal absorption and permeability of the following drugs/compounds have been studied propranol, progesterone; testosterone, naloxone and bupernorphine, ergotamine tartarate, sulbenicillin; cefazolin, cephacetrile, phenol red, salicylic acid, aminopyrin, and bucolome, insulin; enkephalins, enviroxime, dobutamine; clofilium tosylate, glucagon, cyclo-(-Pro-phe-D-Trp-Lys-Thr-Phe) and horseradish peroxidase; sodium guiazulene-3-sulfonate, calcitonin; secretin, nicardipine, substance P, etc.

Rabbit Model

The in vivo rabbit model for nasal drug delivery is outled as follows: Rabbits weighing approximately 3 kg are either anaesthetized or maintained in the conscious state depending on the purpose of the experiment.

The rabbit is anaesthetized by an intramuscular injection of a combination of ketamine and xylazine. The drug solution is delivered by nasal spray into each nostril, while the rabbit's head is held in an upright position. During the study, the rabbit breaths normally through the nostrils and body temperature is maintained at 37° C

with the use of heating pad The blood samples are collected via an indwelling catheter in the marginal ear vein or artery according to the experimental protocol

As an animal model for nasal absorption studies, the rabbit combines several advantages

1 It is relatively cheap, readily available and easily maintained in laboratory settings

2 It permits pharmacokinetic studies as with large animals (monkey)

3.The blood volume is large enough (300 ml approx) to allow frequent blood sampling (1-2 ml)

Thus it permits full characterization of the absorption and determination of pharmacokinetic profile of a drug.

The rabbit model described here has been used in studying the nasal absorption and nasal controlled delivery of progesterone and its hydroxyl derivatives

Dog Model

The in vivo dog model for nasal absorption studies is briefly outlines as follows The dog is either anaesthetized or maintained in the conscious state depending on the purpose of the experiment In the anaesthetized model, the dog is anaesthetized by i.v. injection of sodium thiopental and maintained with sodium Phenobarbital A positive pressure pump provides ventilation through a cuffed endotracheal tube, and a heating pad keeps the body temperature at 37-38^{0}C The blood samples are collected from the jugular vein according to the experimental protocol

The dog model has been used in studying the nasal absorption of propranol, insulin, and other drugs

Sheep Model

The in vivo sheep model for nasal drug delivery is basically similar to that described for the dog model. Male in-house bred sheep are selected for their lack of nasal infectious diseases.

The sheep model has been used in studying the nasal absorption of insulin, metkephamid, and other drugs.

Monkey Model

The in vivo monkey model for nasal absorption studies is briefly outlines as follows: Monkeys (approx 8 kgs) are anaesthetized, tranquilized or maintained in the conscious state as per the experimental purpose The money is tranquilized by intramuscular injection of ketamine hydrochloride or anaesthetized by intravenous injection of sodium Phenobarbital The head of the monkey is held in upright position and a drug solution is administered into each nostril Following administration, money is placed in a supine position in a metabolism chair for 5-10 minutes Throughout the course of study money breaths normally through the nostrils The blood samples are collected via an indwelling catheter in the vein according to the experimental protocol.

The monkey model has been used in studying the nasal absorption of insulin. luteinizing hormone releasing hormone, nicardipine, and other compounds

2.2.42 Ex Vivo Nasal Perfusion Models

Surgical preparation is the same as that is for in vivo rat model. During the perfusion studies, a funnel is placed between the nose and reservoir to minimize the loss of drug solution The drug solution to be evaluated is placed in the reservoir, which is maintained at 37°C, and is circulated through the nasal cavity of the rat by means of a peristaltic pump The perfusion solution passes out from the nostrils, through the funnel, and flows into the reservoir again. The drug solution in the reservoir is stirred constantly and the amount of drug absorbed is then determined by measuring the drug concentration remaining in the perfusion solution. Because of the experimental condition, the possible loss of drug activity due to stability, such as the loss of peptides and proteins by proteolysis, aggregation, and other factors must be considered.

The ex vivo nasal perfusion model described has been performed in studying the nasal absorption of salicylic acid, aminopyrin, and phenol red, leucin enkephalin, sodium benzoate, sodium barbital, sodium Phenobarbital. sodium secobarbital, L-tyrosine and propranolol hydrochloride; hydralazine, insulin and polyethylene glycol 4000 and other drugs.

Using rabbit as the animal model, the ex vivo nasal perfusion model can also be used for studying the pharmacokinetics of drugs following nasal absorption.

2.3 CHITOSAN

Over recent years, the nasal delivery of challenging drugs, such as peptides and proteins and polar molecules such as morphine and migraine compounds has been greatly improved using an approach that is not based upon 'classical' surfactant enhancers, but upon a cationic polysaccharide called chitosan Chitosan is deacetylated chitin, and chitin is the second most abundant polysaccharide in the world Below a pH value of approximately 7 0, chitosan is water-soluble and, because of its cationic nature, can bind with mucosal surfaces and with mucin, the latter occurs through an interaction between the positively charged amine groups on the chitosan molecule and the negatively charged sialic acid groups on mucin This interaction leads to bioadhesion and a reduced mucociliary clearance.

The application of chitosan as a nasal drug delivery system to facilitate the absorption of peptide and protein drugs was first introduced by Illum (1992) . In a later publication it was reported that chitosan promoted the passage of insulin and salmon calcitonin across the nasal mucosa in rat and sheep models (Illum et al., 1994) The mechanism of action of chitosan can be attributed to its bioadhesive properties and its ability to transiently open tight junctions in the membrane (Artursson et al , 1994. Schipper et al., 1997, Dodane et al., 1999) Chitosan has been shown by several research groups to be a mucoadhesive material This characteristic is most likely due to an ionic interaction of the positively charged amino groups of the D-glucosamine units of chitosan with the negative sialic acid groups of mucin or other negatively charged groups of the mucosal membrane (Lehr et al., 1992) . The latter group showed that the interactions between chitosan–mucin were highly pH dependent with the strongest interaction at pH values where both the sialic acid units and the chitosan amino groups were well ionised It was also shown recently, in a rat intestinal loop study by He et al. (1998) that chitosan microspheres were highly mucoadhesive. compared to a control microsphere preparation, in terms of binding to the intestinal wall. Aspden et al. (1995, 1997) evaluated the effect of chitosan on mucociliary clearance, ex vivo, using the frog palate model and human nasal turbinate tissue. In both studies chitosan was found to transiently decrease mucociliary clearance, clearance rates returned to normal after removal of the chitosan. The effect of chitosan on the clearance of a saccharine tablet from the nasal cavity was investigated in human volunteers 1 hr after chitosan administration and after 7 days repeated

administration There was no significant difference between the saccharine clearance times in the chitosan treated humans and the control (Aspden et al, 1997), demonstrating that any effect of chitosan on the mucociliary function is transient

Recently, Soane et al. (1999) evaluated the nasal clearance characteristics of a range of formulations, including chitosan solution and chitosan microspheres, in human volunteers It was found that the half- time of clearance of chitosan solution was almost doubled; 41 min compared with the solution control half-time of 21 min The chitosan microspheres were found to be cleared even more slowly from the nasal cavity, with a clearance half-time of 84 min

However, interestingly, chitosan has another and more dramatic effect in terms of providing improved nasal drug absorption. Chitosan can alter the paracellular transport of drugs by direct effect on the tight junctions between cells It has been shown that the presence of chitosan at a mucosal surface can lead to a transient opening of the tight junctions, and this has been demonstrated in CaCO-2 studies, where measurements of transepithelial resistance, mannitol transport and histological measurements have been made (Kotze et al . 1999) The opening of the tight junctions occurs for a period of approximately 15 minutes and could allow molecules as large as growth hormone (20,000 Da) to pass from the nasal lumen into the circulation For drugs with molecular weights below approximately 10,000 Da, the use of chitosan can lead to an improvement in bioavailability of from 5–10-fold It has been demonstrated that this chitosan effect with various polypeptides, that include desmopressin, insulin, leuprolide, calcitonin, PTH, CCK-8, as well as with polar compounds for the treatment of migraine, such as alniditan, and analgesic agents such as morphine These studies have been performed in an ovine model and in man (Roon et al., 1998) Chitosan is, by its very nature, a high molecular weight material that is not itself absorbed. Chitosan is non-toxic and has a reversible effect on ciliary function. Therefore, chitosan represents a new approach to improving the transmucosal delivery of challenging molecules.

2.4 CARBOPOL

The polyacrylic acid polymers (carbomers and polycarbophil) are widely used in attempts to formulate mucoadhesive drug delivery systems for application to various mucosal sites While reports of in vitro mucoadhesive performance of these polymers abound

(Lueßen et al , 1994; Park and Robinson, 1985, Ponchel et al , 1987, Vidgren et al , 1992; Mortazavi and Smart, 1993), there have been few reports that evaluated their in vivo nasal mucoadhesion performance (Vidgren et al , 1991) A polyacrylic acid gel bioadhesive system improved the absorption of insulin and calcitonin in rats (Morimoto et al , 1985) In another study (Callens and Remon, 2000) , Insulin was administered nasally to rabbits with drum-dried waxy maize starch (DDWM) or maltodextrins with different DE-values and Carbopol 974 P In freeze-dried powder form, bioavailabilities obtained with the powder formulations containing DDWM/Carbopol 974 P (5 and 10%) were significantly higher ($p<0.05$) than those containing maltodextrins-Carbopol 974 P mixtures The bioavailability of the powder formulation containing DDWM and 10% Carbopol 974 P was significantly higher (14 4%) than the bioavailability of the same mixture containing 5% Carbopol 974 P (9 9%) The bioavailability, t(max) and C(max) values of the formulation with 5% Carbopol 974 P were significantly higher in comparison with the formulation without Carbopol 974 P 10% The study concluded that Carbopol 974 P was required when maltodextrins were used in order to obtain a significantly higher bioavailability compared with the formulations without Carbopol 974 P Also, Freeze-drying seemed a prerequisite for a good bioavailability from the powder formulation as well as the ratio of insulin versus bioadhesive powder (1 IU and 2 IU/mg of bioadhesive powder)

2.5 PULMONARY DRUG DELIVERY

The therapeutic benefits of drug inhalation have been appreciated for several decades for the treatment of respiratory diseases, such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and pulmonary infections, as well as for the systemic delivery of anesthetic agents (Anonymous, 1946, Brewis et al., 1995; Camps, 1929; Dale et al , 1987; Graeser et al , 1935) By direct targeting of locally acting drugs to the lungs, a high local concentration of the drugs at the target site, rapid onset of drug action, lower systemic exposure, and consequently reduced side-effects can be achieved (Lipworth, 1996; van den Bosch et al , 1993)

The use of the inhaled route outside the respiratory therapeutic area is today uncommon. However, the advantageous drug absorption characteristics of the lung, e g. the highly vascularized respiratory mucosa, large absorptive surface area, thin air-blood barrier, and the relatively low enzymatic activity in the lung, have attracted attention to pulmonary delivery as a potential alternative for systemic administration of drugs with

poor oral absorption (Anttila et al , 1997, Patton, 1996, Wall et al , 1993) For instance, several investigations regarding inhalation of therapeutic peptides, proteins, oligonucleotides, and vaccines, which are subjected to poor enzymatic stability and low permeation across biological membranes, have been reported (LiCalsi et al , 1999, Niven, 1995; Russell et al , 2001, Skyler et al , 2001) In addition, pulmonary drug delivery is currently evaluated for the delivery of analgetic drugs, such as morphine, for which a rapid onset of drug action is of significant therapeutic importance (Dershwitz et al , 2000)

The efficacy of an inhaled drug is determined by its absorption across the lung barrier and on the location of the pharmacological site of action For locally acting drugs, the absorption into the systemic circulation may imply the removal and consequently the termination of action of the drug in the lung On the other hand, for systemically acting drugs, the absorption profile of the drug from the lung may determine the onset, intensity, and duration of action of the drug (Taylor G , 1990). Although inhalation is a well established means for drug administration, drug absorption kinetics in the lung has not been subjected to extensive research Yet, investigations of the absorption rate and bioavailability of pulmonary delivered drugs in relation to the drugs' molecular properties are important to aid the design of new inhaled drugs for local and systemic action

2.5.1 MAJOR COMPONENTS OF THE LUNG - BARRIERS TO DRUG ABSORPTION

As one of the primary interfaces between the organism and the environment, the respiratory system is constantly exposed to airborne particles, potential pathogens, and toxic gases in the inspired air (Plopper, 1996) As a result a sophisticated respiratory host defense system, present from the nostrils to the alveoli, has evolved to clear offending agents (Twigg, 1998) The system comprises mechanical (i e air filtration, cough, sneezing, and mucociliary clearance), chemical (antioxidants, antiproteases and surfactant lipids), and immunological defense mechanisms and is tightly regulated to minimize inflammatory reactions that could impair the vital gas-exchange (Nicod, 1999; Twigg, 1998)

From a drug delivery perspective, the components of the host defense system comprise barriers that must be overcome to ensure efficient drug deposition and absorption from the respiratory tract

2.5.11 Epithelium

The airway epithelial cells provides a tight ciliated barrier that clears the airways from debris trapped in the airway mucus, prevents indiscriminant leakage of water and solutes into the airways, secretes components for the airway lining fluid and mucus layer; repairs injuries to the epithelium, and modulates the response of inflammatory cells, vessels, and smooth muscle (Rennard et al , 1991) The epithelium lining the tracheobronchial airways is composed of seven different cell types, i e. basal cells, goblet cells, ciliated cells, brush cells, serous cells, Clara cells, and neuroendocrine cells (Plopper, 1996) A variety of migratory cells such as lymphocytes, leukocytes, and mast cells are also present in the epithelium (Plopper, 1996) The epithelium lining the terminal bronchioles is columnar or cuboidal and is composed of ciliated cells and Clara cells (Plopper, 1996) In the alveolar region, four cell types are present the epithelial type I and II cells, alveolar brush cells (type III) and alveolar macrophages (Ma et al , 1996, Plopper, 1996) The squamous type I cell covers approximately 96% of the alveolar surface area and has an average cell thickness of 0 26 um Characteristically the alveolar type I cell has a large cytoplasmic volume and displays only sparse cellular organells most of which are located in the perinuclear region of the cells (Crapo et al., 1982) These morphometric features are favorable for drug transport About 3% of the alveolar surface is covered by the much smaller cuboidal type II cells, which synthesize and secrete surface active materials (Mason R J et al , 1998).

The apical membranes of the epithelial cells are joined by tight junctions that divide the cell membranes into the functionally distinct apical and basolateral domains (Summers, 1991) The tight junctions are highly dynamic structures that act as barriers to fluid flow and control the transport of ions and solutes through the intercellular space (Summers, 1991) The heterogenous composition of the lung epithelium results in a large variation of tight junctional forms with variable tightness (Godfrey, 1997, Schneeberger, 1980).

2.5.12 Endothelium

The lung is unique among tissues in that about 40% of its total cellular composition is capillary endothelium, which is the largest capillary endothelial surface in the body

(Simionescu, 1991) The alveolar-capillary endothelium has specialized organell-free domains to provide a particularly thin (from 200 nm down to 30-35 nm) barrier for gas exchange (Simionescu, 1991) Furthermore, the endothelial cells have a relatively large number of endocytotic vesicles (Schnitzer, 2001). The endothelial cells are joined by tight junction with few parallel arrays of contacts, which renders them leaky when the hydrostatic pressure increase (Plopper, 1996, Simionescu, 1991)

2.5.13 Alveolar macrophages

The alveolar macrophages are found on the alveolar surface These phagocytic cells play important roles in the defense mechanisms against inhaled bacteria and particles that have reached the alveoli (Haley et al , 1991) Particles deposited in the lung parenchyma of rabbits and rats have been demonstrated to be phagocytized by alveolar macrophages within a few hours (Brain et al., 1984, Takenaka et al., 2001). The macrophages are cleared from the alveoli to the bronchioles by the lining fluid, and then from the airways by the mucociliary escalator (Jeffery, 1995)

2.5.14 Interstitium and basement membrane

The interstitium of the lung, the extracellular and extravascular space between cells in the tissue, contains a variety of cells (fibroblasts, myofibroblasts, pericytes, monocytes, lymphocytes, plasma cells), collagen, elastic fibers, and interstitial fluid (Plopper, 1996) Its main role is to separate and bind together the specific cell layers in the tissue The main drainage pathway for the interstitial fluid is the lymphatic vessels. The outer border of the interstitium is defined by the epithelial and endothelial basement membranes (Weibel et al , 1991). The basement membrane modulates the movement of fluid, molecules, particles, and cells from the air space and blood into the interstitium (Weibel et al , 1991) However, plasma proteins and most solutes are thought to diffuse relatively unhindered through it (Patton, 1996)

2.5.15 Lymphatic system

The pulmonary lymphatic system contributes to the clearance of fluid and protein which has filtered from the vascular compartment into the lung tissue interstitium and helps to prevent fluid accumulation in the lungs (Puchelle et al , 1995). The lymphatic vessels are present in the interstitium near the small airways and blood vessels, but not in the alveolar walls (Leak et al., 1983). The leaky lymphatic endothelia allow micron-sized particles (e.g. lipoproteins, plasma proteins, bacteria, and immune cells) to pass freely

into the lymph fluid (Patton, 1996) The flow rate of the lymphatic fluid is normally very slow (1/500 relative the blood flow), but is increased at high pulmonary venous ressure (Patton, 1996) The lymph is filtered through regional lymph nodes and returned to the venous blood circulation at the right jugular and subclavian veins

2.5.16 Epithelial lining fluid

Solid drugs particles delivered to the respiratory tract need to be wetted and dissolved before they can exert their therapeutic activity Although the humidity in the lung is near 100%, the volume of the epithelial lining fluid is small (Wiedmann et al , 2000). The thickness of the lining fluid in the airways is estimated to 5-10 ^m and is gradually decreased along the airway tree until the alveoli, where the thickness is estimated to be about 0 05-0.08 um (Patton, 1996) The volume and composition of the epithelial lining fluid is determined by active ion transport and passive water permeability of the respiratory epithelium (Puchelle et al , 1995) However, due to the inaccessibility and small volume available, the composition of the epithelial lining fluid is not fully known Like the gastric mucosa, the airway mucosa is coated with a layer of phospholipids, which in association with mucins lubricate and protect the epithelium from offending agents (Girod et al., 1992; Puchelle et al , 1995) Phospholipids and proteins in bronchial secretions inhibit the adhesion of cilia to the mucus gel and accelerate ciliary beat frequency (Morgenroth et al., 1985) Bacteriostatic and bactericidal proteins present in the lining fluid, e g IgA, lactoferrin, and lysozyme, are synthesized and secreted by submucosal gland cells and participate in the airway antibacterial defense (Puchelle et al , 1995) In the alveolar region, the surface fluid consists of a thin biphasic layer of plasma filtrates overlaid by a monolayer of pulmonary surfactant (Patton, 1996)

2.5.17 Surfactant

The airway and alveolar lining fluids are thus covered by at least a monolayer of lung surfactant projecting the fatty acid tails into the air space (Patton, 1996). Consequently, interactions between the phospholipids in the lung surfactant and inhaled drugs have been reported. For instance, lung surfactant was shown to enhance the solubility of glucocorticosteroids, which may affect the residence time of the steroid in the lung (Wiedmann et al . 2000) Furthermore, strong interaction of the polypeptides ditirelix and cyclosporin A with phospholipids have been demonstrated and has been suggested

to limit the absorption from the lung, thus leading to a prolonged retention of the drugs in the lungs (McAllister et al., 1996) The use of exogenous surfactant as a vehicle for pulmonary drug delivery has been suggested as a means to enhance the spreading of the drug within the lungs (Van' t Veen et al , 1999) However, in a study with intratracheally instilled Tc-99m-tobramycin in rats it was concluded that the exogenous surfactant increased the lung clearance rate of Tc-99m-tobramycin (Van't Veen et al , 1999) In another study, a decrease in bactericidal activity of tobramycin and gentamicin through binding to lung surfactant was demonstrated in vitro (Van't Veen et al., 1995). These results reflect a complex interaction between drugs and lung surfactant, which should be considered in drug development

2.5.18 Mucociliary clearance

The residence time of an inhaled drug in the lungs depends on the site of deposition. A significant proportion of the drug reaching the lungs from an inhaled aerosol is entrapped in the mucus in the conducting airways The ability of the drug to penetrate the mucus barrier depend on particle charge, solubility, lipophilicity, and size (Bhat et al , 1995; Rubin, 1996) For instance, reduced transports across respiratory mucus layers have been demonstrated in vitro for corticosteroids (Hashmi et al., 1999) and antibiotics (Lethem, 1993).

2.5.19 Pathophysiological changes

Inflammatory lung diseases or repeated mucosal injury, may result in chronic structural changes to the airways (Redington, 2001) The sequestration of drugs (e g. amines) in the lung tissue has been reported to be altered with lung injury and disease, such as inflammation, due to the changes in lung tissue composition (Audi et al., 1999; Pang et al., 1982).

Inflammatory lung diseases, such as asthma and chronic bronchitis, are associated with an impaired mucociliary clearance and hyperplasia of submucosal glands and goblet cells leading to a hypersecretion of mucus and obstruction of the airways (Lethem, 1993; Samet et al., 1994). As a consequence of the airway obstruction, a proximal shift in the airway deposition pattern of inhaled therapeutic aerosols is observed (Rubin, 1996).

There are conflicting results in the literature on the effect of inflammation and allergic reactions on the airway permeability. Some investigations state that the permeability from the air space into the systemic circulation is increased during lung inflammation

(Folkesson et al , 1991, Hogg, 1981, Howite et al 1989), whereas other investigators have demonstrated an unchanged or even decreased airway absorption explained by an instantaneous epithelial restitution in response to epithelial injury (Greiff et al , 2002, O'Byrne et al , 1984, Persson et al , 1997) An increased epithelial permeability of hydrophilic compounds i e terbutaline (Mw 225 Da), ^{99m}Tc-labeled diethylene triamine penta-acetate (^{99m}Tc-DTPA, Mw 492 Da), and ^{111m}In-labeled biotinylated DTPA (Mw 1215 Da) has been demonstrated in smokers as compared to non-smokers (Jones et al , 1980, Mason G.R. et al , 2001, Schmekel et al , 1991)

2.5.2 PARTICLE DEPOSITION

The respiratory tract can be considered as a filter that removes particles from the inspired air (Heyder et al , 2002) The effectiveness of the filter depends on particle properties (e g size, shape, density, and charge), respiratory tract morphology, and the breathing pattern (e g airflow rate and tidal volume) (Heyder et al., 2002) These parameters determine not only the quantity of particles that are deposited but also in what region of the respiratory tract the particles are deposited. As the cross-sectional area of the airways increases, the airflow rate rapidly decreases, and consequently the residence time of the particles in the lung increases from the large conducting airways towards the lung periphery (Schulz et al , 2000) The most important mechanisms of particle deposition in the respiratory tract are inertial impaction, sedimentation, and diffusion (Figure 5) Inertial impaction occurs predominantly in the extrathoracic airways and in the tracheobronchial tree, where the airflow velocity is high and rapid changes in airflow direction occurs (Schulz et al , 2000). Generally, particles with a diameter larger than 10 ^m are most likely deposited in the extrathoracic region, whereas 2- to 10-um particles are deposited in the tracheobronchial tree by inertial impaction (Schulz et al., 2000). A long residence time of the inspired air favors particle deposition by sedimentation and diffusion (Heyder et al., 2002) Sedimentation is of greatest importance in the small airways and alveoli and is most pronounced for particles with a diameter of 0.5-2 µm (Schulz et al., 2000). Ultrafine particles (<0.5 in diameter) are deposited mainly by diffusional transport in the small airways and lung parenchyma where there is a maximal residence time of the inspired air (Heyder et al., 2002). The relationship between particle size and total respiratory tract deposition has been demonstrated to be similar among species (Schlesinger, 1985).

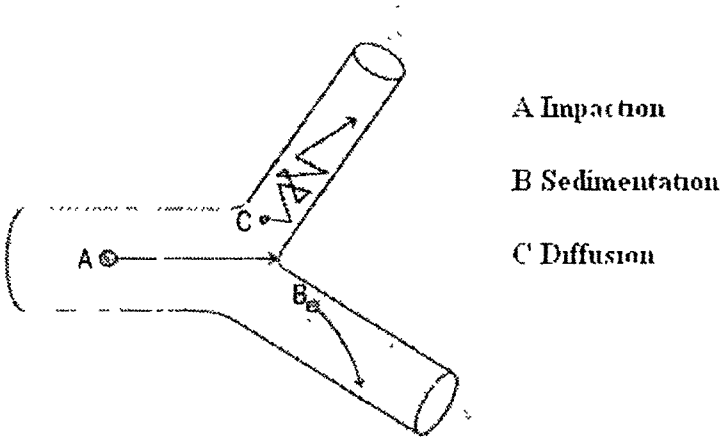


Figure 2.1. Mechanisms of particle deposition in the airways

Table 2.4: Factors that may affect the pulmonary absorption rate and bioavailability

Device and Formulation	Drug	Physiology
particle properties (size, density, shape, charge)	dissolution rate	breathing pattern
deposition pattern	solubility	blood flow
excipients	lipophilicity	airway morphology
concentration	molecular weight	surface area
osmolarity	charge	mucociliary clearance
viscosity	hydrogen bonding potential	lung surfactant
pH	aggregation/complex binding	alveolar macrophages
dose size/volume	conformation	epithelial permeability
	chemical stability	endothelial permeability
	enzymatic stability	transporter proteins
		enzymatic/metabolic activity
		disease
		tissue composition (drug sequestration)

2.5.3 DELIVERY DEVICES

Inhaled therapeutic aerosols are generated by different devices that aim to deliver an aerosol to the lower airways. Inhalation devices can be classified into 3 categories: pMDIs, DPIs, and nebulizer inhalers (Dolovich, 1999, Hickey, 1989). Aerosol generators are characterized (Gonda, 1988) using (1) the output (mass of drug delivered per unit time), (2) the distribution of the agent in different aerodynamic size fractions, and (3) intradevice and inter-device reproducibility of operation.

2.5.31 Propellant-Driven Metered-Dose Inhalers

pMDIs are the most frequently prescribed aerosol delivery system because they are effective and convenient for a large proportion of patients. The fundamental components of pMDIs are an actuator, a metering valve, and a pressurized container that holds the micronized drug suspension or solution, propellant, and surfactant. The high vapor pressure propellant supplies the energy for dispersion in these delivery systems. The limitations of these devices are (1) poor coordination between actuation and inhalation by some patients, (Epstein et al., 1979) and (2) the release of aerosol particles as large particles at a very high velocity (100 km/h). This results in a high oropharyngeal impaction of particles, with approximately 80% of the dose depositing in the oropharynx and only 10% in the pulmonary airways (Dolovich et al., 1981). To overcome the necessity for patient coordination usually required with these devices, breath-actuated pMDIs have been designed. These are essentially similar to conventional pMDIs, with the exception that the dose delivery is triggered by the patient's inspiratory flow (Gupta et al., 1991). The need of spacer and auxiliary devices for optimizing drug delivery from pMDIs has been reported (Sciarea et al., 1978; Dolovich, 1997). Attaching a spacer to the pMDI mouthpiece ensures that the emitted droplets become smaller and have reduced velocity before they are inhaled. The spacer acts as a holding chamber in which the large particles are filtered off, resulting in a reduction of the dose to the patient and impaction losses on the posterior wall of the oropharynx. Consequently, the dose deposited in the oropharynx by such devices is smaller, but the dose delivered to the pulmonary region is the same as or higher than that of a pMDI without a spacer (Newman et al., 1981).

2.5.32 Dry Powder Inhalers

DPIs are the most recent development in respiratory therapy The majority of these devices are breath-activated inhalers that rely on the patient's inspiratory flow to deaggregate and deliver the drug for inhalation, thereby eliminating the requirement of inhalation coordination inherent in pMDI use However, with DPIs there is the need to generate at least moderate inspiratory flow in order to accomplish effective drug delivery The drug in a DPI is in the form of a finely milled powder in large aggregates, either alone or in combination with some carrier substance, commonly lactose (Byron et al , 1990) Most of the particles are initially too large to be carried into the lower airways, but the turbulent air stream created in the inhaler during inhalation causes the aggregates to break up into primary particles sufficiently small to be carried into the lower airways Therefore, the deposition pattern depends on the inspiratory flow generated by the patient. A very low inspiratory flow is likely to move the dose from the inhaler into the patient's mouth, with very low deposition in the pulmonary airways Shear, turbulence, and mechanical intervention may be used to aid in the dispersion of aerosols from dry powders (Malem et al , 1981) Dry powder generation is often hindered by aggregation of the small particles, (Brown, 1987) which is in turn exacerbated by the hygroscopic nature of the drug (Ferron, 1977) and its electrostatic charge. The reduction of powder hygroscopicity and electronic charge may enhance the future prospects of aerosol powder formulation

2.5.33 Nebulizers

Nebulization is of increasing interest because it offers opportunities for novel techniques as well as roviding a potential means of administration for aqueous formulations of biomolecules (Atkins et al., 1994). The most frequently used methods of nebulization are the air jet and ultrasonic devices (Mercer, 1973). The air jet nebulizer produces a stream of high air velocity that causes liquid to spray as a mist. Ultrasonic nebulizers utilize high frequencies to convert liquid into a fine mist (Gupta, 1991) A wide range of droplet size distributions are produced by both types of generators, depending on the brand, the operating conditions, and the composition of the liquid being nebulized (Mercer, 1973; Phipps, 1987, Lewis, 1984). Nebulizers produce smaller droplets than do pMDIs, and these smaller droplets penetrate more easily to the small airways (Gupta, 1991)

2.5.4 BIOLOGICAL MODELS FOR ASSESSMENT OF PULMONARY DRUG ABSORPTION

Several models are available for preclinical investigations of pulmonary drug absorption and deposition The complexity of the models range from permeability screening experiments in cell culture models to in vivo pharmacokinetic analyses in animals The design of the experiments comprises both selection of the most relevant biological model for the specific issue, and the selection of a drug delivery system that is appropriate for the amount of test material available and that can selectively deposit a defined dose of the drug to the intended lung region Combinations of in vitro and in vivo models are needed to elucidate the mechanisms, rate, and extent of absorption, as well as the distribution, metabolism and elimination of a drug after pulmonary administration

2.5.41 In vivo animal models

In vivo pharmacokinetic experiments in animals provide data on the fate of a drug and its metabolites in the body by assessment of the drug concentration in plasma or tissues

In the absence of a significant amount of human absorption data, accurate in vivo pharmacokinetic investigations in animals are important to establish in vitro-in vivo relationships For determination of the pulmonary absorption rate and bioavailability, plasma is sampled at predetermined time points after pulmonary drug administration and analyzed for drug content (Adjei et al , 1992; Krondahl et al , 2002, Taljanski et al., 1997).

An intravenous dose may be administered as reference For investigations of the retention of the drug in the lung tissue, or first-pass pulmonary uptake, or both, drug concentrations in lung tissue are assessed (Brown et al , 1983, Drew et al , 1981, Jendbro et al , 2001). The applied pulmonary administration procedure should be carefully selected to deliver the dose with high precision regarding both dose quantity and deposition pattern. A disadvantage of the in vivo models is that the animals often need to be anesthetized during drug administration to the lungs and at blood sampling. The effect of anesthesia on physiological functions should thus be considered in the design of the experiments For instance, the use of volatile anesthetics has been demonstrated to increase the alveolar epithelial permeability (ChangLai et al , 1999; Wollmer et al., 1990) and to destabilize surfactant (Evander et al., 1987; Wollmer et al., 1990) Anesthesia may also impair the mucociliary clearance (Patrick et al., 1977).

2.5.42 Isolated and perfused lungs

By the use of isolated and perfused lung models, lung-specific pharmacokinetic events can be investigated without the contribution of systemic distribution, metabolism, and elimination In these models, the structural and cellular integrity of the lung tissue, the permeability barriers, interaction between different cell types, and biochemical activity are maintained (Mehendale et al , 1981) Procedures for lung perfusion have been described for rats, guinea pigs (Ryrfeldt et al , 1978), and rabbits (Anderson et al , 1974) Compared to in vivo models, the isolated and perfused lung models provides certain advantages, such as careful control of the ventilation and perfusion of the lung, facilitated administration of drugs to the airway lumen or vascular circulation, easy sampling of perfusate and lavage fluid, as well as easy determination of mass-balance. Hence, the design of the experiments can be adapted to specifically address issues regarding absorption, tissue sequestration, and metabolism The main drawback of the lung models is that the limited viability of the preparation (about 5 hours) (Bassett et al , 1992, Fisher et al , 1980) prevents investigations of slow pharmacokinetic processes Isolated and perfused lung models have successfully been applied to investigate drug dissolution and absorption (Niven et al , 1988; Tronde et al , 2002), mechanisms of absorption (Sakagami et al., 2002a), disposition (Audi et al , 1998, Ryrfeldt et al., 1989), and metabolism (Dollery et al , 1976, Gillespie et al , 1985, Longmore, 1982, Tronde et al , 2002)

2.5.43 Cell culture models

The inaccessibility and heterogeneous composition of the airway epithelium makes it difficult to mechanistically evaluate pulmonary cellular integrity and physiological functions. For investigations of drug transport mechanisms, precise dosing and sampling, as well as defined local drug concentration and surface area of exposure, are important parameters that need to be controllable and reproducible Therefore, a variety of airway and alveolar epithelial cell culture models of animal and human origin have been established as in vitro absorption models (Elbert et al., 1999; Foster et al., 2000; Morimoto et al , 1993; Winton et al , 1998; Yamashita et al., 1996) The models include both cell lines (airway) and primary cell cultures (airway and alveolar). The primary cell cultures more closely resemble the native epithelia, but are less reproducible and more time-consuming to work with compared to the cell lines, which make them less suitable for permeability screening purposes. Two immortalized human bronchial epithelial cell

lines, the Calu-3 and 16HBE14o-, have been suggested as suitable models to investigate the airway epithelial barrier function (i e , tight junction properties) (Wan et al , 2000, Winton et al , 1998) The Calu-3, adenocarcinoma epithelial cells of serous origin from the bronchial airways, comprise a mixed phenotype of ciliated and secretory cells (Mathias et al , 2002) and form tight, polarized and well differentiated cell monolayers with apical microvilli in an-liquid interface culture (Foster et al , 2000, Mathias et al , 2002) The cell line has recently been applied in some experiments investigating airway drug transport mechanisms (Borchard et al , 2002, Florea et al , 2001; Hamilton et al , 2001a, Hamilton et al , 2001b, Mathias et al , 2002, Pezron et al , 2002). At present there is, to our knowledge, no characterized epithelial cell line available for investigations of the alveolar barrier functions Instead, alveolar type II cells, isolated from normal human lungs, rats and rabbits, in primary cultures have been demonstrated to differentiate into type-I-like cells and to form tight epithelial barriers morphologically similar to the in vivo alveolar epithelium (Elbert et al , 1999, Matsukawa et al , 1997, Shen et al , 1997). These models have been used for several investigations of alveolar transport (Dodoo A.N et al., 2000a, Elbert et al , 1999, Matsukawa et al., 1996; Morimoto et al , 1993; Saha et al , 1994, Shen et al , 1997)

2.6 DRY POWDER INHALERS

DPI technology is rapidly expanding to address a broadening therapeutic need as well as market opportunity Characteristics of the ideal DPI system will include most or all of the following attributes

- Simple and comfortable to use,
- Compact and economical to produce,
- Highly reproducible fine-particle dosing.
- Reproducible emitted dose,
- Physically and chemically stable powder;
- Minimal extrapulmonary loss of drug, with low oropharyngeal deposition, low device retention, and low exhaled loss;
- Multidose system;

- Powder protected from external environment and can be used in all climates and protected from moist exhaled air,
- Overdose protection, and
- Indicate number of doses delivered and/or remaining

2.6.1 FACTORS INFLUENCING DPI FORMULATION DESIGN

2.6.11 Physical properties of powders:

Optimization and control of flow and dispersion (deaggregation) characteristics of the formulation is of critical importance in development of DPIs These properties are governed by adhesive forces between particles, including Van der Waals forces, electrostatic forces and the surface tension of absorbed liquid layers (Hinds, 1982).

The forces are influenced by several fundamental physicochemical properties including particle density and size distribution, particle morphology (shape, habit, surface texture) and surface composition (including absorbed moisture) (Hickey et al, 1990). Inter-particle forces that influence flow and dispersion properties of inhalation powders are particularly dominant in the micronized or microcrystalline powders (particles smaller than 5 μm). Hickey et al. (1994) reviewed the factors influencing the dispersion of dry powders as aerosols. Several cohesive and adhesive forces are exerted on particle characteristics such as size, shape, rugosity and crystalline form and powder characteristics such as packing density and equilibrium moisture content. Buckton, 1997 has reviewed particle surface characteristics and several studies have measured the adhesion forces in inhalation powders (Podczek, 1996) Peart and co-workers (1996) measured electrostatic charge interactions from Turbuhalers and drug powders and the results suggest that the inhaler itself and the deaggregation mechanisms influenced the charging phenomena. Electrostatic effects in DPIs have been extensively studies by Mazumder et al (1998) and powder flow properties have also been studied (Dawson et al, 1998).

Further particle characteristics have been studied such as the crystallization and amorphous content of inhalation powders (Phillips et al, 1996; Buckton, 1998) and the measurement of their surface properties by inverse gas chromatography (Thielmann et al, 2002) and computer aided image analysis to plot a Facet Signature (Kaye, 1996).

2.6.12 Drug carrier:

Optimization and control of particle-particle and particle-inhaler interactions is of critical importance in the development of efficient DPIs A paradoxical situation exists in powder formulations – drug particles should be less than 5 μm aerodynamic diameter to ensure efficient lung deposition, but should also exhibit acceptable flow properties required for accurate dose metering Thus, micronized powders are often blended with 'coarse' inert carriers e g lactose, glucose or alternatively pelletized as loose agglomerates to improve powder flow Blending the drug with a carrier has a number of potential advantages, such as increasing the bulk of the formulation An additional benefit that may be gained by the use of a carrier such as lactose is the taste/sensation on inhaling, which can assure the patient that a dose has been delivered Many studies have examined the properties of lactose particles and their interaction with drug particles as part of the process to optimize DPI performance (Patel, 2000) Lactose and other sugars have been studied and used and modification of these materials may allow further formulation optimization Modifications to the lactose surface have been proposed that would improve the surface characteristics (reduce the rugosity) of the material Ganderton (1992) claims that the reducing rugosity increases the percentage of respirable particles in the conventional powder inhalers. Zeng and coworkers (1999) has found that the addition of fine lactose particles (mass median diameter 6 96 μm) increased the fine particle fraction of Salbutamol sulphate from a powder formulation delivered by a Rotahaler. They suggested that this may be because of the fine particles occupy possible drug binding sites on the larger lactose particles. Lucas et al. (1998) demonstrated a similar performance modifying effect with a model protein, albumin and a high-dose agglomerated preparation of Nedocromil Sodium. Other studies have looked at similar effects of lactose size fractions and agglomerates (Boerefijn et al, 1998) The properties of lactose such as particle size and surface morphology (Clark et al, 2000) had a profound effect on the fine particle fraction of the generated aerosol Other excipients, like sugars, have also been studies to establish their preformulation characteristics Braun et al. (1996) used two grades each of α-lactose monohydrate and dextrose monohydrate with Disodium Cromoglycate and generated aerosols using a unit-dose device, the Microhaler (Pearce, 1989).

2.6.13 Particle engineering:

The most commonly used method for improving the flowability, fillability, and dispersibility of small cohesive particles is blending the drug with excipient particles, most commonly lactose, of considerably larger particle size The objective of the mixing process is to produce an ordered powder in which the small particles attach themselves to the surface of larger "carrier" particles The challenge is to ensure that the force of adhesion between the drug and carrier is strong enough to withstand segregation during blending and product storage and weak enough to allow separation of the drug particles from the carrier surface on aerosolization (Moren, 1990, Zanen et al , 1992) The final product performance of a powder blend in a DPI is ultimately dependent on the individual drug and carrier properties as well on the process by which they are blended (Kassem et al., 1989, Ganderton et al , 1992) Small changes in carrier morphology can result in significant variations in the dose received by a patient (Kassem, 1990) Micronization has been used for the past 50 years to produce small particles for inhalation therapy However, only in recent years have batch-to-batch reproducibility and stability problems been associated with the technique Stability issues typically derive from changes to the varying quantities of amorphous material that are produced by the micronizing process on the surface of the resulting particles (Ward et al , 1995). In addition, micronization can cause decomposition of some materials (Rogerson, 1996)

The issues associated with micronization are forcing many companies to investigate alternative methods of producing small particles. Spray drying, a process typically used in the production of coarser (up to 500 μm) food, pharmaceutical, and industrial powders, can also be used to prepare microparticulate powders for DPIs (Venthoye, 1997; Vidgren et al , 1987, Chawla, 1993; Chawla, 1994)

In one study (Chawla et al, 1994), spray-dried Salbutamol Sulfate was seen to perform as well as micronized material Vidgren et al. (1988) have shown that spray-dried particles of Disodium Cromoglycate have better (at least in vitro) aerodynamic properties (a higher fraction of dose in a smaller size range) than micronized material Other techniques such as pelletization (Bell, 1975, Wetterlin, 1987), Lyophilization and supercritical fluid technology (York and co workers, 1996) were also employed.

2.6.2 FILLING AND PACKAGING

The greatest challenge faced in developing packaging systems for dry powders relate to maintaining dispersibility in packaging, which can be affected by compression and electrical charge. Compression of the drug powder, which can be a consequence of excessive handling, can result in an unintended increase in drug concentration. The small drug particles are also vulnerable to alteration in electrical charge, which can result from the motion of particles, against both themselves and the packaging equipment, and from the unintended absorption of water by the drug powder. Drug powders can be packaged either in unit dose or in reservoir systems, each of which has certain advantages (Table 2.5).

2.6.3 RELEASE AND STABILITY TESTING PARAMETERS

Various dry powder attributes are assessed at release and on stability. These include physical characteristics such as powder appearance, content uniformity, delivered dose uniformity, and particle size distribution. Chemical attributes that may be assessed include drug content, purity, and identity as well as the water content of a powder. Dry powders may also undergo microscopic evaluation for foreign particulate matter, unusual agglomeration, and particle size. Microbial limits also should be examined, including the total aerobic, yeast, and mold counts. The presence of specific pathogens should be ruled out. The dry powders also may be dissolved to test for pH level. In addition, certain compendial requirements for content and delivered-dose uniformity should also be measured.

The USP and European Pharmacopoeia (EP) propose that the total aerobic count not exceed 100 CFU/g, that the total yeast count and mold count not exceed 10 CFU/g, and that no specific pathogens be detectable. Specifications for the other attributes should be based on the intended use and the historical performance of the product. As with other dosage forms, specifications must be met throughout the intended shelf life of the product.

Table 2.5: Primary packaging for DPI drug formulation

DOSING SYSTEM	ADVANTAGES	DISADVANTAGES
UNIT-DOSE	Simpler, cheaper device, less prone tomalfunction Protects powder up to the time when it willbe delivered to the patient as an aerosol	Patient must handle and load individual unit-dose packages into the device before dosingDose titration is limited to dose-quantity available from drug supplier (similar to pills)
MULTIDOSE	More convenient for the patient	The device becomes more complex because means to load multiple doses are requiredAlso, means for displaying number of doses left arerequiredDevice may be more prone to malfunction owing tojamming or improper indexing
RESERVOIR	Multidose and dose titration easy to implement, Convenient	Powder not generally well protected after reservoir is opened, physical and/or chemical characteristics may deteriorate with time, Biological contamination may be an issue, Metering of the dose is carried out by the device, which increases the device complexity, metering often is not adequately controlled because the physical characteristics of the powder are often unknown at the time of dosing

The International Conference on Harmonization (ICH) has identified stability requirements for room temperature storage and testing intervals. It recommends that dry powders be stored at 25°C and 60% RH for real-time conditions, at 40°C and 75% RH for accelerated conditions, and 30°C and 60% RH if significant change is observed at accelerated conditions. The ICH recommends testing samples every 3 months for the first year, every 6 months for the second year, and yearly thereafter. In addition to these requirements, the FDA suggests a storage condition at 25°C and 75% RH if significant change is observed at the accelerated condition. Six-month data would be required at the time of the New Drug Application (NDA) submission, and the study must cover 1 year.

2.7 LIPOSOMES

Liposomes are microscopic vesicles composed of phospholipids bilayers surrounding aqueous compartments as described by Bangham et al (1965). Presently, liposomes are one of the most extensively investigated systems for controlled delivery of drug to the respiratory tract. Liposomes offer various benefits for drug administration to the respiratory tract like

- Have relatively low toxicity, as they are prepared with phospholipids endogenous to the lung as surfactants
- Can be prepared with wide range of size, ranging from 20 nm to 1 mm
- Can incorporate both hydrophilic and lipophilic drugs
- Their ability to solubilize poorly water soluble substances, facilitate their nebulization
- They serve as biodegradable pulmonary reservoir with prolonged pulmonary residence times. They decrease mucociliary clearance due to their surface viscosity.
- Can be exploited as a targeting device to individual cell population with in the lung, specifically to the infected or immunologically impaired alveolar macrophages and the lung epithelium.

However, their use also encounters certain limitations such as

- Only potent drugs can be used since a patient can endure only a modest amount of particulate and lipid burden in the lung per dose.
- Poor storage stability of the liposomes, but this can be overcome to a large extent by lyophilization.

- Liposomes preparations are difficult to administer except by nebulization but stability upon nebulization is a concern
- The cost factor is a serious constraint

2.7.1 COMPOSITION OF LIPOSOMES

2.7.11 Phospholipids

Liposomes can be formed from many different phospholipids (Tyrrell et al, 1976) Phosphatidyl choline (PC), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) are widely used natural phospholipids. Other synthetic phospholipids which are used to prepare liposomes include, dipalmitoyl phosphatidyl choline (DPPC), dimyristoyl phosphatidylcholine (DMPC), dioleoyl phosphatidylcholine (DOPC), distearyl phosphatidyl choline (DSPC) etc (New 1990 and Lasic 1998)

2.7.12 Steroids

Cholesterol does not, by itself, form bilayers structures, but it can be incorporated into phospholipid membranes in high concentrations. Being an amphipathic molecule, cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface, and aliphatic chain aligned parallel to the acyl chains in the center of bilayers (Betageri 1993)

2.7.13 Antioxidant

All the liposomes undergo auto-oxidation even in the presence of trace amounts of oxygen and this process is accelerated by elevated temperature, light, metal ions and some solutes Incorporation of α-tocopherol into liposomes has been reported (Hunt and Tsang, 1981) to prolong the characteristic induction phase of auto oxidation Addition of 0.1 mole% of α-tocopherol roughly doubles the induction time relative to liposomes containing no α-tocopherol. it was established that α-tocopherol suppresses the oxidation of PC liposomes by scavenging both, the aqueous radicals attacking from outside of the membrane and lipophilic radicals within the membranes (Etsuo, 1989)

2.7.14 Other Non-structural Components

Charge inducers such as Diacyl glycerol, Stearylamine and dicetyl phosphate have been incorporated into liposomes so as to impart either a negative or a positive surface

charge to these structures Many single chain surfactants of number of single and double chain lipids having fluorocarbon chains and also compounds like quaternary ammonium salts and dialkyl phosphates (Ringdorf et al , 1988) can also be used to form liposomes

2.7.2 TYPE OF LIPOSOMES

Different types of liposomes can be prepared and are classified by the size and structure A multilamellar vesicle (MLVs) consists of numerous concentric bilayers separated by aqueous spaces and range up to 15 μm in diameter Vesicles consisting of a single bilayer encompassing a central aqueous compartment are referred to as small unilamellar vesicles (SUVs), which range upto 100 nm in diameter and large unilamellar vesicles (LUVs) ranging from 100 to 500 nm in diameter

2.7.3 METHODS OF LIPOSOMES PREPARATION

There are at least fourteen Major published methods for making liposomes (Ostro MJ and Cullis, 1989, Martin, 1990) The seven, most commonly employed methods are, Lipid film hydration method (Bangham et al , 1965) Ethanol injection method (Batzri and Korn, 1973) , Ether infusion method (Deamer and Bangham, 1976) , Detergent dialysis method (Kagawa and Racker) , French press method (Barenholz et al , 1976) , Rehydration-dehydration techniques (Shew and Deamer, 1985) , Reverse phase evaporation method (Szoka and Papahadjopoules, 1978)

2.7.4 LIPOSOMES AS A NASAL DELIVERY SYSTEM

The relationship between the rigidity of liposomal membrane and the absorption of insulin after nasal administration of liposomes modified with the enhancer containing Insulin was studies in rabbits (Muramatsu et al , 1999)) The rigid liposomal membranes render liposomes stable and thus protect the Insulin from enzymatic degradation Soyabean derived sterol (SS) or its stearyl glucoside (SG) was employed as an enhancer. Dipalmitodyl phosphatidylcholine (DPPC) liposomes modified with SG had increased the fluidity of the hydrophobic group of the liposome bilayer compared with the liposomes modified with Chol (CH) or SS, however, the fluidity of the polar group of the liposome bilayer was decreased at 37°C. This indicates that the fluidity of the hydrophobic group of the liposomal bilayer is responsible for the increase of liposomal leakage and the stability of the liposomes. When insulin was administered nasally as solution in rabbits, no hypoglycaemic effect was evident The

administration of insulin contained in DPPC/SG liposomes with high fluidity lead to a high glucose reduction over a period of 8 hours however, DPPC/SS and DPPC/CH liposomes with low fluidity caused low glucose reductions These results indicate that liposomes modified with SG can be a promising carrier for nasal delivery of drugs

The loading and leakage characteristics of the desmopressin-containing liposomes and the effect of liposomes on the nasal mucosa permeation and anti-diuresis of desmopressin were investigated (Law et al , 2001) Higher loading efficiency of desmopressin for positively charged liposomes than negatively charged liposomes was obtained; and neutral liposomes resulted in a similar loading efficiency as that of positively charged liposomes Greater leakage of desmopressin from negatively charged liposomes than from positively charged and neutral liposomes was shown The increase of permeability of desmopressin through the nasal mucosa indicated positively charged liposomes>negatively charged liposomes>solution It was suggested that the enhanced contact time of positively charged liposomes with negatively charged nasal mucosa led to a high local desmopressin concentration on the penetration site to promote an effective penetration of desmopressin through the nasal mucosa The desmopressin anti-diuresis result after intranasal administration was in good agreement with the permeability result in the order of positively charged liposomes>negatively charged liposomes>solution One of the mechanisms for the explanation of the best result on the enhancement of anti-diuresis for positively charged liposomes may be the bioadhesive effect of the liposomes on the negatively charged nasal mucosa.

The nasal administration of large protein molecules, such as G-CSF and erythropoietin, can also be achieved via the nasal routes However, not surprisingly, the quantities delivered will be less than those achieved for molecules of lower molecular weight, such as calcitonin and insulin

2.7.5 LIPOSOMES AS PULMONARY DRUG DELIVERY SYSTEM

One of the perceived benefits of liposomes as a drug carrier is based on their ability to alter favorably the pharmacokinetic profile of the encapsulated species and thus provide selective and prolonged pharmacological effects at the site of administration Administration of liposomes to the respiratory tract is particularly attractive because of the accessibility of the lung as a target organ, the compatibility of liposomes and

lung surfactant components, and the need for sustained local therapy following inhalation Consequently, numerous studies have explored the effect of liposomal encapsulation on the distribution and fate of compounds administered directly to the lung by either intratracheal instillation or inhalation

The range of drugs that has been investigated for pulmonary delivery via liposomes parallels the general interest in liposomes as drug carriers for anticancer and antibiotic agents, peptides and enzymes (Table 5 6)

Table 2.6: Potential Therapeutic Applications of Liposomes Based Systems in Lung Delivery

DRUG CLASS	DRUG	REFERENCES
Anticancer agents	Cytosine arabinoside	Juliano and McCullough, 1980
Antibiotics	Pentamidine Enviroxime Benzylpenicillin	Gupta and Hickey. 1991 Jobe and Ikegami, 1987, Garcon et al , 1989) Mihalko et al , 1988
Oxygen scavengers	Superoxide dismutase Glutathione	Padmanabhan et al., 1985 Jurima-Romet and Shek, 1991
Bronchodilators	Metaproterenol Bitolterol mesylate Salbutamol	McCalden, 1990 McGurk et al , 1987 Jurima-Romet et al , 1990
Steroids	Hydrocortisone-21-octanoate Stanozolol	Jurima-Romet et al., 1990 McGurk et al , 1986
Antiallergics	Sodium cromoglycate Oxytocin	Abra et al , 1984 Mihalko et al., 1988

2.7.51 Formulation factors Influencing Drug Release

The rate of drug release from liposomes is a critical factor in determining the duration of activity The pulmonary absorption of liposomal carboxyfluorescein has been demonstrated to be lipid dose dependent with higher amount of phospholipid showing

higher rate of absorption (Woolfrey et al., 1988). Similarly negatively charged vesicles were absorbed twice as fast in comparision to neutral vesicles. Composition and size of liposomes also affect the absorption profile (Abra et al., 1990). The presence of cholesterol and phospholipids with saturated hydrocarbon chains increased the drug residence time within the lung. Amphipathic compounds like α-tocopherol improve the retention of liposomally entrapped drug in the rat lung following intratracheal instillation (Suntres and Shek, 1994). The spreading of liposomes at the alveolar surface increases by the inclusion of phosphatidyl glycerol, potentially decreasing vesicle stability and accelerated drug release (Oyarzun et al., 1980).

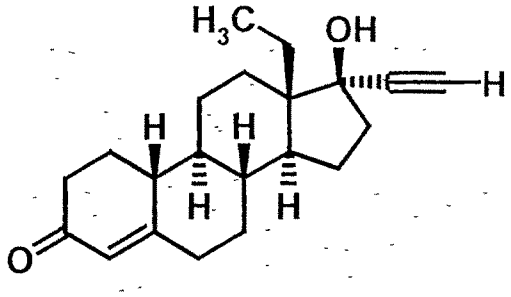
2.7.52 Performance of Liposomes during Aerosolization:

If liposomes are intended to act as multiunit slow release reservoirs in the lung or as target-selective pulmonary drug carriers, they must retain their integrity during aerosolization. It has been hypothetized that shear forces during the aerosolization process may transiently disrupt phospholipid bilayers leading to loss of encapsulated drug (Gilbert et al., 1988). During aerosolization of liposomes the drug loss may occur along with the change in average vesicle size (Taylor et al., 1990). The leakage of drug from the liposomes reaches maximum as the average liposome size approaches the droplet size (Niven et al., 1991). The loss in encapsulated drug can also be attributed to the physical shearing of lipid (Gilbert et al., 1988).

2.8 DRUG PROFILES

2.8.1 LEVONORGESTREL (BAN, USAN, rINN):

Levonorgestrelum, D-Norgestrel, Wy-5104 (-) 13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one



Levonorgestrel

2.8.11 Physical Properties

Ph Eur A white or almost white crystalline powder Practically insoluble in water, slightly soluble in alcohol; sparingly soluble in dichloromethane Protect from light

USP 25 A white or practically white, odourless powder, practically insoluble in water, slightly soluble in alcohol; soluble in chloroform Protect from light.

2.8.12 Mechanism of action

Like other Progesterones, LN inserts its contraceptive effect by thickening cervical mucus and also inhibiting ovulation

2.8.13 Pharmacokinetics

Levonorgestrel is rapidly and almost completely absorbed after administration by mouth, and undergoes little first pass hepatic metabolism It is highly bound to plasma proteins: 42-62% to sex hormone binding globulin and 30-56% to albumin. The proportion bound to sex hormone binding globulin is higher when it is given with an estrogen LN and Norgestrel are metabolized in the liver to sulfate and glucuronide conjugates, which are excreted in the urine and to a lesser extent in the faeces.

2.8.14 Side effects

Irregular bleeding, mastalgia, headaches, amenorrhoea, breast cancer, cervical neoplasia, and rarely hepatocellular carcinoma. Pregnancy, unexplained vaginal bleeding, and breast cancer—preclude use of the method

2.8.15 Uses

Levonorgestrel (LN) has been used for many years both alone (in low doses) in the progestogen only pill (POP) and in combination with estrogen in COC preparations for contraception in females. Implants are also available

2.8.16 Methods of Estimation

UV Spectrophotometry

Levonorgestrel can be analyzed in pharmaceuticals at absorbance maxima of 240 nm in 85% ethanol/methanol in water. Conversion to active glyoxalyl derivative, which produces absorption maxima at about 244 nm and 318 nm was described. Other methods are also been described

Colorimetric analysis:

Reaction with INH reagent leads to development of coloured species with absorption maxima at about 380 nm. This method is also stability indicating

Other reagents used are Dinitrophenylhydrazine, Blue Tetrazolium, 2,6-Di-tert-butyl-p-cresol

Fluorometric Analysis

Sulfuric acid-induced fluorescence with an emission λmax of 520nm with an excitation λmax of 460 nm was used. In other method sodium hydroxide and potassium tert-butoxide were used to produce fluorogens. These methods were suitable for measurement up to nanogram levels

Titrimetric Analysis:

LN can be titrated in silver nitrate in tetrahydrofuran with sodium hydroxide

Polarographic Analysis:

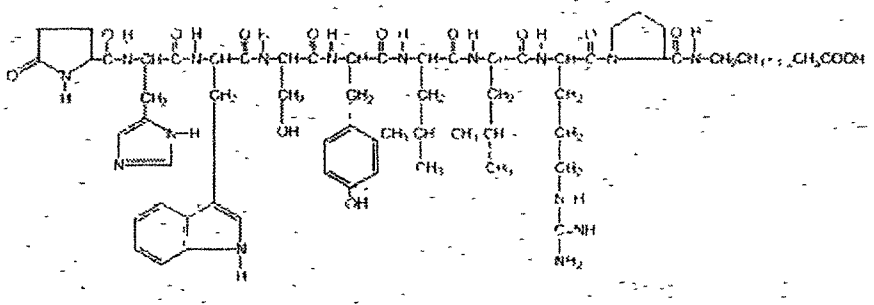
LN can be estimated by polarography in alkaline isopropanol

Chromatographic Analysis:

Various chromatographic techniques including Thin-Layer Chromatography, Gas chromatography, Column Chromatography have been used for estimation of LN

2.8.2 LEUPROLIDE ACETATE

Leuprolide acetate is a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone (GnRH or LH-RH). The analog possesses greater potency than the natural hormone. The chemical name is 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt) with the following structural formula.



2.8.21 Physical Properties

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol

2.8.22 Mechanism of Action

Leuprolide is a synthetic analog of gonadotropin releasing hormone (GnRH) acting mainly on the pituitary gland in humans. Continuous treatment produces initial stimulation (3-4 days), then suppression of hormones to castrate or post-menopausal levels (2-4 months). In males, the net effect is a reduction of testosterone to castration levels within two to four weeks. In females, both ovarian estrogen and androgen synthesis are inhibited.

2.8.23 Pharmacokinetics

LEU is not active when given orally but is well absorbed following subcutaneous or intramuscular injection. In vitro binding to human plasma proteins ranged from 43%

to 49% In healthy male volunteers, a 1 mg bolus of leuprolide administered intravenously revealed that the mean systemic clearance was 7 6 L/h, with a terminal elimination half-life of approximately 3 hours based on a two-compartment model

2.8.24 Side Effects

Adults LEU may cause nausea, vomiting, hot flashes, night sweats, bone pain, swelling of feet and ankles, headache, or difficulty urinating the first few days as your body adjusts to the medication LEU may cause reduced sexual desire Sometimes it may also cause rapid heartbeat, chest pain, breathing difficulties, fever, chills, painful urination, testicular or prostate pain (men), persistent irritation at the injection site Children - LEU may cause general pain, acne, irritation at injection site, vaginal inflammation or discharge (females) The medication may rarely cause swelling of hands or feet, weight gain, headache, nervousness, drowsiness, nausea, vomiting, gingivitis, or trouble swallowing

2.8.25 Uses

LEU is a synthetic hormone When it is used, the body stops producing testosterone hormones in males and estrogen hormones in females When the medication is stopped, hormone levels return to normal Leuprolide is used in the treatment of prostate cancer in men, early puberty in children, and anemia (due to uterine fibroid tumors) in women LEU may also be used for ovarian or breast cancer in women

2.8.26 Methods for Estimation (Akwete and Hsu, 1993)

Ultraviolet Spectroscopy

UV spectra of Leuprolide acetate gave absorption maxima at 240, 280, and 289 nm with molar absorptivities 14,360, 6662, and 6536, in 0 1 N NaOH, respectively, The absorption maxima in 0 1% HCl were at 278 and 288 nm with molar absorptiviteis of 6347 and 4713, respectively.

Bioanalysis

The methods are based on receptor binding techniques (Marshall and Odell, 1975). LH release (Vale and Grant, 1974)

Chromatographic Analysis:

HPLC

Chromatographic purity method using BioSil ODS-5S column and Nucleosil 10 column was reported The methods are sensitive for detection of impurities at 0 1-0 2% especially for those eluting prior to an immediately following LPU

TLC

The TLC procedure was carried out using a solvent system consisting of chloroform methanol 32% acetic acid (60:45 20) and sprayed with 1% potassium iodide/1% soluble starch solution on Silica gel GF_{254} plates

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