OPTIMIZATION OF SYSTEMIC NASAL DELIVERY OF PROGESTERONE USING POLYACRYLIC ACID BASED GELS

THESIS

Submitted to

The Tamilnadu Dr. M.G.R. Medical University,

Guindy, Chennai-600032, Tamilnadu, India

As a partial fulfillment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

(Faculty of Pharmacy)

Submitted by

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CERTIFICATE

This is to certify that the thesis titled 'Optimization of Systemic Nasal Delivery of Progesterone using Polyacrylic Acid Based Gels' submitted to The Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai-600032, Tamilnadu, India as a partial fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY (Faculty of Pharmacy) was completely carried out independently by Mrs. Grace Rathnam, M.Pharm., during 2005-2009 under my guidance and supervision. The work embodies research observations, which were performed with utmost care and precaution. This work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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(DR. R. ILAVARASAN)

DECLARATION

I hereby declare that the thesis titled 'Optimization of Systemic Nasal Delivery of Progesterone using Polyacrylic Acid Based Gels' submitted to The Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai, Tamilnadu, India as a partial fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY (Faculty of Pharmacy) was completely carried out by me during 2005-2009 at C.L.Baid Metha College of Pharmacy, Thorapakkam, Chennai, under the guidance and supervision of Dr. N.Narayanan M.Pharm., Ph.D., Joint Director of Medical Education (Pharmacy), Directorate of Medical Education, Kilpauk, Chennai-600010. This work is original and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

GRACE RATHNAM

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ABBREVIATIONS USED

AUC	:	area under curve
BKC	:	benzalkonium chloride
BDDS	:	bioadhesive drug delivery systems
β-CD	:	beta cyclodextrin
BP	:	bioadhesive polymer
CD	:	cyclodextrin
CDs	:	cyclodextrins
C_{max}	:	maximum concentration
D	:	diffusion coefficient
DSC	:	differential scanning calorimetry
EDTA	:	ethylenediamine tetra acetic acid
FTIR	:	fourier transform infrared spectroscopy
g	:	grams
h	:	hours
HPβ-CD	:	hydroxypropyl beta cyclodextrin
IN	:	intra nasal
J_{ss}	:	steady state flux
K_p	:	permeation coefficient
m.wt	:	molecular weight
µg	:	microgram
MA	:	mucoadhesive
ng	:	nanogram
PAA	:	polyacrylic acid
PBS	:	phosphate buffer saline
PXRD	:	powder X-ray diffraction
SEM	:	scanning electron microscopy
SFD	:	spray freeze dried
SBEβ-CD	:	sulfobutyl ether beta cyclodextrin
t_{max}	:	time to maximum concentration
UV	:	ultraviolet
IgA	:	immunoglobulin A
DNA	:	deoxyribonucleic acid
ICH	:	International Conference on Harmonisation
LDH	:	lactate dehydrogenase
ALP	:	alkaline phosphatase
AMP	:	cyclic adenosine monophosphate
SSBG	:	sex steroid binding globulin
CBG	:	corticosteroid binding globulin
CYP1A	:	Cytochrome P450, family 1, subfamily A
CYP2A	:	Cytochrome P450, family 2, subfamily A
CYP2E	:	Cytochrome P450, family 2, subfamily E

CONTENTS

In the past one decade, the use of nasal cavity as a route for drug delivery has been an area of great interest to the pharmaceutical industry, especially for systemic acting drugs that are difficult to deliver via routes other than injection. Certain drugs[1] are delivered to the nasal cavity because this is their intended site of action; these are administered as nasal drops or sprays for a local effect. Such drugs in clinical use include decongestants, antibiotics and mucolytics.

The nasal cavity can also be exploited as a route of entry into the systemic circulation, either because the absorption profile of the drug is appropriate to its clinical application, e.g. a fast onset of action for the treatment of migraine with sumatriptan and for those compounds which cannot be given orally because they are destroyed in the gastrointestinal fluids, metabolized in the wall of the gastrointestinal tract or undergo extensive biotransformation by the liver during their first passage around the circulation. This route is also used for some unconventional drug molecules, like those included in the "new biotherapeutics" such as peptides, polypeptides and DNA which in the absence of an alternative non–invasive route of delivery, are usually given by injection. These molecules are unlikely to realize their full clinical potential unless the patient can easily and conveniently self–administer the drug and hence this goal has led to the investigation of various transmucosal routes for drug delivery including the buccal, pulmonary, rectal and nasal routes.

1. STRUCTURE AND PHYSIOLOGY OF THE NASAL CAVITY

The nasal cavity[2] extends 12 -14 cm from the nostrils to the nasopharynx and is divided in two, laterally, by the nasal septum (Fig.1)

1.1 Physiological structure

The nasal vestibule has the smallest cross–sectional area in the respiratory tract (approximately 0.3 cm^2 on each side) and extends from the entrance of the nostrils, which are guarded by vibrissae (hairs) to the anterior ends of the inferior turbinates. The lining of the vestibule changes from skin at the entrance, to squamous epithelium and then to ciliated columnar secretory epithelium at the turbinates. The area from the anterior ends of the turbinates to the anterior portion of the nasopharynx constitutes the main nasal passage. Here the walls of the nasal

septum are folded to create the turbinates and meatuses (air spaces). The narrow width of the meatueses (0.5–1.0 mm) helps to maintain contact between the air stream and the epithelium lining the nasal passage. The olfactory region of the nose is located towards the roof of the nasal cavity and is lined with non–ciliated neuro–epithelium. The remainder of the main nasal passage is lined with pseudo stratified columnar secretory epithelium, consisting of basal cells, goblet cells and columnar cells, which may be ciliated or unciliated. Microvilli are found on the columnar cells which increase the surface area available for absorption.

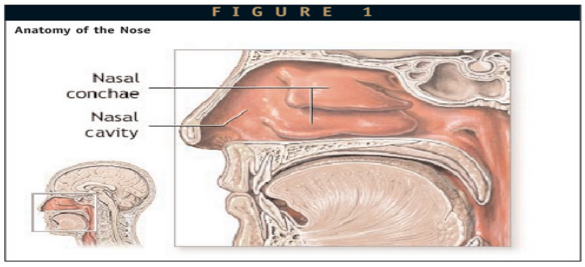


Figure 1. Structure of the nasal cavity

The epithelial cells in the nasal vestibule are stratified, squamous and keratinized with sebaceous glands. Pseudostratified columnar epithelial cells (Fig.1a) interspersed with goblet cells, seromucus ducts and the openings of subepithelial seromucus glands cover the respiratory region (the turbinates).

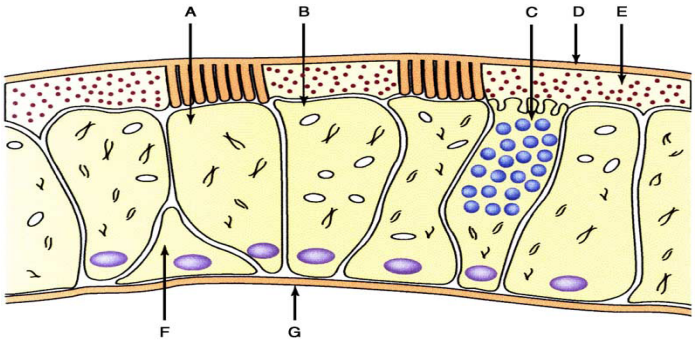


Fig. 1a Cell types of the nasal epithelium showing ciliated cell (A), non-ciliated cell (B), goblet cells (C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane

The nasal mucosa is highly vascular, superficial and deep layers of arterioles supply the lamina propria and between the venules and capillaries there are numerous sinuses or venous lakes which are linked to erectile tissue, particularly in the middle and inferior turbinates, which enable the airway to widen or narrow. This automatically controlled vasculature of the nasal tissue, in combination with its rich supply of secretory cells, is of importance in the modification of inspired air.

The submucosal glands[3] which secrete the greater quantity of nasal mucus, comprise both mucus cells, secreting the mucus gels and serous cells producing a watery fluid from the nose to the nasopharynx. A mucus layer[4], resulting from nasal and lacrimal gland secretions as well as plasma transudate, is present on the nasal passage epithelium. The pH of secretions ranges from 5.5 to 6.5 and 5.0 to 6.7 in adults and children, respectively. The mucus consists of an outer viscous layer of mucin (gel) and a watery (sol) layer located along the mucosal surface. Glycoproteins, particularly mucin, are responsible for the gel-like appearance of the mucus. Lysozymes, enzymes and immunoglobulins in addition to other proteins may also be found in the mucus. The production[5] of IgA by both the adenoid tissue and the nasal mucosa contribute significantly to the immune protection against inhaled bacteria and viruses. Approximately 3% of the mucus consists of these proteins while the remainder is made of 90–95% water and 1–2% salt. Each day about 1500 to 2000 ml of mucus is produced and covered with a new mucus layer approximately every 10 min.

1.2 Mucociliary clearance (MCC)

Mucociliary clearance contributes to the defense mechanisms of the body by entrapping potentially hazardous substances, such as dust and microorganisms, within the viscoelastic mucus blanket lining the nasal passages (Fig.2). The mucus is then propelled by the claw–like tips of the cilia, beating in a co–ordinated manner within the periciliary fluid, towards the nasopharynx where the mucus and any entrapped particulates is either swallowed or expectorated. In the nasal cavity mucus is moved by the cilia at a rate of approximately 10 cm min^{-1} and clearance of the bulk of the mucus from the nose to the nasopharynx occurs over10-20 min.

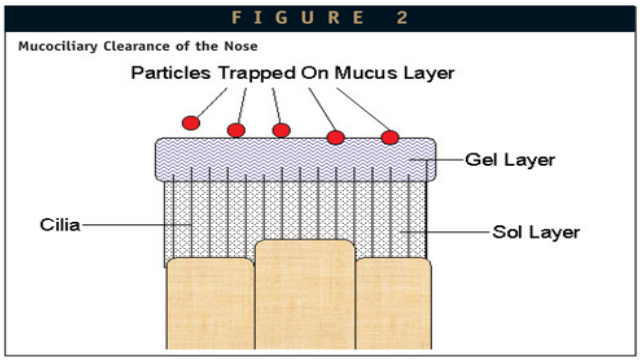


Figure 2. Mucocicliary clearance of the nose

Efficient mucociliary clearance depends on a successful relationship between the

- Cilia
- Periciliary fluid
- Mucus

Changes in any of the above three parameters can alter the characteristics of clearance and those patients with compromised clearance, such as those affected with conditions such as cystic fibrosis or ciliary dyskinesia appear to be more susceptible to chronic respiratory infections.

1.2.1 Cilia

Cilia are hair like protrusions on the epithelial cell surface. There are approximately 300 cilia per cell and each cilia is 5-10 µm long and 0.1-0.3 µm wide and beats at a frequency[6] of about 20 Hz. Although there is a lot of inter-individual differences in MCC rate, this has been estimated at 6 mm/min.

1.2.2 Periciliary Fluid

Periciliary fluid is a watery, ionic solution, maintained by transepithelial ion transport that provides an environment within which the cilia are able to beat

1.2.3 Mucus

The nasal epithelium like many other epithelia in contact with the external environment is covered by a number of important physiological roles.

- It entraps substance entering the nasal cavity and participates in the removal of particulates via mucociliary clearance; this process protects the underlying mucosa.
- The capacity of mucus to hold water permits the humidification of the inspired air and also aids heat transfer, since water is a better conductor of heat than air.

Respiratory mucins are high molecular weight glycoproteins, long, linear, apparently flexible threads which vary in length from 0.5 to 10 μm. They are composed of sub-units (monomers) each about 500 nm in length. The monomer is comprised of a protein "backbone" core, with alternating oligosaccharide-rich regions (approximately 100 nm in length) and "naked" regions of folded protein stabilized by disulfide bonds.

Mucus possesses both solid-like (elastic) and liquid-like (viscous) attributes simultaneously and is therefore termed as viscoelastic gel. The viscoelastic properties arise from the non-covalent interactions (entanglements) between the predominantly anionic mucin molecules, although weak hydrogen–bonding and ligand–like attractions between protein regions of adjacent molecules may also play a role. Gel properties are effected by:

- mucin size
- mucin polydispersity
- the type of mucins present

It is widely held that mucus should possess specific rheological properties for clearance from the airway to occur. Any alteration in mucus rheology that compromise clearance can predispose the individual to airway disease and infection. Xenobiotic-metabolizing enzymes are present in the nasal mucosa. In animals, these

enzymes are found in greater quantities in the olfactory epithelium compared to the respiratory epithelium of the nose. In human, this distinction remains unclear owing to difficulty in obtaining olfactory epithelium. In the respiratory mucosa of human, the concentration of cytochrome P450 enzymes is 25 pmol/mg of microsomal protein. Isoforms of the P450 enzyme that have been identified in human thus far include CYP1A, CYP2A, and CYP2E. Carboxylesterases, glutathione S-transferases, and rhodanese enzymes have also been detected in the human nasal mucosa.

2. PHYSIOLOGICAL FACTORS AFFECTING NASAL BIOAVALIABILITY

The main factors affecting the absorption of drugs from the nasal cavity are physiological in origin.

2.1 Area

The total surface area of both nasal cavities is about 160 cm^2 . The area available for absorption is enhanced by:

- The convolutions of the turbinates
- The microvilli present on the surface of the ciliated and unciliated cells of the respiratory epithelium.

2.2 Blood supply

The nasal mucosa is highly vascular. This property facilitates its physiological role in heat exchange and also potentially drug absorption. The rich blood supply means that drugs absorbed via the nasal route have a rapid onset of action, which can be exploited for therapeutic gain.

2.3 Contact time and mucociliary clearance

The length of time the drug is in contact with the absorbing tissue will influence how much drug crosses the mucosa. In the nasal cavity this is influenced

by the rate at which the drug is cleared from the absorption site by mucociliary clearance and by metabolism. While the mucociliary clearance of deposited particles is advantageous if the particles are likely to be hazardous, the clearance of a deposited drug is clearly not beneficial if it prevents absorption. The normal half time of clearance in human is about 20 min. Therefore strategies to increase the nasal bioavailability of drugs that are poorly absorbed from the nasal mucosa can be aimed either at increasing the nasal membrane permeability or increasing the contact time for absorption by decreasing the mucociliary clearance rate[7]. The clearance of a drug formulation from the nasal cavity is influenced by its site of deposition. The site of deposition in the nasal cavity profoundly affects the rate of mucociliary clearance of a drug moiety.

- Particles deposited on ciliated regions (for example, the turbinates) of the mucosa are immediately available for clearance.
- Particles deposited on non– ciliated regions (for example, the anterior of the nasal cavity) will move more slowly (such particles will land on mucus which is being dragged through the cavity from nasal absorption).
- Particles which deposit on the nasopharyngeal regions will be swallowed immediately and are therefore not available for nasal absorption.

2.4 Disease

The rate of mucociliary clearance can be affected by the pathophysiological condition of the nasal cavity and this will also affect the rate of clearance of administered drug. Such conditions include rhinitis, the common cold, hay fever, sinusitis, asthma, nasal polyposis, Sjogren's and Kartagener's syndromes. In addition, environmental factors such as humidity, temperature and pollution can also affect the rate of nasal clearance.

2.5 Enzymatic activity

The nasal secretions possess a wide range of enzymes and enzymes are also present in the epithelium of the nasal cavity. It has been suggested that the low

bioavailability of some nasally administered peptides result from their enzymatic degradation in the nasal cavity.

2.6 Mucus barrier

In addition to its involvement in mucociliary clearance, mucus can also affect drug delivery by interacting directly with nasally administered drugs by:

- Acting as a physical barrier to drug diffusion, and /or
- Binding to drugs.

3. Transport routes and mechanisms

The organization and architecture of epithelial mucosa restrict drug permeation across the epithelial barrier to two main routes

- The paracellular route : between adjacent epithelial via the mechanisms of passive diffusion or solvent drag
- The transcellular route: across the epithelia cells, which can occur by any of the mechanisms namely, passive diffusion, carrier mediated and endocytic processes.

4. FORMULATION FACTORS AFFECTING NASAL BIOAVAILABILITY

4.1 Physicochemical factors associated with the drug

For nasal drug delivery, it has been suggested that mechanisms of absorption exist, based on the physicochemical properties of the drug.

- A fast rate, which is dependent on the lipophilicity of the drug
- A slower rate, which is dependent on molecular weight.

Thus, lipophilic drugs such as a propanalol, progesterone, 17β-oestradiol, naloxone and testosterone are absorbed rapidly and completely from the nasal cavity. In

contrast, their oral bioavailabilities range from 25% for propanalol to less than 1% for progesterone.

4.1.1 Concentration

In the majority of cases the absorption of the drug of interest across the nasal mucosa is via passive diffusion (paracellular or transcellular). As such, the rate of absorption will be affected by the concentration of drug in solution at the absorbing membrane

4.1.2 Buffer Capacity

Nasal formulations are generally administered in small volumes ranging from 25 to 200 μL with 100 μL being the most common dose volume. Hence, nasal secretions may alter the pH of the administrated dose. This can affect the concentration of un-ionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH *in situ*.

4.1.3 Osmolarity

Drug absorption can be affected by tonicity of the formulation. Shrinkage of epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity. Low pH has a similar effect as that of a hypertonic solution

4.1.4 Gelling Agents or Gel-Forming Carriers

Increasing solution viscosity may provide a means of prolonging the therapeutic effect of nasal preparations. A drug carrier such as hydroxypropyl cellulose is effective for improving the absorption of low molecular weight drugs but does not produce the same effect for high molecular weight peptides. Use of a combination of carriers is often recommended from a safety (nasal irritancy) point of view.

4.1.5 Solubilizers

Aqueous solubility of drug is always a limitation for nasal drug delivery in solution. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol (saturated polyglycolyzed C_8- C_{10} glyceride) can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as hydroxy propyl beta cyclodextrin that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers.

4.1.6 Preservatives

Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. Mercury containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in nasal systems[8].

4.1.7 Antioxidants

A small quantity of antioxidants may be required to prevent drug oxidation. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxy toluene and tocopherol. Usually, antioxidants do not affect drug absorption or cause nasal irritation. Chemical/physical interaction of antioxidants and preservatives with drugs, excipients, manufacturing equipment and packaging components should be considered as part of the formulation development program.

4.1.8 Humectants

Many allergic and chronic diseases are often connected with crusts and drying of mucous membrane. Certain preservatives/ antioxidants among other excipients are also likely to cause nasal irritation especially when used in high quantities. Adequate intranasal moisture is essential for preventing dehydration. Therefore, humectants can be added especially in gel-based nasal products.

Humectants avoid nasal irritation and are not likely to affect drug absorption. Common examples include glycerin, sorbitol and mannitol.

4.1.9 Chemical Form

The chemical form of a drug can be important in determining absorption. For example, conversion of the drug into a salt or ester form can alter its absorption. Itwas observed that *in situ* nasal absorption of carboxylic acid esters of l-tyrosine was significantly greater than that of l-tyrosine[9].

4.1.10 Polymorphism

Polymorphism is known to affect the dissolution rate and solubility of drugs and thus their absorption through biological membranes[10]. It is therefore advisable to study the polymorphic stability and purity of drugs for nasal powders and/or suspensions.

4.1.11. Molecular Weight

A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 Daltons. Absorption decreases significantly if the molecular weight is greater than 1000 Daltons except with the use of absorption enhancers.

4.1.12 Particle Size

It has been reported that particle sizes of nasal powders greater than 10 µm are deposited in the nasal cavity. Particles that are 2 to 10 µm can be retained in the lungs and particles of less than 1 µm are exhaled.

4.1.13 Solubility & Dissolution Rate

Drug solubility and dissolution rates are important factors in determining nasal absorption from powders and suspensions. The particles deposited in the nasal

cavity need to be dissolved prior to absorption. If a drug remains as particles or is cleared away, no absorption occurs

5. ADVANTAGES AND DISADVANTAGES OF NASAL DRUG DELIVERY[1]

5.1 Advantages

The general advantages of the nasal cavity for drug delivery include:

5.1.1 Large surface area

The nasal cavity offers a relatively large surface area (approximately 160 cm^2) for drug absorption.

5.1.2 Rich blood supply

The highly vascular surface of the nasal mucosa ensures rapid adsorption and onset of action, as well as the maintenance of 'sink' conditions

5.1.3 Low metabolic activity

The metabolic activity of the nasal cavity towards peptides and proteins is less than that of the GI tract, making this route an attractive alternative to the oral delivery of these moieties. In contrast to the oral route, this route avoids degradation in the intestinal wall or the liver, prior to the drug reaching the systemic circulation.

5.1.4 Accessibility

The nasal cavity offers a readily accessible surface for drug delivery, obviating the need for complex delivery devices to enable the drug to reach its absorption site. Thus devices for nasal delivery are simpler in design than those intended to deliver drugs to, for instance, the alveolar region of the lung and are non-invasive requiring the simple instillation of drops or sprays.

5.1.5 Ease of administration

Nasal devices, such as metered-dose nasal sprays are simple for the patient to use and might be expected to be more acceptable to the patient than the use of pessaries or suppositories for the intravaginal and rectal delivery routes respectively.

5.1.6 Intestinal alternative

The nasal route may become a useful alternative to the intestinal route for drug absorption in situations where use of the gastrointestinal route is unfeasible. Examples include:

- Patients with nausea and vomiting.
- Patients with swallowing difficulties and children.
- Drugs that are unstable in the gastrointestinal fluids.
- Drugs that undergo extensive first-pass effect in the gut wall or liver.

5.2 Disadvantages

The disadvantages of the nasal cavity for drug delivery include

5.2.1 Mucociliary clearance

Mucociliary clearance reduces the retention time of drugs within the nasal cavity and thus the opportunity for absorption.

5.2.2 Mucus barrier

Drug diffusion may be limited by the physical barrier of the mucus layer and the binding of drugs to mucin.

5.2.3 Metabolic activity

While the metabolic activity of the nasal cavity towards peptides and proteins is less than that of the GI tract, it should be recognized that the nasal

mucosa and secretions do have the ability to degrade drugs and that measures may be necessary to overcome this.

5.2.4 Limited to potent molecules

For drugs of a high molecular weight (which are thus poorly absorbed), the route is limited only to potent drug molecules, typically those with effective plasma concentrations in the ng/ml $-^{1}$ (or lower) range.

5.2.5 Lack of reproducibility

The major problem associated with intranasal delivery is the question of whether it can provide reliable absorption. Diseases such as the common cold and hay fever are recognized to alter the condition of the nose, either increasing or decreasing mucociliary clearance, or altering the permeability of the absorbing mucosa.

5.2.6 Adverse reactions

Locally irritating or sensitizing drugs must be used with caution in this route. Nasal epithelia and in particular the cilia are highly sensitive and fragile

6. INCREASING THE PERMEABILITY OF THE NASAL EPITHELIUM

6.1 Absorption enhancers

A variety of molecules have been studied in an effort to find a compound which is able to increase the permeability of the nasal epithelium without causing harm. The mechanisms of absorption promotion proposed for the different compounds are numerous and it is likely that more than one mechanism is involved.

6.1.1 Alteration of mucus layer

Agents that decrease the viscoelasticity of mucus, for example anionic and cationic surfactants and bile salts, have been shown to increase absorption.

6.1.2 Alteration of tight junctions

Substances that sequester extracellular calcium ions, which are required to maintain tight junction integrity for example EDTA, bile salts, will cause the tight junctions to open. Thus, paracellular route becomes leakier, permitting increased absorption of substances that use this route.

6.1.3 Reversed micelle formulation

The differing adjuvant activities of various bile salt species relate to their differing capacities to penetrate and self–associate as reverse micelles within the membrane. In reverse micelles, the hydrophilic surfaces of the molecules face inward and the hydrophobic surfaces face outward from the lipid environment. The formulation of reverse micelles within the cell membranes may create an aqueous pore, through which drug moieties can pass.

6.1.4 Extraction by co–micellization

Solubilization of cell membrane lipids, for example the removal of cholesterol by surfactants such as bile salts and polyoxyethylene ethers increases permeability of nasal epithelium.

Cyclodextrins (CDs) are cyclic oligosaccharides showing a polar outer surface and a non polar interior cavity able to include lipophilic guest molecules. This composition allows the formation of inclusion complexes with lipophilic compounds. The mechanisms of action of cyclodextrins include solubilization and stabilization of peptides and proteins, prevention of aggregation and proteolytic degradation and the opening of tight epithelial junctions. CDs are also able to enhance hydrophilic drug absorption by the extraction of specific lipids from biological membranes leading to an increase in membrane permeability and fluidity. The extent of protection and absorption enhancement seems to depend strongly on the nature of of the drug used as well as the CD used.

The following characteristics should be considered in choosing an absorption enhancer[7]

a. The enhancer should be pharmacologically inert at the concentration used.
b. It should be nontoxic, nonirritating and nonallergenic.
c. If the enhancer has any effect it should be completely reversible.
d. Only small amount of the enhancer should be required.
e. It should be compatible with the drug and formulation adjuvants.
f. It should be able to remain in contact with the nasal mucosa long enough to achieve a maximal effect.
g. It should be inexpensive
h. It should not have offensive odor

Classification and examples of nasal drug delivery optimizers

1. Cyclodextrins: The most studied types are α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, methyl cyclodextrin and hydroxypropyl β-cyclodextrin. Only β-cyclodextrin is a compendial substance and is considered for a GRAS (generally recognized as safe) status. This class of absorption optimizers has a lot of potential as it shows the promise for a good absorption enhancement without significant toxicity potential.

2. Fusidic acid derivatives: These primarily consist of sodium salts of fusidic acid. Sodium taurodihydrofusidate (STDHF) is a derivative of fusidic acid.

3. Phosphatidylcholines: They are surface active amphiphilic compounds produced in biological membranes.

4. Microspheres and liposomes: The majority of the work involving these is limited to the delivery of macromolecules.

5. Bile salts and surfactants: Bile salts are the most widely used surfactants for nasal absorption optimization. At relatively low concentrations, 10-20 mmol they are able to improve the absorption of peptides and other

drugs. Commonly used bile salts are sodium cholate, sodium deoxycholate, sodium glycocholate, sodium glycocholate. Possible mechanisms by which these compounds exert their effects include increasing permeability of the membrane structure, inhibition of proteolytic enzymes, and formation of aqueous pore-type transport pathways and solubilization of drugs in aqueous vehicle.

6.2 Increasing contact time at absorption site

Prolonging the contact time between the drug and its absorption site is likely to increase the bioavailability of the drug. Since drugs may be cleared from the nasal cavity by mucociliary clearance, swallowing and / or by metabolism, the inhibition or avoidance of these clearance mechanisms should result in increased absorption. Strategies being investigated include following

6.2.1 Modifying the site of deposition

Although it is assumed that the principal sites for the systemic absorption of intranasally delivered drugs, these are also an area of high mucociliary clearance, especially in the highly ciliated middle and posterior region[11]. Thus drug deposited in the anterior region of the nasal cavity may be expected to clear less rapidly and have a greater opportunity to be adsorbed.

6.2.2 Mucoadhesion as a strategy to improve systemic drug delivery via the nasal route

Owing to the fact that intimate contact between a delivery device and the absorbing cell layer will improve both effectiveness and efficiency of the product, many researches have recently focused on developing bioadhesive drug delivery system (BDDS)[12].The term bioadhesive refers to either adhesion between two biological materials or adhesion between some biological material (including cells, cellular secretions, mucus, extracellular matrix, and so on) and an artificial substrate. Bioadhesives (sometimes also termed mucoadhesives) adhere to biological

substrates such as mucus or tissue. Bioadhesives are proposed to influence drug bioavailability by:

- Decreasing the rate of clearance from the absorption site thereby increasing the time available for absorption.
- Increasing the local drug concentration at the site of adhesion/absorption.
- Protecting the drug from dilution and possible degradation by nasal secretions.

7. Bioadhesion and Mucoadhesion

The process involved in the formation of bioadhesive bonds has been described in three steps:

(1) wetting and swelling of polymer to permit intimate contact with biological tissue,

(2) interpenetration of bioadhesive polymer (BP) chains and entanglement of polymer and mucin chain and

(3) formation of weak chemical bonds in the case of hydrogels.

It has been determined that several polymer characteristics are required to obtain adhesion

(1) sufficient quantities of hydrogen – bonding chemical groups (-OH and –COOH),
(2) anionic surface charges,
(3) high molecular weight,
(4) high chain flexibility, and
(5) surface tensions that will induce spreading into the mucus layer.

These characteristics favor the formulation of bonds that are either mechanical or chemical in nature.

7.1 Theories of Bioadhesion

Several theories have been developed to describe the processes involved in the formation of bioadhesive bonds. These theories have been used as guidelines in engineering possible BDDSs. Some are based on the formation of chemical bonds, while others focus on chemical interactions.

7.1.1 The electronic theory

This is based on an assumption that the bioadhesive material and the glycoprotein mucin network have different electronic structures. On this assumption, when two materials come in contact with each other, electron transfer will occur in an attempt to balance Fermi levels, causing the formation of a double layer of electrical charge at the interface. The bioadhesive force is believed to be due to attractive forces across this electrical double layer.

7.1.2 The Adsorption Theory

This theory states that the bioadhesive bond formed between an adhesive substrate and intestinal mucosa is due to 'Van der waals' interactions, hydrogen bonds and related forces

7.1.3 The wetting theory

The ability of bioadhesive polymers or mucus to spread and develop intimate contact with their corresponding substrate is one important factor for bond formation. The wetting theory, which has been used predominantly in regards to liquid adhesives, uses interfacial tensions to predict spreading and in turn, adhesion.

7.1.4 The diffusion Theory

The diffusion theory suggests that interpenetration of BP chains and mucus polymer chains produce semi permanent adhesive bonds and bond strength is believed to increase with the depth of penetration of the polymer chains. Penetration

of BP chains into the mucus network, and *vice versa*, is dependent on concentration gradients and diffusion coefficients.

7.1.5 The fracture theory

The most useful theory for studying bioadhesive through tensile experiments has been the fracture theory, which analyzes the forces required to separate two surfaces after adhesion. The maximum tensile strength produced during detachment, can be determined by dividing the maximum force of detachment F_m, by the total surface area (A_o) involved in the adhesive interaction,

$$\sigma_m = F_m / A_o$$

8. Polymers used in nasal delivery

The most common polymers used for intranasal delivery are carbopol, cellulose compounds, sodium hyaluronate, polycarbophil, starch, dextran and chitosan[13]. These may be delivered to the nasal passage via several systems including liquid solutions, gel, powder and microspheres. Several factors are important for the effectiveness of a bioadhesive material[14]. Both the chemical class of a polymer and its polymer molecular mass are important to its bioadhesive characteristics. Each class has a critical mass value where adhesiveness is at an optimum level. Also, penetration via the bioadhesive chain is extremely important to its binding ability; therefore, extensive or dense cross-linking is not desired. Similar to having an optimum mass for bioadhesive properties, the polymer concentration at the targeted interface may also affect binding ability. Other considerations for bioadhesive efficacy include the level of hydration and the pH of the environment. Too much hydration will cause excessive swelling of the polymer and subsequently decrease adhesion. Also, changes in the nasal pH may alter the bioadhesive binding effectiveness via alteration of charge potential and interactions. The other most[15] predominant polymer used for bioadhesion is chitosan. It is biodegradable and has low toxicity. Increased[16] absorption obtained with chitosan is the result of both bioadhesion and alteration of the tight junctions. The starch microsphere system was studied for its impact on nasal absorption of desmopressin in sheep[17]. Compared to

the control nasal solution, the starch intranasal system provided significantly shorter time to maximum concentration (T_{max}), higher maximum concentration (C_{max}), increased area under the curve (AUC) and improved bioavailability. The starch microsphere system is considered relatively safe for drug delivery. Other bioadhesive materials have been studied as well. Carboxymethylcellulose was determined to be a safe system for short-term intranasal use. Also, it was found effective for providing sustained release of apomorphine in rabbits[18]. A combination of polymethacrylic acid and polyethylene glycol has been evaluated for its effects on intranasal budesonide delivery in rabbits[19]. Another system, gelatin microspheres, provided desirable absorption of intranasal salmon calcitonin. Additionally, gelatin microspheres[20] demonstrated usefulness for nasal administration of levodopa. An initial fast release was observed followed by a slower release. Additional systems for increasing residence time and absorption following intranasal administration are emulsion formulations[21], liposome[22] and nanoparticles[23].

9. DELIVERY SYSTEMS

The selection of delivery system depends upon the drug being used[7].

Nasal drops: nasal drops are one of the most simple and convenient systems developed. Disadvantage of this system is the lack of dose precision.

Nasal Sprays: Both solution and suspension formulations can be formulated into nasal metered dose pumps and actuators, a nasal spray can deliver an exact dose.

Nasal gels: Nasal gels are high viscosity thickened solutions or suspensions. The advantages are lack of nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing of the formulation, reduction of irritation by using smoothing/emollient excipients.

Nasal powders: The dosage form may be developed if solution and suspension cannot be used due to lack of stability. The advantages are the stability of

the formulation. However, the suitability of the powder formulation is dependent on the aerodynamic properties and nasal irritancy of the active drug and/or excipients.

9.1 Delivery Device

The delivery device[14] for a particular formulation should be based on several factors, including accuracy and dose reproducibility, cost, simplicity of use for the patient, physiochemical characteristics of the drug and chosen dosage form, and protection from microbial contamination. Metered-dose systems are considered the best for dose accuracy and reproducibility. Current delivery devices for liquid and powder intranasal formulations are the following

- Liquid formulation: Instillation catheter, dropper, unit-dose containers, squeeze bottle, pump spray, airless and preservative-free sprays, compressed air nebulizers and metered-dose inhalers.
- Powder formulations: Insufflators, monodose inhalers, multidose inhalers and pressurized metered-dose inhalers.

The delivery device and drug formulation should also be compatible with each other to prevent leaching and absorption, which could pose a risk for toxicity and may affect dose accuracy, respectively. Additionally, a device should be chosen on its ability to store the formulation appropriately without compromising stability.

Table 1: Important Considerations for Intranasal Dosage Forms

Sl.No.	Dosage form	Characteristics
1	Nasal drops	Best for solutions Metering of doses may be inaccurate or inconsistent
2	Solution spray	Metered-dose actuator devices are available Accurate dosing is feasible with spray devices
3	Suspension spray	Metered-dose actuator devices are available Orifice of the actuator may need modification for proper delivery Common dosage form for local effects
4	Powders	May cause irritation and leave gritty sensation Greater expense for drug development
5	Gels	Metered-dose devices are available Postnasal drip and anterior leakage are decreased Used for systemic and local drug delivery
6	Emulsions/ointments	Best for local action Patient acceptability is low Difficult to provide accurate dose

The drug chosen for this work is progesterone because of its limited oral effectiveness, resulting from a high first-pass effect and a brief profile of the drug, progesterone is presented.

PROGESTERONE

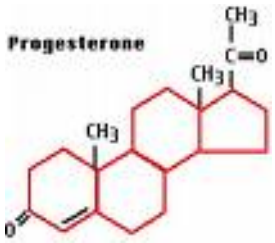
Chemical name : Pregn 4-en-3,20 dione.

Molecular formula : $C_{21}H_{30}O_2$

Molecular weight : 314.5

Description : Colorless crystal or white to almost white crystalline powder.

Molecular structure



Progesterone is the most important progestin in humans. In addition to having important hormonal effects, it serves as a precursor to the estrogens, androgens and adrenocortical steroids. It is synthesized in the ovary, testis and adrenal from circulating cholesterol. Large amounts are also synthesized and released by the placenta during pregnancy. In the ovary, progesterone is produced primarily by the corpus luteum. Normal males appear to secrete 1-5 mg of progesterone daily, resulting in plasma levels of 0.03 µg/dL. The level is only slightly higher in the female during the follicular phase of the cycle, when only a few milligrams of progesterone per day are secreted. During the luteal phase, plasma levels range from 0.5µg/dL to more that 2µg/dL

Progestins are endogenous hormones that produce numerous physiological actions[24]. In women, these include development effects, neuroendocrine action

involved in the control of ovulation, the cyclical preparation of the reproductive tract for fertilization and implantation and major actions on mineral, carbohydrate, protein and lipid metabolism. Progesterone is secreted by the ovary mainly from the corpus luteum during the second half of the menstrual cycle. Secretion actually begins just before ovulation from the follicle that is destined to release the ovum. The formation of progesterone from steroid precursors occurs in the ovary, testis, adrenal cortex and placenta. The stimulatory effect of leutinizing hormone (LH) on progesterone synthesis and secretion by the corpus luteum is mediated by a membrane-bound receptor linked to a G protein-coupled signal transduction pathway that increases the synthesis of cyclic AMP by stimulation of adenyl cyclase.

Physiological and Pharmacological actions

***Neuroendocrine actions*:** Progesterone produced in the luteal phase of the cycle has several physiological effects. It decreases the frequency of the hypothalamic pulse generator and increases the amplitude of LH pulses released from the pituitary.

***Reproductive tract*:** Progesterone released during the luteal phase of the cycle decreases estrogen-driven endometrial proliferation and leads to the development of a secretory endometrium. The abrupt decline in the release of progesterone from the corpus luteum at the end of the cycle is the main determinant of the onset of menstruation. Progesterone also influences the endocervical glands and the abundant watery secretion of the estrogen stimulated structures is changed to a scant viscid material.

***Mammary glands*:** During pregnancy and to a minor degree during the luteal phase of the cycle, progesterone acting with estrogen brings about a proliferation of the acini of the mammary gland. Toward the end of pregnancy, the acini fill with secretions and the vasculature of the gland is notably increased; however only after the level of estrogen and progesterone decrease at parturition does lactation begin.

CNS effect: If the body temperature is carefully measured each day throughout the normal menstrual cycle, an increase of about 1.0 ^{o} F may be noted at midcycle; this correlates with ovulation.

***Metabolic effects*:** Progestins have numerous metabolic actions. Progesterone itself increases basal insulin levels and the rise in insulin after carbohydrate ingestion but it does not normally cause a change in glucose tolerance. Progesterone stimulates lipoprotein lipase activity and seems to enhance fat deposition.

Mechanism of action

The mechanism of action of progesterone is similar to that of other steroidal hormones. Progesterone enters the cell and bind to progesterone receptors that are distributed between the nucleus and the cytoplasmic domains. The ligand-receptor complex binds to a response element to activate gene transcription. The response element for progesterone appears to be similar to the corticosteroid response element, and the specificity of the response depends upon which receptor is present in the cell as well as upon other cell-specific receptor coregulators and interacting transcription factors. The progesterone receptor complex forms a dimer before binding to DNA. There is a single progesterone receptor (PR) gene that produces two isoforms of the progesterone receptor, PR-A and PR-B. The first 164 amino acids of PR-B are missing from PR-A; this occurs by use of two distinct estrogen-dependent promoters in the PR gene. Both PRs have a molecular domain structure common to all members of the nuclear receptor subfamily. Since the ligand-binding domain is identical in both isoforms of PR there is no difference in ligand binding as is seen with the two isoforms of ER.

Absorption, Fate and Excretion

Progesterone itself undergoes rapid first pass metabolism; histologically, this low oral bioavailability limited the administration of the natural hormone to intramuscular injections in oil or to vaginal suppositories and is an impetus to develop 17-α-hydroprogesterone analogs such as medroxyprogesterone acetate and

19-nor steroids for oral use[25]. More recently high dose preparations of micronized progesterone containing small particles (10 μm) suspended in oil and packaged in gelatin capsules have been developed. Although the absolute bioavailability of these preparations is low, effacious plasma levels nevertheless may be obtained. Progesterone is also available in oil solution for injection, as a vaginal gel and as a slow release intrauterine device for contraception. In the plasma, progesterone is bound by albumin and CBG but is not appreciably bound to SSBG. The elimination half life is approximately 5 min, and the hormone is metabolized primarily in the liver to hydroxylated metabolites and their sulfate and glucoronide conjugates, which are eliminated in the urine. Volume of distribution is found to be 17 -29 liter and plasma protein binding is 95-98 %. Presystemic metabolism is noted to be 75% and metabolism is reported to be hepatic. Renal excretion accounts for extensive elimination and plasma half life is 3-6 min.

Therapeutic uses

Progesterone is the main hormone of the corpus luteum and the placenta. It acts on the endometrium by converting the proliferative phase induced by oestrogen to a secondary phase and preparing the uterus to receive the fertilized ovum. It also suppresses uterine mobility and is responsible for the further development of the breasts.

Progesterone has a catabolic action and a slight rise in basal body temperature occurs during the secondary phase of menstruation[26]. Progesterone and other progestins are used in the treatment of functional uterine bleeding. They are also used, often with oestrogen in menstrual disorders and have been given in the treatment of neoplasms of the breast and endometrium. It is also used in habitual or threatened abortion. Progesterone is used for luteal support in *In Vitro* Fertilization (IVF) programs. This is given as i.m injection or as a vaginal gel. Progesterone can be used diagnostically to test for estrogen secretion and for responsiveness of the endometrium. After administration of progesterone for 5 to 7 days to amenorrheic women, withdrawal bleeding will occur if the endometrium had been stimulated by

endogenous estrogens. Combination of estrogens and progestins also can be used to test endometrial responsiveness in patients with amenorrhoea.

Dose[27]

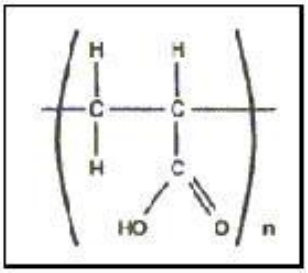
For primary and secondary amenorrhea, 5 to 10 mg a day for 6 to 8 days; in combination with a estrogen for functional uterine bleeding and menorrhagia, 5 to 10 mg a day for the last 6 days of the cycle, or 2 to 10 mg a day for 5 days or until hemostasis occurs or 50 mg followed by 10 mg a day for 4 days. Intrauterine contraceptive system, 38 mg in silicone oil, once a year. In habitual abortion, progesterone has been administered from the start of pregnancy, usually in doses of 5 to 20 mg twice or thrice weekly by intramuscular injection; for threatened abortion doses of 25 mg daily is given until pains and haemorrhage cease then reduced to 10 mg per day.

POLYMER PROFILE

Carbopol polymers

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol[28]. They are produced from primary polymer particles of about 0.2 to 6.0 μm average diameter. The flocculated agglomerates cannot be broken into the ultimate particles when produced. Each particle can be viewed as a network structure of polymer chains interconnected via cross-linking. Carbomers readily absorb water, get hydrated and swell. In addition its hydrophilic nature, its cross-linked structure and its essentially insolubility in water makes carbopol a potential candidate for use in controlled release drug delivery system[29,30].Carbopol polymers are offered as fluffy, white, dry powders (100% effective). The carboxyl groups provided by the acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol polymers have an average equivalent weight of 76 per carboxyl group[31].

The general structure is given below.



Carbopol polymers are manufactured by cross-linking process. Depending upon the degree of cross-linking and manufacturing conditions, various grades of carbopol are available. Each grade is having its significance for its usefulness in pharmaceutical dosage forms. Carbopol 974P and 971P are crosslinked with allyl pentaerythriol and polymerized in ethyl acetate, a GRAS solvent. Polycarbophils are crosslinked with divinyl glycol.

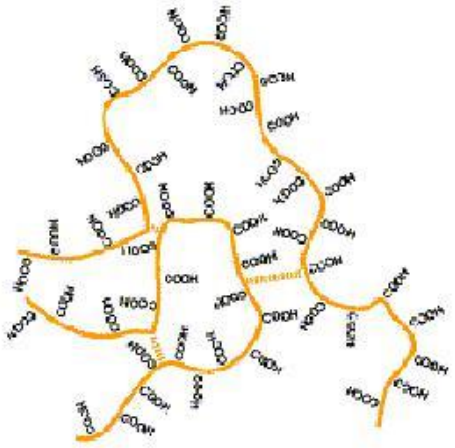


Fig.3. Schematic drawing of a molecular segment of a cross-linked polyacrylic acid polymer

2.1 Physical Properties [32]

The three dimensional nature of these polymers confers some unique characteristics, such as biological inertness, not found in similar linear polymers. The carbopol resins are hydrophilic substances that are not soluble in water. Rather,

these polymers swell when dispersed in water forming a colloidal, mucilage-like dispersion. Carbopol polymers bear very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Because the pKa of these polymers is 6.0 to 6.5, the carboxylate moiety on the polymer backbone ionizes, resulting in repulsion between the native charges, which adds to the swelling of the polymer. The glass transition temperature of carbopol polymers is 105°C (221°F) in powder form. However, glass transition temperature decreases significantly as the polymer comes into contact with water. The polymer chains start gyrating and radius of gyration becomes increasingly larger. Macroscopically, this phenomenon manifests itself as swelling.

2.2 Rheological properties

While the relationships between structure and properties have been of interest both academically and in industry different grades of Carbopol polymers exhibit different rheological properties. This is a reflection of the particle size, molecular weight between crosslinks (Mc), distributions of the Mc, and the fraction of the total units, which occur as terminal, i.e. free chain ends[33-36]. The molecular weights between adjacent crosslinks (Mc) are approximately inversely proportional to the crosslinker density.

2.3 Applications of Carbopol polymers [37-40]

The readily water-swellable Carbopol polymers are used in a diverse range of pharmaceutical applications to provide:

- Controlled release in tablets.
- Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications.
- Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical lotions, creams and gels, oral suspensions and transdermal gel reservoirs.

- Permanent suspensions of insoluble ingredients in oral suspensions and topicals.
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants.

2.4 Bioadhesive Applications [41-43]

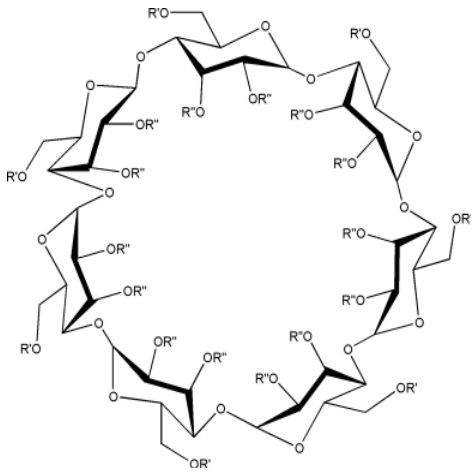
Many hydrophilic polymers adhere to mucosal surfaces as they attract water from the mucus gel layer adherent to the epithelial surface. This is the simplest mechanism of adhesion and has been defined as "adhesion by hydration" Various kinds of adhesive force, e.g. hydrogen bonding between the adherent polymer and the substrate, i.e. mucus, are involved in mucoadhesion at the molecular level. Carbopol polymers have been demonstrated to create a tenacious bond with the mucus membrane resulting in strong bioadhesion.

2.5 Toxicological studies [44]

The carbopols, like other high molecular weight polymers, demonstrate a low toxic and irritation potential based on their physical and chemical properties.

3. CYCLODEXTRINS[45-49]

The structure of cyclodextrin (7 glucose units) is shown.



R′, R″ = H for 'natural' α-, β- and γ-cyclodextrins

R′, R″ = $CH_2CHOHCH_3$ for 2-hydroxypropyl cyclodextrins

The basic closed circular structure of beta cyclodextrin is maintained and the glycosidic oxygen forming the bond between adjacent monomers and the hydrogen atoms lining the cavity of the cyclodextrin impart an electron density and hydrophobic character to the cavity. The hydroxyl groups and the hydroxypropyl groups are on the exterior of the molecule and interact with water to provide the increased aqueous solubility. Cyclodextrins (CDs) are 'bucketlike' or 'conelike' toroid molecules, with a rigid structure and a central cavity, the size of which varies according to the cyclodextrin type. The internal surface of the cavity is hydrophobic and the outside of the torus is hydrophilic; this is due to the arrangement of hydroxyl groups within the molecule. This arrangement permits the cyclodextrin to accommodate a guest molecule within the cavity, forming an inclusion complex. Cyclodextrins may be used to form inclusion complexes with a variety of drug molecules, resulting primarily in improvements to dissolution and bioavailability owing to enhanced solubility and improved chemical and physical stability. The mechanisms by which this solubilization occurs will be due to inclusion complex formation in which the guest and host molecules are in dynamic equilibrium with the complex. The central CD cavity provides a lipophilic microenvironment into which suitably sized drug molecules may enter. No covalent bonds are formed or broken during the drug/CD complex formation and in aqueous solutions, the complexes are readily dissociated. The natural CDs, in particular β-CD, are of limited aqueous solubility meaning that complexes resulting from interaction of lipophiles with these CDs may also be poorly soluble resulting in precipitation of the solid CD complexes from water and other aqueous systems. This is thought to be due to relatively strong intramolecular hydrogen bonding in the crystal lattice. Substitution of any of the hydrogen bond-forming hydroxyl groups, even by lipophilic functions, results in dramatic improvement in their aqueous solubility[50]

3.1 Toxicological considerations [51]

CDs are associated with MW ranging from almost 1000 to over 2000 Da and are hydrophilic with a significant number of H-donors and acceptors and, thus, are not significantly absorbed from the gastrointestinal tract in their intact form. Hydrophilic CDs, namely HPβ-CD and SBEβ-CD, are considered non-toxic at low

to moderate oral and intravenous doses. HPβ-CD is much more water-soluble and more toxicologically benign than the natural β-CD[52,53]. Two major toxic side effects have been reported after systemic[54-56] administration of cyclodextrins: renal toxicity and hemolysis. However the risk of systemic side-effects of cyclodextrins after nasal administration depends on how much CD will be absorbed. After nasal administration of drug-CD complex formulation, only the drug is absorbed by the nasal epithelium but not the CD and its complex. The fraction of CD not absorbed is removed by the nasal mucociliary clearance system which transports it to the esophagus where it is swallowed.

AIM AND OBJECTIVE OF THE STUDY

In view of poor oral bioavailability of progesterone, it is apparent that a serious need exists for the improved delivery of this hormone for greatly enhanced bioavailability as compared to oral administration while at the same time providing relative ease of administration when compared to intramuscular injection. Hence it is the object of this present work to provide a novel drug delivery system for accomplishing the delivery of this natural female hormone and thus avoid the disadvantages inherent in the use of potentially unsafe synthetic progestins. It is yet further aim of the present work to provide a novel dosage form containing progesterone useful in the treatment of conditions such as menopause, menstrual disorder, habitual abortion, etc., which are known to respond to natural hormone.

The foregoing objects are achieved by administration of progesterone as gels adapted for nasal administration. The bioavailability of nasally administered drugs depends on factors such as solubility and dissolution rate. Progesterone is a poorly water-soluble drug, thereby complicating nasal absorption. Hence β-cyclodextrin (β-CD) and hydroxypropyl β-cyclodextrin (HPβ-CD) are used as solubilizer and absorption enhancers as both physical mixtures and inclusion complexes. This study also aims to study the effect of cyclodextrins as absorption enhancer and also its effect when used as physical mixture or as an inclusion complex on the absorption enhancement.

For most absorption enhancers a direct relationship exists between absorption promoting ability and local toxic effect. In many cases marked and irreversible damage has been seen in the nasal epithelium after application. Cyclodextrins are chosen as they are proven to be safe and nontoxic and hence should be suitable for exploitation in nasal products. The aim of the present work is to carry out in-depth toxicological evaluations using various models to assess the safety profile of the gels.

The mechanical properties were characterized by rheological and textural analysis. The rheological profile of gels and their mixtures with mucin was carried

out to study the viscosity enhancement. Textural profile analysis of gels for adhesiveness and hardness was determined.

A bioadhesive force is required between the drug device and the mucosal surface to successfully retain the device and retard the natural clearance processes. Hence it was important to calculate the force of adhesion using rheological method.

In nasal drug delivery it is a prerequisite to investigate the effects of drugs and additives on nasal functioning at an early stage. The self cleaning capacity of the nose, as effective by the ciliary epithelium and necessary to remove dust, allergens and bacteria should not be influenced by the nasal medication. The aim also includes studying the effect of the formulation on mucociliary clearance rate using frog palate method.

The aim of this study also includes the determination of stability profile of the gels as per ICH guidelines.

Literature survey carried out for the present work includes reports on all previous work done by other workers in the field of nasal drug delivery systems. The level of interest in nasal drug delivery has increased substantially as is evident by the number of research activities done on this subject in the past two decades. The objective of their work includes demonstrating the nasal route which provides a viable alternative for many drugs which are presently administered via parenteral and oral routes. The optimization of nasal absorption is achieved by improving formulation excipients, designing various types of nasal dosage forms, using various absorption enhancers and understanding their mechanism of nasal absorption.

The study by Eunsook *et al.*[57] was aimed to formulate and evaluate nasal delivery systems containing ondansetron hydrochloride. In the *in vitro* study, the permeation rate with the addition of 10% polyethylene glycol 300 (PEG 300) to aqueous solution containing 0.01% benzalkonium chloride (BKC) and 10% sulfobutylether β-cyclodextrin sodium salt (SB-CD) was somewhat more rapid up to 1.5 h compared to the addition of 10% PG. Even though cyclodextrins including SB-CD or dimethyl-ß-cyclodextrin failed to show permeation enhancing effects of ondansetron hydrochloride, the addition of 10% SBCD to aqueous solution containing 10% PEG 300 and 0.01% BKC could be a good candidate for ondansetron nasal delivery systems because of its safety profile, stable storage in refrigerator and solubilizing effect.

In this study the suitability of spray drying as a method for the formulation of mucoadhesive microspheres for nasal delivery was evaluated by Rathananand *et al.*[58] The microspheres were produced from mucoadhesive polymers including chitosan salt, hydroxypropylmethylcellulose, hydroxypropylcellulose, sodium alginate and contained levocetirizine dihydrochloride as the model drug.. The conditions of the spray dryer were optimized for production yield and particle size.

Gelatin A microspheres of propranolol hydrochloride for intranasal systemic delivery were developed by Dandagi *et al.*[59] with the aim to avoid first pass metabolism, to improve the patient compliance, to use an alternative therapy to conventional dosage form, to achieve controlled blood level profiles, and to improve

the therapeutic efficacy of propranolol hydrochloride in the treatment of various cardiovascular disorders and as a prophylactic for migraine. Gelatin A microspheres were prepared by emulsion crosslinking method using glutaradehyde as a crosslinking agent. Gelatin and chitosan were used as polymer and copolymer respectively. The data indicated that propranolol hydrochloride release followed Higuchi's matrix and Peppa's model.

Domperidone microspheres for intranasal administration were prepared by Yadav *et al.*[60] emulsification crosslinking technique. Starch a biodegradable polymer was used in preparation of microspheres using epichlorhydrine as cross-linking agent. The formulation variables were drug concentration and polymer concentration and batch of drug free microsphere was prepared for comparisons. Concentration of both polymer and drug affected *in vitro* release of drug.

Novel mucoadhesive chitosan microspheres were developed by Jain *et al.*[61] to explore the possibilities of non invasive delivery of insulin. The mucoadhesive chitosan microspheres were prepared by emulsification method. Glutaraldehyde cross-linked microspheres showed better reduction of blood glucose level than citric acid cross-linked microspheres. The *in vivo* performance of mucoadhesive microspheres showed prolonged and controlled release of drug as compared with the conventional dosage form

The purpose of research by Shelke *et al.*[62] was to evaluate suitability of sorbitan monostearate organogels for nasal delivery of propranolol hydrochloride. The viscosity increase was proportionate to chain length of tween surfactants. The water holding capacity, and hence the electrical conductivity increased with sorbitan monostearate concentration. The release retardant effect of propranolol hydrochloride through sheep nasal mucosa was observed with the order of Tween 20 < Tween 60 < Tween 80. The organogels exhibit useful pharmaceutical properties.

The use of HPβ-CD to increase the water solubility of progesterone in parenteral dosage form was described by Zoppetti *et al.*[63] A precipitate was noticed during ICH stability conditions. This was investigated. The precipitate was

progesterone and residual unmodified β-cyclodextrin. Hence a process was developed to separate residual unreacted β-cyclodextrin from HPβ-CD by formation of insoluble inclusion complex.

The purpose of these studies by Robert *et al.*[64] was to enhance mucosal and systemic antibody production in response to increased local residence time of a whole inactivated influenza virus administered as a dry powder nasal vaccine formulation. Spray-freeze-drying (SFD) particles suitable for nasal delivery were characterized for physico-chemical properties and stability. Mucoadhesive compounds (MA) were characterized for their effects on nasal residence time of vaccine powders in rats. Intramuscular delivery provided equivalent serum antibody titers to intranasal (IN) powder without MA, in the presence of carboxymethyl cellulose, sodium alginate, and hydroxypropyl methylcellulose after initial dosing and all formulations except IN powder with chitosan after boosting. Intra nasal liquid preparations provided equivalent serum antibody titers to all IN powders after the initial vaccination and significantly greater serum antibody titers than IN powder with chitosan after boosting.

Solubility improvement of progesterone using complexes with various cyclodextrins and ternary complexes with PEG 6000 was carried out by Malika *et al.*[65] Complexes with HPβ-CD and PMβ-CD were the most efficient for the solubilization of progesterone with the highest apparent stability constants. Dissolution constant rates were considerably enhanced in PEG 6000.

The goal of this study by Krauland *et al.*[66] was to develop a microparticulate delivery system based on a thiolated chitosan conjugate for the nasal application of peptides. Insulin was used as the model peptide. A mixture of the chitosan-TBA conjugate, insulin and the permeation mediator, reduced glutathione were formulated to microparticles. The microparticles so prepared showed a controlled release of fluorescein isothiocyanate labeled insulin over 6 h. Nasal administered chitosan-TBS-insulin microparticles led to an absolute bioavailability of 7.24%.

The objective of this present study by Varshosaz *et al.*[67] was to develop chitosan bioadhesive gel for nasal delivery of insulin. A nasal perfusion test was used to study the toxicity of 4 absorption enhancers: saponin, sodium deoxycholate, ethylendiamine tetra-acetic acid (EDTA) and lecithin. The gel of 2% medium molecular weight of chitosan with EDTA caused increase in insulin absorption and reduction in glucose level by as much as 46% of the intravenous route.

Mucoadhesive chitosan microspheres of amlodipine besylate (AB) were prepared by Patil and Murthy[68] for nasal administration with the aim of avoiding the first pass effect by simple emulsification crosslinking method. The microspheres were evaluated for physical characteristics such as particle size, particle shape and surface morphology by scanning . Its possibility to avoid first pass metabolism of AB ultimately showed improvement of bioavailability than oral dosage, probably as a consequence of prolonged residence at the absorption site.

In this study Gavini *et al.*[69] attempted the nasal administration of Carbamazepine (CBZ) using microspheres constituted by chitosan hydrochloride (CH) or chitosan glutamate (CG). The microspheres were produced using a spray-drying technique and characterized in terms of morphology (scanning electron microscopy, SEM), drug content, particle size (laser diffraction method) and thermal behaviour (differential scanning calorimetry, DSC). Spray-drying was a good technique of preparation of CBZ-loaded microspheres. The loading of the drug into the polymeric network always led to an increase in the dissolution rate compared to CBZ raw material. The microspheres obtained using chitosan glutamate had the best behaviour both *in vitro* and *in vivo*. They increased the drug concentration in the serum when compared to the nasal administration of the pure drug (C_{max} 800 and 25 ng/ml for microspheres and pure drug, respectively). The results obtained indicated that the loading of CBZ in chitosan glutamate microspheres increases the amount of the drug absorbed through the nose.

In this study by Cetin *et al.*[70] nasal dosage forms solutions, gels and powder of metoclopramide (MTC) were prepared using carbopol 981. The penetration enhancing effect of dimethyl β-cyclodextrin was used in powder and was observed

in *ex vivo* and *in vivo* experiments. The gel formulations were found to have a higher nasal bioavailability than those of the solution and powder and this represented a promising tool for the systemic delivery.

Intranasal delivery systems of sumatriptan using thermoreversible polymer Pluronic F127 and mucoadhesive polymer carbopol 934P (C934P) was developed by Rita *et al.*[71] Formulations were modulated so as to have gelation temperature below 34^{o}C to ensure gelation at physiological temperature after intranasal administration. The results of *in vitro* drug permeation studies across sheep nasal mucosa indicated that effective permeation coefficient could be significantly increased using *in situ* gelling formulation with carbopol 0.3% or greater. Finally histopathological examination did not detect any damage during *in vitro* permeation studies.

This work by Fu-Gen *et al.*[72] involved the preparation of prostaglandin E1-hydroxy propyl-β-cyclodextrin complex and its nasal delivery in rats. Solubility and stability of prostaglandin was improved by the above process. Studies in rats showed a rapid decrease in blood pressure after nasal administration and exhibited a dose-efficacy relationship which showed results similar to intravenous route.

D'Souza *et al.*[73] studied with the objective to formulate insulin gel for intranasal delivery using carbopol and HPMC. The *in vitro* release and hypoglycemic activity was carried out in animal model and healthy human volunteers. The use of bioadhesive gel promoted the prolonged contact between the drug and absorptive site in nasal cavity and also facilitated direct absorption of medicament through nasal mucosa. The study concluded that intranasal gel was a pleasant and painless alternative to injectable insulin.

The present study by Baboota *et al.*[74] was an attempt to form an inclusion complex of rofecoxib with HPβ-CD by the spray-drying to improve the aqueous solubility of the drug, thus enhancing its dissolution rate, thereby leading to a faster onset of action and less gastro intestinal mucosal toxicity. The inclusion complex was found to have improved *in vitro* drug release compared with the pure drug.

Though the physical mixture of rofecoxib with cyclodextrins reduced ulcer formation, it was the spray-dried complex formation approach that minimized gastric ulceration.

Abdolhossein *et al.*[75] investigated nasal absorption of insulin from a carbopol-based nasal spray gel in rabbits. The insulin gel produced a significant hypoglycemic response in rabbits whereas no response was seen following administration of insulin solution.

The aim of this work by Sambhaji *et al.*[76] was to improve absorption and patient compliance by using thermoreversible gels for nasal administration of Vitamin B_{12} using pluronic (PF 127). In the present research work effects of Vitamin B_{12} and gel additives, viz. PF concentration, osmolarity, polyethylene glycol on thermodynamic properties of phase transition at gelation and gel melting was reported. Enthalpy of both transitions remained unchanged with vitamin indicating no interaction with polymer. Benzalkonium chloride decreased gelation onset temperature. Thermodynamic properties of PF 127 gels are significantly altered with polymer concentrations and water soluble formulation.

The primary goal of this work by Brent *et al.*[77] was to evaluate the long-term constant zero-order release of progesterone from a waterborne, *in situ*-gelling, injectable material. The motivation for this is to develop an intrafallopian tube embolization system for contraception. Poly(ethylene glycol) diacrylate (PEGDA, 575 g/mol) or poly(propylene glycol) diacrylate (PPODA, 540 g/mol) as a Michael-type addition acceptor was combined with pentaerythritol-tetrakis (3-mercaptopropionate; a Michael-type addition donor). Cylinders with 25 wt.% load of progesterone exhibited constant release for more than 50 days in both the PEGDA and PPODA systems. In contrast, these in situ-gelling materials reported here can be used to provide zero-order, partition-controlled release of progesterone and enhance the efficiency of an intrafallopian tube embolization system through progesterone release in an injectable, *in situ* forming system.

Shirui *et al.*[78] used melatonin as model drug starch microspheres for intranasal administration prepared by the emulsification cross-linking technique. Nasal clearance of 99mTc labeled starch microspheres was investigated using gamma scintigraphy. It was revealed that more than 80% of the starch microspheres could be detected in the nasal tissue 2 h after administration. The absolute bioavailability of melatonin was 84%.

Effect of chitosan on progesterone release from hydroxypropyl-β-cyclodextrin complexes was studied by Cerchiara *et al.*[79] Progesterone alone and its inclusion complex with HPβ-CD were incorporated into chitosan by spray drying and freeze drying process. Release data from samples showed a significant improvement of the dissolution rate and a controlled release in the presence of chitosan.

The study by Dyer *et al.*[80] was to investigate whether the widely accepted advantages associated with the use of chitosan as a nasal drug delivery system might be further improved by application of chitosan formulated as nanoparticles. Insulin-chitosan nanoparticles were prepared by the iontotropic gelation of chitosan glutamate and tripolyphosphate pentasodium and by simple complexation of insulin and chitosan. The insulin-chitosan solution formulation was found to be significantly more effective than the complex and nanoparticles formulations. Also chitosan powder was the most efficient formulation for nasal delivery of insulin in the sheep model.

This investigation by Karen *et al.*[81] was to see the possibility of correcting the endometrial alterations induced by clomiphene citrate (CC) by vaginal hormonal supplements estradiol and progesterone gel. All the biopsies in the hormone supplementation groups showed complete predecidual changes and were 'in phase' with findings normally made 10 days post ovulation. The addition of vaginal estradiol and progesterone normalized the alterations in endometrial morphology and improved endometrial receptivity in CC cycles and ultimately higher pregnancy rates.

The aim of Fusashi *et al.*[82] was to evaluate the utility of insoluble powder formulation for nasal systemic drug delivery and compare the efficacy of liquid and powder formulations. The nasal absorption of drugs was examined in rats using hydrophilic compounds with various molecular weights (MW) such as phenol red, cyanocobalamin and fluorescein isothiocyanate (FITC)-Dextrans, and several kinds of powders. Intranasal residence time was also compared. Insoluble calcium carbonate (CaCO3) powder formulation provided increased absorption of drugs over the wide range of MW from 354 to 77,000 Da. Furthermore, improved bioavailability of FITC-Dextran (MW 4,400; FD-4) was also achieved with other insoluble powders as well as CaCO3, but not with soluble powders. Insoluble powder formulations improve nasal bioavailability predominantly by retarding drug elimination from the absorption site and appeared to be effective for nasal systemic drug delivery.

The aim of this work by Emilio and Erika[83] was to study the influence of β-cyclodextrin (β-CD) on the biopharmaceutic properties of diclofenac (DCF). To this purpose the physicochemical characterization of diclofenac-β-cyclodextrin binary systems was performed both in solution and solid state. Diffusion experiments showed that DCF diffusion was higher from the saturated drug solution (control) than the freeze-dried inclusion complexes, prepared using different DCF-β-CD molar ratios. However, the presence of the inclusion complex was able to stabilize the system giving rise to a more regular diffusion profile.

Control of drug release from polymeric systems by incorporation of cyclodextrin as physical mixtures, covalently bound conjugates or cross-linking agents was demonstrated by David *et al.*[84] Incorporating cyclodextrins into polymeric matrices increased drug release by improving solubility of drugs, acting as channeling agents, promoting erosion of matrix. Drug release could be reduced by increasing molecular weight due to complexing, reducing concentration of diffusible species and acting as cross linking agent.

A simple approach was presented by Fabiana *et al.*[85] to modulate drug delivery from swellable systems of cross linked polyethylene glycol by using

complexants like cyclodextrins. The effect of complexants were interpreted by means of simple mass balances on diffusing species and the involved relevant parameters was individuated. The application of this strategy to the release of nicardipine from swellable systems by using cyclodextrin as complexant has evidenced the potential of the approach to tailor drug release.

A new spray-drying procedure for the production of nasal powder of cyanocobalamin as an alternative to the conventional freeze-drying method was described by Garcia *et al.*[86] The drying method, either the new spray drying or the conventional freeze-drying, was less important. Interestingly, an inverse correlation was found between water uptake and drug diffusion. The highest absorption enhancement was observed with cellulose microcrystalline powders, which provided a 25% mean absolute bioavailability followed by crosspovidone and dextran microspheres formulations with mean bioavailability values of 14% and 7% respectively.

Of the several routes available for mucosal immunization, the nasal route was particularly attractive because of ease of administration and the induction of potent immune responses, particularly in the respiratory and genitourinary tracts. However, adjuvants and delivery systems were required to enhance immune responses following nasal immunization. This review by Michael and Hagan[87] focussed on the use of microparticles as adjuvants and delivery systems for protein and DNA vaccines for nasal immunization. In particular was discussed the author's own work on poly (lactide co-glycolide) (PLG) microparticles with entrapped protein or adsorbed DNA as a vaccine delivery system. The possible mechanisms involved in the enhancement of immune responses through the use of DNA adsorbed onto PLG microparticles were also discussed.

Illum *et al.* [88] reviewed studies conducted on nasal vaccination: a non-invasive vaccine delivery method that holds great promise for the future. For some situations, the nose could provide a suitable route for priming and boosting (for example in children) while in other situations, the nasal route could be more appropriate for boosting.

This paper by Illum *et al.*[89] investigated the effect of starch microspheres on the absorption enhancing efficiency of various enhancer systems in formulations with insulin after application in the nasal cavity of sheep. The enhancers studied were lysophosphatidylcholine, glycodeoxychlolate and sodium taurodihydroxyfusidate, a bile salt derivative. The bioadhesive microspheres were shown to increase synergistically the effect of the absorption enhancer on the transport of the insulin across the nasal membrane.

In this study by Callens and Remon[90] insulin was administered nasally to rabbits as a dry powder formulation which consisted of maize starch or maltodextrin and carbopol 974P prepared by freeze drying. Carbopol 974P was required when maltodextrin was used in order to get a significantly higher bioavailability compared to formulation without carbopol. Freeze drying seemed a prerequisite for good bioavailability.

Carboxymethyl cellulose (CMC) powder formulation of apomorphine was prepared by Michael *et al.*[91] by lyophilization and characterized with respect to the *in vitro* and intranasal *in vivo* release of apomorphine in rabbits. This was compared with apomorphine release from degradable starch microspheres DSM and lactose as well as *in vivo* absorption after subcutaneous injection. *In vitro* release of apomorphine from CMC was sustained unlike that of DSM and lactose. Drug release increased with drug loading. The sustained plasma level of apomorphine by CMC was achieved with relative bioavailabilities equivalent to subcutaneous injection.

Efficacy of luteal support from single daily administration of 8% progesterone gels was tested in 43 women in an *In Vitro* Fertilization program with historical pregnancy rates >50% by Schoolcraft *et al.*[92] Results were compared with similar group receiving 50 mg i.m. progesterone. Total pregnancy rates, clinical pregnancy rates and live birth rates were similar for both groups. Gels offered an appreciable improvement as it provided an efficient luteal support option that avoided painful injections.

Caludia and Randall[93] examined several potential polymer platforms for their *in vitro* protein release, relative bioadhesive properties and induction of cytokine release from respiratory epithelium. Starch, alginate, chitosan and carbopol microparticles containing bovine serum albumin (BSA) were prepared by spray drying method. Polarized Calu-3 cell sheets were used to evaluate relative bioadhesion, enhancement of protein transport and induction of cytokine release *in vitro*. Starch and alginate microparticles released protein more rapidly but were less adhesive to Calu-3 cells than chitosan and carbopol microparticles. Protein transport was enhanced from carbopol gels and chitosan microparticles.

Kouji *et al.*[94] prepared microparticles of novel, bioadhesive graft copolymers of polymethacrylic acid and polyethylene glycol (P(MAA-PEG)). The aims of this study were to investigate the uptake and release kinetics of budesonide from P(MAA-PEG) *in vitro* as well as the pharmacokinetics following nasal administration of the polymer contain budesonide. The release of budesonide from the polymer swollen in 25% ethanol solutions obeyed classical Fickian release behavior after an initial rapid drug burst.

Inclusive complexes of hydrocortisone and progesterone with β-cyclodextrin or 2-hydroxypropy1–β-cyclodextrin. were prepared by Cavalli *et al.* [95] The inclusion complexes were incorporated in two types of solid lipid nanoparticles (SLN). Using the β-cyclodextrin complexes the incorporation of the more hydrophilic drug hydrocortisone was higher than that of progesterone. Release of hydrocortisone and progesterone from SLN was lower when they were incorporated as inclusion complexes than as free molecules.

Takenaga *et al.* [96] examined the application of various microparticle resins for the nasal delivery of insulin in rabbits. Intranasal administration of human insulin mixed with fractionated sodium polystyrene sulfonate powder caused a rapid increase of the plasma insulin level while intranasal administration of insulin alone caused little increase.

Delivery of nasal powders of granulated cyclodextrin by insufflation was studied by Alessia DA *et al.*[97] in order to find the relationship between powder properties and delivery behavior. Particle size was the main parameter affecting nasal powder delivery, both as to the amount of dose sprayed and the aspect of cloud produced. Powder of around 100 microns in size showed useful insufflation characteristics for nasal delivery.

Lena and Peter[98] studied the influence of osmolarity on nasal absorption of insulin from the thermogelling polymer ethyl (hydroxyethyl) cellulose (EHEC). Insulin 3 IU/kg body weight, given intranasally in a hypo osmotic thermogelling system, containing EHEC, m-cresol and glycerol was more efficient in lowering plasma glucose than insulin delivered in iso- and hyper osmotic gels.

Pereswetoff and Edman[99] studied the effects of the particle size of dextran microspheres on the nasal absorption and the localization of insulin in the spheres in rats. The freeze-drying process was used to load insulin into the microspheres which significantly affected the integrity and the surface properties of the spheres. Spheres with insulin on the surface were more effective in promoting insulin absorption than were those with insulin distributed within the dextran matrix. Particle size tended to influence the kinetics of the insulin effect curve.

Daniela *et al.*[100] conducted a study on the nasal administration of progesterone powder formulations in New Zealand rabbits. Formulations were prepared by adding either co-ground or co-lyophilized progesterone/cyclodextrin to hydrophilic excipients. Nasal bioavailability of the powder mixtures containing co-ground and co-lyophilized progesterone/cyclodextrin were 34% ad 19% respectively, serum peaks were higher and more rapidly achieved following nasal application in comparison with oral administration.

Schipper *et al.*[101] studied the nasal absorption of progesterone in male Wistar rats, using dimethyl-β-cyclodextrin (DMβ-CD) as an absorption enhancer. DMβ-CD solubilized steroids which were poorly water soluble by formation of inclusion complexes. The systemic bioavailabilities of progesterone with DMβ-CD, and a

progesterone suspension were compared. DMβ-CD increased the bioavailability of progesterone from 18 % for the suspension to 58 % for the inclusion complex. The bioavailabilities of estradiol and progesterone were high, 59% and 67% respectively. They were not significantly different from those achieved after separate administration. The combined preparation had only a mild effect on the ciliary beat frequency of human nasal adenoid tissue *in vitro*.

The effect of administration for 10 days of nasal spray of progesterone along with oral conjugated estrogens on the serum level and the endometrium for 4 weeks to post menopausal women was studied by Cicinelli *et al.*[102] Repetitive nasal spray administration led to increase in progesterone serum levels and when the estrogen stimulation was adequate secretory changes in the endometrium was seen.

Ting *et al.*[103] described the spray drying and spray desolvation for the generation of poly vinyl alcohol (PVA) micorparticles intended for nasal administration. Spray drying method produced microparticles of appropriate size distribution but which were hollow and therefore unsuitable for nasal delivery due to rapid clearance. Desolvation by spraying PVA solution onto acetone produced solid collapsed spheres with desired size for nasal deposition.

Microspheres of both starch and dextran cross-linked with epichlorohydrine, functioned as an enhancer for the absorption of insulin in rats and were prepared by Edman *et al.*[104] Starch microspheres were more effective than dextran spheres in inducing a decrease in blood sugar. A conceivable hypothesis with regard to the mechanism of action could be that the epithelial mucosa was dehydrated, with a reversible shrinkage of the cells thus giving a physical separation of the intercellular junctions.

In the present study by Schipper *et al.*[105] the effect of different concentrations of dimethyl β-cyclodextrin DMβ-CD on the absorption of intranasally administered insulin in rats was studied. Administration of insulin with 5 % DMβ-CD resulted in a bioavailability of 100%. The minimal concentration of DMβ-CD required to improve the insulin absorption was 2%. Increasing the

concentration to 3, 4, and 5% resulted in more pronounced insulin absorption. DMβ-CD decreased the cilliary beat frequency of both chicken embryo trachea and human adenoid tissue *in vitro*.

Cicinelli *et al.*[106] investigated the bioavailability of progesterone administered by nasal route in 10 healthy menopausal women. A dose of 11.2 mg was administered and physiological circulating progesterone levels of 2 ng/ml were achieved within 2 min after nasal administration and lasted for 6 h and returned to basal value after 12 h. The effectiveness appeared to be due to the high solubility in oleic carrier.

This paper by Illum *et al.*[107] described an assessment of the potential of using bioadhesive microspheres as a nasal delivery system for biosynthetic human growth hormone in sheep. The microsphere system was used alone and in combination with a biological surfactant, lysophosphatidylcholine (LPC). It was absorbed to only a very low extent as a solution. Rapid and a much higher absorption was seen when it was administered as in combination with the microspheres and LPC as an enhancer.

A new formulation for nasal absorption containing 17β-estradiol (E2) by dimethyl β-cyclodextrin (DM-CD) as a solubilizer and absorption enhancer was described by Walter *et al.*[108] Nasal administration of this E2-DMCD formulation gave a significantly higher E2 absorption than an E2 suspension in both rabbits and rats.

The utility of the nasal route for the administration of progesterone and 17 beta estradiol was studied in rats by Basarshi-Nassar *et al.*[109] The results indicated that both the steroids were rapidly absorbed from the nasal cavity. The bioavailability of 14C radio labeled progesterone administered nasally was found to be 100%. Nasal administration of 17 beta estradiol resulted in significantly higher blood levels.

Diane *et al.*[110] investigated the influence of mucosal route and penetrant hydrophilicity on the *in vitro* absorption of a model lipophilic compound, progesterone in ovariectomised rabbits. The rate and extent of mucosal absorption

decreased as penetrant hydrophilicity increased for the nasal, rectal and vaginal route. The absorption characteristics of a lipophilic compound, such as progesterone was influenced by the properties of both the mucosa and the drug.

The pharmacokinetics of progesterone was studied by Anand *et al.*[111] in blood and CSF of adult ovariectomized rhesus monkeys after administration as iv injection, iv infusion and nasal spray. The bioavailability was greater when it was infused or sprayed nasally. The bioavailability was enhanced by extending the duration over which the steroid was delivered into the systemic circulation.

Jian *et al.*[112] carried out a study to evaluate the potential of aminated gelatin as a nasal absorption enhancer for peptide drugs. The hypoglycemic effect after intranasal administration of insulin with aminated gelatin significantly increased compared with that after intranasal administration of insulin in phosphate buffer saline, indicating that aminated gelatin effectively enhanced the nasal absorption of insulin. In contrast neither kind of native gelatin showed any absorption-enhancing effect.

Nasal products need to be evaluated for their local tolerance and toxicity profile. A number of researchers have developed different validation procedures and applications using different animal models.

Studying the nasal mucociliary clearance system is important in order to optimize nasal drug delivery, with respect to both safety and the amount and rate of absorption.

Strong mucoadhesion would most likely produce changes in the rheological properties of the interfacial region, strengthening the weakest component of the adhesive joint. This has led several authors to suggest that rheological synergism between mucin–polymer or mucus–polymer mixtures can be used as an *in vitro* parameter to determine the mucoadhesive properties of materials.

The rheological characteristics of aqueous dispersion of four types of pectin and their mixtures with mucin were investigated by Nartaya *et al.*[113] using a simple

viscometric method with a Brookfield viscometer, and were compared to the known mucoadhesive chitosans and Carbomer 934P. The viscometric method introduced the parameter, viscosity enhancement, which was an empirical determinant of the absolute force of bioadhesion. The higher the molecular weight, the greater the viscosity enhancement indicating the rheological synergism. The force of bioadhesion was also found to be dependent on molecular weight of pectin, its initial viscosity and environmental pH. These results were in agreement with an already reported *in-vitro* test on gastrointestinal mucosa. This corroborated the use of pectin as a mucoadhesive polymer for gastrointestinal mucoadhesive drug delivery system.

The interaction between pectin and mucin was investigated by Pornsak and Nathaya[114]by comparing the viscoelastic properties of mucin-pectin mixtures, using a small strain oscillatory rheology against those of pure components. All pectins showed rheological synergism when mixed with mucin as evidenced by an increase in dynamic moduli and a decrease in loss tangent. The interaction between pectin and mucin related to the physical entanglements of gel network and dependent on pectin type, pectin concentration, mucin concentration and dispersion medium

The purpose of these studies by Shah and Maureen[115] was to identify the rheological properties of polyacrylic acid gels necessary for optimal reductions in mucociliary clearance. The mucociliary transport of two bioadhesive polyacrylic acid polymers, polycarbophil and carbopol was assessed *in vitro* by measuring their clearance rates across explants of ciliated bovine tracheal tissue. The viscoelastic properties of polymer gels were measured in the presence of mucus using controlled stress rheometry.

The purpose of this research by Shah and Donovan[116] was to compare the visco elastic properties of several neural and anionic polysaccharides polymers with their mucociliary transport rates (MTR) across explants of ciliated bovine tracheal tissue to identify rheological parameters capable of depicting the extent of reduction in mucociliary transport. Anionic polysaccharides were more efficient at decreasing the mucociliary transport rate than were the neural polymers and a concentration

threshold where no further decrease in MTR occurred with increasing polymer concentration was observed for neural polysaccharides.

As preservatives may impair mucociliary clearance, the aim of this work was to systematically study their time-dependent effect on the ciliary beat frequency (CBF) in human nasal epithelial cells (HNEC). CBF was determined using a high-speed digital imaging method by Mallants *et al.*[117]. Five preservatives were selected including benzalkonium chloride, phenylethyl alcohol, methylparaben, propylparaben and chlorbutol. The authors were interested in the effect of these preservatives on CBF after single and repetitive exposure. Methylparaben (0.0033%), propylparaben (0.0017%) and chlorbutol (0.005%) did not impair CBF, neither after a single short-term exposure period, nor after a single long-term exposure period. Long-term exposure to benzalkonium chloride (0.1%), phenylethyl alcohol (0.125%) and a combination of methyl- and propylparaben (0.0033 and 0.0017%) significantly decreased CBF. After a short-term exposure period, CBF recovered for phenylethyl a alcohol and the combination of methyl- and propylparaben. Benzalkonium chloride decreased CBF non-reversibly

The effect of temperature and ionic strength on small deformation rheology has been investigated for mixed gels of mucin and alginates by Catherine *et al.*[118] Based on these investigations it was proposed that mixed alginate gels were maintained by both enteropolymeric mucin-alginate interactions and homopolymeric mucin-mucin interactions

The aim of this work by Giulia *et al.*[119] was to investigate the gelation properties of carbopol 971 and 974 polymeric system in water soluble cosolvents such as glycerine and PEG 400. Rheological properties of PEG and glycerine samples were compared with similar systems in water by performing oscillatory analysis and measuring elastic modulus G' and G". The results showed that carbopol in PEG after heating to gels that show a satisfactory rheological behaviour. The elastic modulus was greater than the viscous one showing a remarkable elastic character and the performed frequency sweeps showed a typical spectrum for a gel like structure.

The objective of this study by Bounmany *et al.* [120] was to compare the effects of buffered hypertonic and buffered normal saline nasal spray on mucociliary clearance and nasal airway patency. Study design was double blind trial with subjects acting as their own control. Buffered hypertonic and buffered normal saline nasal spray significantly improved mucociliary clearance and should therefore be beneficial in conditions such as rhinitis and sinusitis which were associated with disruption of mucociliary clearance. However these sprays did not appear to affect the nasal airway.

The aim of this study by Kazunori *et al.*[121]was to investigate the effect of highly water soluble cyclodextrins (CD) on the histological integrity of nasal mucosa. *In vivo* single and repeated nasal exposure studies were performed using male Wistar rats. Light microscopy and scanning electron microscopic studies were used. 20% randomly methylated β cyclodextrin showed severe damage on the integrity of nasal mucosa. 10% hydroxyl propyl β cyclodextrin showed no mucosal damage. Less than 10% CD solutions did not produce gross tissue damage.

The objective of this study by Adriaens and Remom[122] was to evaluate an alternative mucosal irritation test using the slug *Arion lusitanicus* as test organism. The effect of 28 reference test samples on the mucosal tissue of slugs was determined by the amount of mucus produced, the reduction in body weight and the release of proteins from the body wall. The data of the mucosal irritation test were compared with the available *in vivo* Draize scores for eye irritation (MMAS). The amount of mucus produced and the reduction in body weight caused by a 60 min contact period were significantly correlated with the MMAS scored for eye irritation. However the mucus produced was the best endpoint to classify the chemicals into 3 categories corresponding to EU classification (NI, R36, R41).

The possible adverse effects of a powder formulation containing drum-dried waxy maize starch and carbopol 974 (90/10) on the nasal mucosa of rabbits and foot mucosa of slugs after multiple administration was carried out by Callens *et al.*[123]. The effect of the formulation was measured in rabbits by the release of proteins and LDH and also histopathology. In slugs the factors considered were change in body

weight, amount of mucus produced, release of proteins, LDH and ALP. Twenty four hours after the powder administration the release of the marker molecules was comparable with the negative controls. The results indicated that the effect of the powder on the mucus was negligible.

A rheological method by Hagerstrom *et al.*[124] to measure mucoadhesion for two ion-sensitive polymers, Carbopol 934 and Gelrite (deacetylated gellan gum) in a simulated physiological environment using two commercially available mucin was developed. The elastic modulus for a polymer / mucin mixture was compared with the elastic modulus for the polymer alone, and an increase in the elastic modulus for the mixture compared to the polymer was interpreted as a positive interaction caused by mucoadhesion. In this study the influence of polymer concentration, type of mucin and experimental rheological factors, such as gap width were examined. Carbopol 934 interacted more strongly with the bovine submaxillary gland mucin than with the porcine gastric mucin.

The ability of the mucoadhesive polymers to produce a large increase in resistance to deformation when incorporated into a mucus gel relative to when the mucus gel and test materials were evaluated separately at the same concentration termed as rheological synergism had been used as a measure of the strength of the mucoadhesive interaction by Flemming *et al.*[125] Rheological synergism was evident for a range of materials with known mucoadhesive properties. This behavior was marked with materials known to be of high mucoadhesive strength even when fully hydrated.

In this study, the thermal behaviour of bioadhesive polymer hydrogels, a mucin gel and their mixtures, were investigated using thermorheological and thermogravimetric analysis by Slobodanka and Duncan[126]. Three poly(acrylic acid (PAA) polymers with different cross-linking status (Carbopol 974P, Carbopol 971P and Noveon AA-1) were selected on the basis of their good bioadhesive properties. Rheological scans from 10-90°C were performed in the oscillatory mode. A marked increase in elastic modulus (G') with temperature was detected above 50°C in all mucin-containing samples, as opposed to the pure gels which showed a flat

response. The thermal behaviour of the Noveon AA-l/mucin mixture did not show the same trends on mixing with mucin as did the Carbopol systems, indicating that the type of cross-linking agent (a tetrafunctional entity, as opposite to a bifunctional one in the Carbopols) may have had an impact.

The immunological implications of intranasal drug delivery in dextran microspheres and a thermogelling ethyl (hydroxyethyl) cellulose (EHEC) system were studied by Morath and Edman[127]. The amount of IgA and IgG in plasma and nasal washing from rats administered with equine myoglobin in the two nasal delivery systems were not significantly different from the controls throughout the experiment. The intranasal administration generated neither specific IgA nor IgG antibodies in plasma or in nasal washing.

Use of an erythrocyte model to investigate the membrane activity of enhancers of intranasal absorption was carried out. Enhancers were ranked according to the haemolytic activity using a erythrocyte model by Chandler *et al.*[128]

A simple viscometric method was used by Emad and Gallo[129] to quantify mucin-polymer bioadhesive bond strength, viscosity components of bioadhesion, force of bioadhesion. Validity of the technique and the effect of ionic charge, polymer conformation and rate of shear on Viscosity component of bioadhesion ηb and Force of bioadhesion F were discussed. Data generated by this method were in agreement with other methods.

The review by Bhise *et al.*[130] focussed on the various bioavailability barriers in nasal drug delivery and the strategies to improve the bioavailability of nasal dosage forms. The noninvasiveness and self administrative nature of nasal delivery attracted the formulation scientists to deliver protein and peptide compounds. Despite of all the advantages of nasal drug delivery, the bioavailability of nasally administered products, especially for protein and peptide molecules was affected by many barriers such as physiological barriers, physicochemical and formulation barriers.

The current review by Henry *et al.*[131] provides an in-depth discussion of therapeutic aspects of intranasal (IN) delivery including consideration of the intended indication, regimen and patient population, as well as physicochemical properties of the drug itself. Case examples were provided to illustrate the utility of IN dosing. It was anticipated that the present review would prove useful for formulation scientists considering IN delivery as a delivery route.

The goal of all drug delivery systems was to deploy medications intact to specifically targeted parts of the body through a medium that could control the therapy's administration by means of either a physiological or chemical trigger. Different approaches to deliver insulin including transdermal, transmucosal, pulmonary route using dry aerosol and inhalers, smart hydrogels, nasal delivery and oral delivery were discussed by Varshosaz[132].

The nasal route could be particularly important for drugs used in crisis management such as for pain and for centrally acting drugs where the pathway from the nose to brain might provide a faster and more therapeutic effect. This article by Sarsija and Baskaran[133] focused on newer developments and strategies for nasal delivery along with nasal absorption mechanism

Michael *et al.*[134] reviewed the background of nasal mucoadhesive drug delivery with special references to the biological and pharmaceutical considerations for nasal mucoadhesive drug administration. Applications of nasal mucoadhesives for the delivery of small organic molecules, antibiotics, proteins, vaccines and DNA were also discussed

Biodegradable polymer and particulate carriers have been shown to be of considerable potential for the delivery of peptides, proteins and DNA in animal models. In the context of vaccine delivery to the upper and lower respiratory tracts, the use of mucoadhesive agents offered a strategy for the facilitation of increased residence time and increased vaccine efficacy. Experimental data were presented by Oya *et al.*[135] that demonstrated the potential of muco- and bioadhesive agents in combination with liposomes for intranasal delivery of tetanus toxoid in mice.

Some of the interesting findings and applications of cyclodextrins (CDs) and their derivatives in different areas of drug delivery and gene delivery was reviewed by Challa et al.[136] The article highlighted the important CD applications in the design of various novel delivery systems like liposomes, microspheres, microcapsules, and nanoparticles. Some important considerations in selecting CDs in drug formulation such as their continuing ability to find several novel applications in drug delivery were expected to solve many problems associated with the delivery of different novel drugs through different delivery routes.

A review by Andreas[137] on thiolated polymers or designated thiomers which were mucoadhesive basis polymers displayed thiol bearing side chains. Based on thiol/disulfide exchange reactions and /or a simple oxidation process disulfide bonds were formed between such polymers and cysteine rich sub domains of mucus glycoproteins building up the mucus gel. Due to the immobilization of thiol groups on mucoadhesive basis polymers, their mucoadhesive properties were 2-up to 140-fold improved. The higher efficacy of this new generation of mucoadhesive polymers in comparison to the corresponding unmodified mucoadhesive basis polymers was verified via various *in vivo* studies.

Stephen *et al.*[138] reviewed the *in vivo* testing which had been recommended for establishing bioavailability (BA) and bioequivalence (BE) by radionuclide imaging methods. A combination of *in vitro* testing and radionuclide imaging could help to define the most appropriate standards for BA and BE assessment, for locally acting and systemically acting nasal products.

Davis and Illum[139]described the basic concepts for the transmucosal delivery of drugs and in particular the use of nasal route for delivery of challenging drugs such as polar low molecular weight drugs and peptides and proteins. Strategies for the exploitation of absorption enhancers for the improvement of nasal delivery were discussed. Selected enhancer systems such as cyclodextrins, phospholipids, bioadhesive powder systems and chitosan were discussed. Methods for the assessing irritancy and damage to the nasal membrane from the use of absorption enhancers

for the improved nasal delivery of vaccines were reported with reference to recent clinical phase I/II studies.

Illum[140] discussed the problems associated with nasal drug delivery and how it was possible, sometimes by means of quite simple concepts, to improve transport across the nasal membrane. It was feasible to deliver efficiently challenging drugs such as small polar molecules, peptides and proteins and even the large proteins and polysaccharides used in vaccines or DNA plasmids exploited for DNA vaccines

A review by Illum[141] of recent developments and research strategies for nasal drug delivery was presented. Nasal absorption, nasal physiology, nasal absorption enhancement, new nasal delivery systems and the discovery that drugs could be transported directly from the nose to the brain via the olfactory pathway were discussed.

To better understand the basis for nasal drug absorption and factors that could influence it, various factors were reviewed by Michael *et al.*[142] The anatomy and physiology of the nasal cavity, as well as ciliary beating and mucociliary clearance as they related to the nasal drug absorption were reviewed. The experimental models used in nasal drug delivery research were also reviewed.

A variety of different types of nasal vaccine systems was described to include cholera toxin, microspheres, nanoparticles, liposomes, attenuated virus and cells and outer membrane proteins (proteosomes). The review by Illum *et al.*[143] described the work on the use of the cationic polysaccharide, chitosan as a delivery system for nasally administered vaccines.

Davis[144] reviewed the nasal route for vaccination especially for the prophylaxis of respiratory diseases. Vaccination via the respiratory tract was reviewed and the deposition and clearance of antigens in the deep lung and nose were described and contrasted.

Merkus *et al.*[145] reviewed the use of various cyclodextrins as excipients including their toxicity in nasal drug delivery. Review of the use of various cyclodextrins as excipients including their toxicity in nasal drug delivery.

A review by Morath[146] on types of microspheres was reported. The building materials of the microspheres such as starch, dextran, albumin and hyaluronic acid and the bio-availability of several peptides/proteins were improved in different animal models. The residence time in the cavity was considerably increased for microspheres compared to solutions. Microspheres also exert a direct effect on the mucosa, resulting in the opening of tight junctions between the epithelial cells.

This article by Gonda *et al.*[147] reviewed the mathematical models of deposition and disposition of drugs administered into the human cavity for systemic delivery. The modeling of the disposition kinetics included drug release from carrier, translocation within the nasal cavity and into the gastrointestinal tract, drug decomposition and drug absorption during the transit through the nose and the gastrointestinal tract.

A review of the optimization of systemic nasal drug delivery with pharmaceutical excipients were discussed by Behl *et al.*[148] The classification and use of nasal drug delivery optimizers like cyclodextrins, fusidic acid derivatives, phosphotidylcholines, microspheres, liposomes, bile salts and surfactants were discussed.

The development of delivery systems for challenging drug molecules was discussed by Stanley and Illum[149] with particular reference to the products of biotechnology and unmet medical needs

Kublika and Vidgrenb[150] reviewed the many drug delivery devices for nasal application of liquid, semisolid and solid formulations in respect to their deposition in the nasal cavity. The site of deposition and the deposition area depended on several parameters which were related to the delivery device, such as mode of administration, particle size of the formulation and velocity of the delivered particles.

Jerome and Artursson[151] reviewed the current knowledge on the regulation of tight junction permeability which was used as a platform for an analysis of the mechanisms of some common absorption enhancers.

From the foregoing literature, application of gels for nasal delivery of drugs has not been investigated in detail. It was also understood that though a number of researchers have worked on the effectiveness of cyclodextrins as nasal absorption enhancers for many drugs including progesterone a lacuna exists. This lacuna is noticed in the lack of research work in the area of effect of various methods of addition of cyclodextrins to the preparations. Hence the present work attempted to address these issues.

SCOPE AND PLAN OF THE WORK

The overall scope of the thesis was to formulate gels for a lipophilic sex hormone, progesterone having a suitable composition including bioadhesive polymers and absorption enhancers for transmucosal administration for systemic delivery of the drug through nasal route. The efficacy and effectiveness of the intranasally administered progesterone gels are studied by various *in vitro* and *in vivo* methods.

More specifically the plan of work is as follows

1. Preformulation studies
   - (i) Identification of the drug
   - (ii) Compatibility study of progesterone with excipients
   - (iii) Calibration curve of progesterone
   - (iv) Stability study of progesterone in nasal mucosal extract
   - (v) Membrane activity study of polymers and absorption enhancers

2. Progesterone-cyclodextrin complex
   - (i) Preparation of progesterone – cyclodextrin (β-CD and HPβ-CD) physical mixtures and inclusion complexes
   - (ii) Characterization of the inclusion complex
     - (a) Fourier transform infra red spectrophotometry (FTIR)
     - (b) Differential scanning calorimetry (DSC)
     - (c) Powder X-ray diffraction (PXRD)
     - (d) Scanning electron microscopy (SEM)
     - (e) Drug Content
     - (f) Aqueous solubility studies
     - (g) Dissolution profile

3. Mucoadhesive gels of progesterone
   - (i) Preparation of mucoadhesive gels as per 3^2 factorial design using three polyacrylic acid polymers (carbopol 974, carbopol 971 and

polycarbophil) at three different concentrations incorporating either β-CD or HPβ-CD as absorption enhancers.

(ii) Determination of drug content of the gels

(iii) Determination of drug release measurements and diffusion coefficient

(iv) *Ex vivo* permeation study and measurement of permeability co-efficient

(v) Determination of drug release pattern

(vi) Bioavailability studies in rabbits

(vii) Statistical optimization results

4. Toxicological studies

(i) Experimental procedure using slugs

(ii) Experimental procedure using rabbits

(iii) Histological studies

5. Mucociliary clearance study

(i) Determination of mucociliary clearance rate using frog palate explant

6. Mechanical properties of gels

(i) Determination of force of adhesion of gels

(ii) Texture profile analysis of gels

7. Accelerated stability studies as per ICH guidelines

List of chemicals used

Chemicals used	Make
Progesterone I.P	Gift sample from AIN Medicare (I) Pvt.Ltd, Chennai, India
Beta-cyclodextrin	Rolex Laboratory, India
Benzalkonium chloride	S.D.Fine Chemicals, India
Carbopol 974 – P NF	Noveon Inc.,
Carbopol 971 – P NF	Noveon Inc.,
Dibasic sodium phosphate	Qualigens Fine Chemicals, India
Hydrochloric acid	Qualigens Fine Chemicals, India
(2-hydroxy propyl) beta cyclodextrin	Sigma – Aldrich Chemicals, India
Methanol	Hong Yang Chemical Corporation, India
Mucin type I-S	Sigma – Aldrich Chemicals, India
Monobasic potassium phosphate	Qualigens Fine Chemicals, India
Monobasic sodium phosphate	Qualigens Fine Chemicals, India
Potassium bromide	S.D.Fine Chemicals , India
Potassium chloride	Qualigens Fine Chemicals, India
Propylene glycol	S.D.Fine Chemicals , India
Polycarbophil AA-1	Noveon Inc.,
Sodium chloride	Qualigens Fine Chemicals, India
Triethanolamine	S.D.Fine Chemicals , India

List of instruments used

Instrument	Make
Cone and Plate Viscometer	R.E.L. , Research Equipments London Ltd.,
Differential Scanning Calorimetry	TA Instruments, DSC 2010
Electronic Balance	Schimadzu
Fourier Transform Infra Red Spectrophotometer	Schimadzu ABB Bomem, MB Series
Franz Diffusion Cell	Orchid Scientific
Humidity Chamber	Sigma Instruments
Micro Pipettes	Bio-Era Medicare Scientific
Magnetic Stirrer	Remi Equipments
Probe Sonicator	Vibra Cell VCX 500, Sonics & Materials Inc.,
pH Meter	Digisum Model 2001, Sri Shanthi Enterprises
Powder X-ray Difractometer	PAN Analytical, X'per PRO
Scanning Electron Microscope	Hitachi Model S-3000H
Temperature Controlled Shaking Water Bath	Sub Zero Laboratory Instruments
Texture Profile Analyzer	QTS-25 Texture Analyzer, Brookefield
UV-Visible Spectrophotometer	Schimadzu UV-1601
Viscometer	DV-E, Brookefield

The drug progesterone was identified according to the certificate of analysis given by the supplier and analyzed by IR spectroscopy. The infrared (IR) spectrum obtained was compared with that of the standard as given in the Indian Pharmacopoeia.

1.1 Compatibility studies

Excipient compatibility studies are an important part of any preformulation screening for dosage form development. This study would indicate the excipients to be avoided and form a part of preliminary stability assessment. 200 mg each of the drug and the excipients (carbopols and polycarbophil) were blended[152] and kept in closed vials at 50^{o}C for 7 days. The samples were taken for FTIR and DSC studies.

Samples (about 1% w/w) were mixed with potassium bromide powder and compressed to a 12 mm disc by a hydraulic press at 10 tons compression force for 30 s. Samples were analyzed in a FTIR Spectrophotometer (Shimadzu ABB Bomem) in the region 4600 to 400 cm^{-1}. DSC analysis of the solid complex, pure drug and cyclodextrins were performed using a Differential Scanning Calorimeter (TA Instruments, DSC 2010). All the samples (5-10 mg) were heated in crimped aluminum pans at a scanning rate of 10^{o} C/min in the temperature range 25^{o} to 300^{o} C.

1.2 Calibration curve of progesterone

1.2.1 Linearity and Range

20 mg of progesterone[153] was accurately weighed and dissolved in sufficient methanol to produce 100 ml. This stock solution was diluted with pH 6.2 phosphate buffer to get concentrations from 2 to 14 µg/ml. The absorbance of a 1 cm layer of this diluted solution was measured at 240 nm. The absorbance was plotted against concentration.

1.2.2 Precision

Precision can be defined[154] as 'the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample.'

Repeatability[3] is the precision of a method under the same operating conditions over a short period of time. This involves multiple measurements of the same sample (different preparations) under the same conditions. Precision is expressed by Relative Standard Deviation (RSD) or Coefficient of Variation (CV) of a data set. RSD (%) = 100 SD/x, where SD is the standard deviation, x is the average of n measurements. Intra assay precision was performed using concentrations at three levels, 5, 10 and 15 µg/ml. Each concentration was analyzed in triplicate (n=3) and % RSD calculated.

1.3 Stability test of progesterone in nasal mucosa extracts of sheep

Freshly excised sheep nasal mucosa[57] was mounted on Franz diffusion cell and 15 ml of phosphate buffer, pH 6.2 was filled in the receiver compartment. After 4 h of extraction while stirring, extract solutions were collected. Progesterone was added at a concentration of 200 µg to the nasal mucosa extract and incubated at 37^{o}C up to 4 h. The amount remaining at predetermined time interval (1, 2, 3 and 4 h) were analyzed spectrophotometrically.

1.4 Membrane activity of carbomers, polycarbophil and cyclodextrins

A major limiting factor associated with the addition of absorption enhancers[145] and other excipients to a formulation for nasal administration was the potential toxicity to the nasal mucosa. Nasal absorption enhancers are required to be non-irritating, non-toxic and non-allergenic or at least to have immediate reversible effects.

Erythrocytes are commonly used as a model system for investigations into membrane interactions. They were available readily in large amounts and their lysis

is easily measured by colorimetric determination of haemoglobin release. The relative membrane activity of different enhancing agents identified in this way provided information about their biological effects.

1.4.1 *Preparation of erythrocyte stock suspension*

A stock suspension of erythrocytes was prepared on the day of the experiment. 4.5 ml of blood was collected from the marginal ear vein of four rabbits in a 15 ml plastic test tube containing 0.63 ml of trisodium citrate solution used as an anticoagulant. The tube was carefully inverted a few times to ensure mixing. It was then immediately centrifuged at 1500 rpm for 5 min and the supernatant removed. The erythrocytes were washed by the addition of an equal volume of phosphate buffer saline, pH 7.0 (PBS), centrifuging at 1500 rpm for a further 5 min and removal of the supernatant. This washing procedure was repeated three more times. A 2.0 ml sample of the washed cells was then added to the 15.0 ml PBS to produce the erythrocyte stock suspension which was used in experiments within 3 h of preparation.

1.4.2 *Preparation of the test solutions*

The solutions were prepared in PBS by dilution of a stock solution. They were prepared in concentrations at which they are going to be used in the experiments.

Table 2: Concentration of test solutions for membrane activity study

Sl.No.	Substance	Concentration mg/ml
1	Carbopol 974 solution	1
2	Carbopol 971 solution	1
3	Polycarbophil solution	1
4	B-CD	5
5	HPβ-CD	5
6	Propylene glycol (PG)	1
7	Tween 40	1
8	Tween 80	1

1.4.3 *Erythrocyte incubation procedure*[128]

A 0.5 ml aliquot of the stock erythrocyte suspension was added to 7 ml of the test solution in a test tube. The test tube was carefully inverted three times to ensure complete mixing and then incubated in a water bath at 37^{o}C for 10 min. The incubation mixture was then centrifuged at 1800 rpm for 2 min and the absorbance of the supernatant measured at 543 nm against a PBS blank. Each incubation was carried out in duplicate. Deionised water and PBS were used as control and were incubated with erythrocytes in duplicate.

The absorbance measurement from the PBS control incubations A_{PBS}, reflected minimum haemoglobin release under the experimental conditions. Values from test incubations A_{test} were corrected for this background haemolysis using the A_{PBS}. Total haemolysis was assumed to occur with deionised water A_{dw}. % of relative haemolysis was calculated using the formula,

$$\% \text{ haemolysis} = [A_{test} - A_{PBS}] / A_{dw} \times 100$$

2. PROGESTERONE-CYCLODEXTRIN PHYSICAL MIXTURES AND INCLUSION COMPLEXES

2.1 Physical mixtures of progesterone and CDs

The physical mixtures of progesterone and β-CD and HPβ-CD were obtained by thoroughly mixing the various components together with a spatula. The following physical mixtures were prepared by this method

1. Progesterone/ β-CD in molar ratio 1:1
2. Progesterone/HPβ-CD in molar ratio 1:1

2.2 Preparation of solid inclusion complexes of Progesterone and CDs

The following solid inclusion complexes containing cyclodextrins were prepared[65,145].

1. Progesterone/ β-CD complex: molar ratio 1:1
2. Progesterone/HPβ-CD complex: molar ratio 1:1

Progesterone–β-CD and progesterone- HPβ-CD inclusion complexes were prepared by freeze drying technique[155,156]. Accurately weighed β-CD and HPβ-CD were first dissolved in distilled water. Progesterone was added to these solutions in carefully predetermined molar ratio of 1:1. The entire solutions were stirred with a magnetic stirrer for 7 days at room temperature. The resulting solutions were filtered through 0.22 m filter. The filtrates were frozen at -40°C for 10 h in a Telstar L-3 freeze drier and then a vacuum was applied to obtain a pressure of 0.05 mbar. Temperature was increased from -40°C to 0°C in a 37 h cycle and after 2 h at this temperature it was increased to 30°C over 6 h. Vials containing the freeze dried products were plugged immediately after removal from the freeze dryer.

The preparation yield for the inclusion complex was calculated using the formula

$$\% \text{ Yield} = \frac{\text{Product (w)}}{\text{Drug (w) + CD (w)}} \times 100$$

3. CHARACTERISATION OF THE INCLUSION COMPLEX OF PROGESTERONE WITH β-CD AND HPβ-CD

3.1 Fourier transform infra red spectrophotometry (FTIR)

Infrared spectrophotometry has been employed as a useful tool[79] to identify the drug-excipient interaction. Samples (about 1% w/w) were mixed with potassium bromide powder and compressed to a 12 mm disc by a hydraulic press at 10 tons compression force for 30 s. Samples were analyzed in a FTIR Spectrophotometer (Shimadzu ABB Bomem) in the region 4600 to 400 cm^{-1}. Complex formation was evaluated by comparing the IR spectra of the solid complex and of the drug.

3.2 Differential Scanning Calorimetry (DSC)

DSC has been one of the most widely used calorimetric techniques[79] to study the solid state interaction of drug with cyclodextrins. DSC analysis of the solid complex, pure drug and cyclodextrins were performed using a Differential Scanning Calorimeter (TA Instruments, DSC 2010). All the samples (5-10 mg) were heated in crimped aluminum pans at a scanning rate of 10^{o} C/min in the temperature range 25^{o} to 300^{o} C.

3.3 Powder X-ray diffraction (PXRD)

PXRD technique[157] has been extensively used to study interaction between drug and cyclodextrins. The diffraction studies were carried out in a powder X-ray diffractometer (PAN Analytical, X'per PRO) using germanium monochromated Cu K (2.2 KW max) radiation in transmission mode. The samples were rotated during data collection to reduce orientation effects. PXRD pattern of solid complex, pure drug and cyclodextrins were recorded between $2\theta = 5$ to 50^{o} at 40 kV and 30 mA.

3.4 Scanning Electron Microscopy (SEM)

The impact of complexation on the drug was studied using a scanning electron microscope (Hitachi Model S-3000H). SEM was used to assess[158] the microscopic aspects of the drug, the cyclodextrins and the complexes formed. Although this method is not a very conclusive method to confirm complex formation, nevertheless it helps to assess the existence of a single component in the preparations obtained.

3.5 Drug Content

An accurately weighed amount of the complex was dissolved in methanol and assayed for progesterone spectrophotometrically at 240 nm against blanks prepared using same concentration of the ingredients except drug in the solvent. The amount of progesterone was calculated using a validated calibration curve (R^2 = 0.9996), repeatability coefficient of variation CV = 0.33 %.

3.6 Aqueous Solubility studies

CDs interact with poorly water soluble[156] compounds to increase their apparent solubility. The mechanism by which this solubilization occurrs is by inclusion complexation formation in which the guest and host molecules are in dynamic equilibrium with the complex. CD intervention is most applicable to Class II and IV compounds and the use of CDs could alter the properties of these classes of drugs so that they become Class I like in behavior.

The aqueous solubility of the compounds i.e., pure drug, progesterone/CDs physical mixture and progesterone/CDs inclusion complexes were determined at 25^{o}C in distilled water. Solubility was measured by shaking a well dispersed solute in excess (50 mg) in water (100 ml) until equilibrium was attained. Solute and solvent[159] were placed in well stoppered conical flasks and agitated at room temperature continuously for 24 h. After 24 h the solution was filtered. It was suitably diluted and absorbance was measured at 240 nm. The amount of progesterone was calculated using a validated calibration curve (R^2 = 0.9996) and repeatability coefficient of variation (CV) = 0.33 % (Table 8)

3.7 Dissolution

Dissolution studies of progesterone– CDs physical mixtures and inclusion complexes were carried out using rotating method with a USP XXII apparatus 2. The dissolution test was performed by spreading the powder samples, equivalent to 10 mg of progesterone over the dissolution medium consisting of 900 ml of distilled water maintained at 37^{o}C. Absorbance was measured at 240 nm for progesterone quantification. The dissolution tests were carried out for 60 min, at time intervals of 10 min and the results were subsequently computed with a standard calibration curve of the drug. A correction was made for volume loss after each sample was discarded. The dissolution experiments were conducted in triplicate.

Model fitting of the release profiles were carried out using four different models viz. Zero order, First order, Higuchi matrix and Peppas model. The percentage dissolution efficiency (% D.E) and Mean Dissolution Time (MDT) were

calculated. Dissolution efficiency has been used for the characterization of dissolution profiles. % DE is well suited to make quantitative comparisons amongst different formulations. Mean dissolution time (MDT) was calculated from the dissolution data using the following equation (Mockel and Lippold)[160] $MDT = (n/n+1)\, k^{1/n}$ where, n = release exponent and k = release rate constant

4. MUCOADHESIVE GELS

The bioadhesive properties[75] of polyacrylic acids (PAA) are well recognized. Three of the polyacrylic acids polymers selected in this study were carbopol 974, carbopol 971 and polycarbophil. PAA polymers were delivered as dry flocculated powders consisting of primary particles with an average diameter of about 0.2 μm. Each primary particle contained a network of cross linked polymer chains and will swell up to 1000 times their volume in contact with water to form a continuous gel when exposed to a suitable pH. Polycarbophil is a homopolymer of acrylic acid cross linked with divinylglycol. Carbopol is a copolymer of acrylic acid and a long-chain alkyl methacrylate cross-linked with allylethers of pentaerythritol. In most liquid systems carbopols and polycarbophil required neutralization by a base to induce sufficient increase in viscosity. Neutralization of the polymers caused increase in ionization of the carboxyl functionalities and the resulting intramolecular repulsion results in the polymer chains taking on an extended conformation with increased polymer entanglements. This lead to increase in gel viscosity.

4.1 Preparation of gels[145]

Full factorial design

Factorial design is an optimization technique, where all the factors were studied in all possible combinations. This technique was considered most efficient in estimating the influence of individual variables (main effects) and their interaction using minimum experimentation. A 3^2 full factorial design was constructed. The dependent variables selected for this study were concentration of the polyacrylic acid polymer (X_1) that is carbopol 974, carbopol 971 and polycarbophil at 1%, 1.5% and 2% and method of addition of cyclodextrins (X_2) that is no addition, as a

physical mixture with drug and inclusion complex with drug. The factor X_1 was selected on the basis of the preliminary studies carried out before implementing the experimental design. The dependent variables studied were diffusion co-efficient (D), apparent nasal permeability co-efficient (K_p) and bioavailability (F). The level of individual variables and values of codes are shown in Table 3 and 3a. The composition for the various formulations is shown in Tables 4-9. PAA was used as the mucoadhesive polymer. Propylene glycol was used as the solubilizer for the drug and benzalkonium chloride was the preservative at 0.01%. Total quantity of 10 g of formulation was prepared for each batch. The formulations were designated codes as described below

Table 2a: Formulation codes

PAA	CDs	F
C_1-carbopol 974	B: β-cyclodextrin	F 1,4,7-without cyclodextrins
C_2-carbopol 971	H: hydroxy propyl β-cyclodextrin	F 2,5,8-with cyclodextrins as a physical mixture with drug
P-polycarbophil		F3,6,9-with cyclodextrins as an inclusion complex with drug

In case of formulations F1, F4 and F7 progesterone was dispersed in propylene glycol and sonicated (Vibra Cell VCX 500, Sonics & Materials Ins.,) for 5 min. Required quantity of PAA was wet blended thoroughly with water. This was then suspended in water and allowed to swell. The polymeric dispersions was stirred in a magnetic stirrer for 60 min to which benzalkonium chloride (BKC) solution and the drug was incorporated in a drop wise manner and the pH was then adjusted to 7.0 by addition of triethanolamine. In case of formulations F2, F5 and F8 progesterone along with cyclodextrin was dispersed in propylene glycol and water mixture and sonicated (Vibra Cell VCX 500, Sonics & Materials Ins.,) for 5 min. Required quantity of PAA was wet blended thoroughly with water. This was then suspended in water and allowed to swell. The polymeric dispersions were stirred in a magnetic stirrer for 60 min to which BKC solution and drug dispersion was incorporated in a drop wise manner and the pH was then adjusted to 7.0 by addition

of triethanolamine. In case of formulations F3, F6 and F9 progesterone-inclusion complex was dispersed in propylene glycol and water and sonicated (Vibra Cell VCX 500, Sonics & Materials Inc.,) for 5 min. Required quantity of PAA was wet blended thoroughly with water. This was then suspended in water and allowed to swell. The polymeric dispersions were stirred in a magnetic stirrer for 60 min to which BKC solution and the drug complex dispersion was incorporated in a drop wise manner and the pH was then adjusted to 7.0 by addition of triethanolamine.

Table 3: A 3^2 full factorial design and level of independent variable

Sl.No.	Trial No.	Coded values	
		X1	X2
1	F1	-1	-1
2	F2	-1	0
3	F3	-1	1
4	F4	0	-1
5	F5	0	0
6	F6	0	1
7	F7	1	-1
8	F8	1	0
9	F9	1	1

Table 3a: Values of the codes used.

Sl.No	Coded values	Independent variables	
		X_1	X_2
1	-1	1%	Nil
2	0	1.5%	Physical mixture
3	1	2%	Inclusion complex

X_1 is the % concentration of poly acrylic acid polymers

X_2 is the method of addition of cyclodextrins in the gels

Table 4: Composition of the nasal gels prepared using carbopol 974

Ingredients	C_1F1	C_1BF2	C_1BF3	C_1F4	C_1BF5	C_1BF6	C_1F7	C_1BF8	C_1BF9
Progesterone (mg)	250	250		250	250		250	250	
complex equivalent to drug (mg)	-	-	250	-	-	250	-	-	250
β-CD (mg)	-	902	-	-	902	-	-	902	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Carbopol 974 (mg)	100	100	100	150	150	150	200	200	200
BKC 100 mg/ 100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10	10	10	10

Table 5: Composition of the nasal gels prepared using carbopol 971

Ingredients	C_2F1	C_2BF2	C_2BF3	C_2F4	C_2BF5	C_2BF6	C_2F7	C_2BF8	C_2BF9
Progesterone (mg)	250	250		250	250		250	250	
Complex equivalent to drug (mg)	-	-	250	-	-	250	-	-	250
β-CD (mg)	-	902	-	-	902	-	-	902	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Carbopol 971 (mg)	100	100	100	150	150	150	200	200	200
BKC 100 mg/100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10	10	10	10

Table 6: Composition of the nasal gels prepared using polycarbophil

Ingredients	PF1	PBF2	PBF3	PF4	PBF5	PBF6	PF7	PBF8	PBF9
Progesterone (mg)	250	250		250	250		250	250	
Complex equivalent drug(mg)	-	-	250	-	-	250	-	-	250
β-CD (mg)	-	902	-	-	902	-	-	902	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Polycarbophil (mg)	100	100	100	150	150	150	200	200	200
BKC 100 mg/100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10	10	10	10

Table 7: Composition of the nasal gels prepared using carbopol 974

Ingredients	C_1HF2	C_1HF3	C_1HF5	C_1HF6	C_1HF8	C_1HF9
Progesterone (mg)	250		250		250	
complex equivalent to drug (mg)	-	250	-	250	-	250
HPβ-CD (mg)	1183	-	1183	-	1183	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5
Carbopol 974 (mg)	100	100	150	150	200	200
BKC 100 mg/100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10

Table 8: Composition of the nasal gels prepared using carbopol 971

Ingredients	C_2HF2	C_2HF3	C_2HF5	C_2HF6	C_2HF8	C_2HF9
Progesterone (mg)	250		250		250	
Complex equivalent to drug (mg)	-	250	-	250	-	250
HPβ-CD (mg)	1183	-	1183	-	1183	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5
Carbopol 971 (mg)	100	100	150	150	200	200
BKC 100 mg/100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10

Table 9: Composition of the nasal gels prepared using polycarbophil

Ingredients	PHF2	PHF3	PHF5	PHF6	PHF8	PBF9
Progesterone (mg)	250		250		250	
Complex equivalent drug(mg)	-	250	-	250	-	250
HPβ-CD (mg)	1183	-	1183	-	1183	-
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5
Polycarbophil (mg)	100	100	150	150	200	200
BKC 100 mg/100 ml (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Water q.s (g)	10	10	10	10	10	10

4.2 Determination of drug content

50 mg of the gels were dissolved in methanol and 1 ml of this stock solution was diluted to 100 ml using phosphate buffer, pH 6.2 and absorbance was measured at 240 nm against blanks prepared using same concentration of the CDs and polymer in the solvent. The amount of progesterone was calculated using a validated calibration curve (R^2 = 0.9996) and repeatability coefficient of variation (CV) = 0.33 %.

4.3 Drug release measurements and determination of diffusion coefficient

Drug release from gels was measured[68] by the USP paddle method using containers with a fixed volume of 6 cm^3 covered with a mesh. The containers [58, 61,161] containing gels equivalent to 5 mg of progesterone were immersed in 200 ml phosphate buffer, pH 6.2 at 32^oC and stirred at 20 rpm. The tests were conducted at 32^oC to simulate the physiological temperature of the nasal cavity. The stirring rate was chosen to give adequate convection and to minimize surface erosion of the gels. A sample of 5 ml was collected at various time intervals and for each removed sample, fresh medium was added in order to keep the volume of dissolution medium constant. The concentration of progesterone after suitable dilution was determined by spectrophotometry at 240 nm. The amount of drug released plotted against square root time was a straight line during the initial period, and the diffusion coefficient was calculated from the slope of the graph.

Drug release kinetics

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equations like zero order (% release vs. t), first order (log % release vs. t) and Higuchi model (M_t/M_∞vs. $t^{1/2}$). In order to define a model which would represent a better fit for the formulation, drug release data was further analyzed by Peppas equation, $M_t/M_\infty=kt^n$, where M_t is the amount of drug released at time t and M_∞ is the amount released at time ∞, thus the M_t/M_∞ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of

drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

The release data was evaluated by the model-dependent (curve fitting) method using the PCP Disso V2.08 software (Pune, India).

4.4 *Ex vivo* permeation studies

The *ex vivo* permeation study was carried out by following the procedure described by Steffen Lang *et al*[162]. Tissue with nasal mucosa was excised from the noses of freshly slaughtered sheep. After removing the skin, tissue containing nasal mucosa was cut off with a sharp knife from the frontal part of the nasal conch (conchae nasals dorsales) above the *os incisivum* starting from the incisura nasoincisiva. The excised tissue was stored on ice during transport to the laboratory. At no more than 30 min after the excision, the mucosa was separated from the underlying cartilage by blunt stripping using a pair of tweezers. Samples were taken and inserted into the Franz diffusion chambers, the apical side of the tissue typically facing the donor compartment. Franz diffusion cells with an area of 1.01 cm^2 and a final volume of 15 ml were used to evaluate the progesterone release profiles from gel formulations in a closed system. The gel equivalent to 5 mg of progesterone was placed on the upper side of the nasal mucosa. The donor half cell was then carefully placed on top of the receptor half cell and clamped. The donor and the receiver compartment containing phosphate buffer (pH 6.2):ethanol (3:1) used as the diffusion medium to maintain sink conditions were kept in intimate contact and the temperature was maintained at 32^oC. The whole assembly was kept on a magnetic stirrer and stirred continuously. The samples were withdrawn at definite time intervals and equal amount of the phosphate buffer was replaced. The cumulative amount of progesterone permeated per unit area was plotted against time, and the slope of the linear portion of the plot (mg/min) was used as the steady state flux (J_{ss}). The apparent[15] nasal permeability coefficient K_p (cm/s) was calculated using the equation $K_p = J_{ss} \times 1/ [60\times C_v]$ in which J_{ss} is steady state flux (mg/cm^2min), C_v is the total donor concentration of the formulation (mg), 60 is the conversion of minutes to seconds.

4.5 Histopathological evaluation of the mucosa

Each piece of mucosa was carefully removed from the diffusion chamber, rinsed with phosphate buffer pH 6.2 after the *ex vivo* release experiments. Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections were cut using a rotary microtom and stained with hematoxylin and eosin. Sections were examined under light microscope, to detect any damage to the tissue during *ex vivo* permeation by a pathologist blinded to the study. The mucosa, fixed directly after isolation at the slaughter house was used as a control.

4.6 Bioavailability studies in rabbits

Rabbits of female sex were chosen for the study. The use of rabbits[142] offers a number of advantages. They were suitable for both pharmacokinetic and pharmacodynamic studies. Additionally, repeated experiments could be performed on the same animal after an appropriate drug wash out and recovery period. This would reduce the cost as well as variability in the data. The larger entrance into the nasal cavity (orifice) compared with rats enabled easy administration of the prepared formulations. Pharmacokinetic experiments could be performed without the need for anaesthesia, especially as some anaesthetics inhibit MCC which affect drug absorption. The total blood volume (approx. 300 ml) allowed for repeated sampling. The respiratory mucosa is pseudostratified and columnar ciliated epithelium with goblet cells.

White female rabbits[73] with a mean weight of 3±0.5 kg were acclimatized for 2 weeks before the study. Approval was obtained from the Institutional Animal Ethics Committee (No.IAEC/XII/08/CLBMCP/2008). The animals were fasted overnight prior to drug administration with free access to water. A two week wash out period was allowed between experiments. The formulations were administered intranasally at a dose[110] of 80 µg/kg body weight into each nostril. The animals, five in each group were kept conscious during the experiments and permitted to breathe normally through the nostrils.

Group 1 received an intravenous injection of parenteral solution (80 µg/kg body weight) of progesterone to calculate the absolute bioavailability. Group 2 – 10 received C_1F1 to C_1BF9. Group 12 – 17 received C_1HF2, C_1HF3, C_1HF5, C_1HF6, C_1HF8 and C_1HF9. Due to their low solubility, solution of progesterone i.v injection was prepared in sterile normal saline containing 10% v/v ethanol[110]. This preparation was coded as IV. A suspension of progesterone as complex with HPβ-CD and 0.05% carbopol 974 was prepared and this was coded as NS (Group 11).

To determine plasma progesterone concentration for pharmacokinetic analysis, blood samples were withdrawn from the marginal ear vein at predetermined time intervals of 05, 15, 30, 60, 120 and 240 min after administration of the drug. Plasma was separated by centrifugation at 2000 rpm and samples were stored at 5^{o}C until assay. Progesterone drug content was determined by using a competitive solid phase enzyme immunoassay (Progesterone EIA Biomerieux Italia, I-Rome).

A non compartmental pharmacokinetic model was used to analyze the plasma drug concentration-time profiles. The area under the serum concentration vs. time curves (AUC) up to the last data point at 4 h were calculated using the linear trapezoidal method. AUCs were corrected for basal levels of progesterone by subtraction of the mean blank AUC. The pharmacokinetic parameters area under the concentration-time curve from 0 to 240 min (AUC), peak concentration (C_{max}) and time to reach peak concentration (t_{max}) after both intravenous and intranasal administration were calculated independently of kinetic models by trapezoidal rule and from raw data. The absolute bioavailability following intranasal administration was determined by dividing the intranasal AUC by the intravenous AUC. The pharmacokinetic analysis was carried out using PK Solutions 2.0 (Summit Research Services, USA) software.

4.7 Statistical optimization of results

Several formulation and processing variables are found to affect the gel properties. The effect of several factors and their interactions could be determined

simultaneously by factorial design experiments. In the present work, a two factor three level (3^2) factorial design was employed to investigate the effect of factors such as different concentrations of polyacrylic acid polymers and method of addition of cyclodextrin on diffusion coefficient (D), permeability coefficient (K_p) and bioavailability (F). The independent variables selected were concentrations of polyacrylic acid polymers and method of addition of cyclodextrin. The response variable studied were Y_1, diffusion coefficient (D), Y_2, permeability coefficient (K_p) and Y_3, bioavailability (F). Three different levels of each independent variable were selected for the study. The three levels for polymer concentration chosen were 1%, 1.5% and 2%. The three levels chosen for the method of addition were nil addition, physical mixture and inclusion complex. The mathematical relationships containing only significant factors influencing each response were generated using multiple linear regression and analyzed on software PCP-RSM and Design Expert 7.1 trial (Statease Statistical Software Package).

5. TOXICOLOGICAL STUDIES

The safety[145] of nasal formulations can be determined by their direct effects on the nasal epithelium. i.e. local toxicity. The possible toxicological effects induced by multiple nasal administration of gels was investigate using the various models viz. slug mucosal irritation test, rabbit nasal irritation test and histological evaluation. The advantages of these methods are as follows:

Slug mucosal irritation test: This method using slugs is an excellent alternative to the use of vertebrates which is strongly recommended to be replaced by lower organisms for ethical and economic considerations.

Rabbit nasal irritation test: The advantages of this method are the possibility of using non-anaesthetized and non-sedated animals and the possibility of a repeated dose administration with determination of marker compounds.

Histological study: A histological evaluation of the nasal epithelium of the rabbits completed the study as it is a standard method for evaluation of cytotoxicity. The samples selected for the slug mucosal irritation test were the gels prepared with

polymer in the concentration of 2 % along with cyclodextrins as physical mixtures. This allows for exposure of higher concentration of polymer along with drug and CD to be exposed to nasal mucosa.

Preparation of 5% BKC gels for positive control

5% benzalkonium chloride (BKC) gels were used as the positive control since it was known that high concentrations of BKC cause toxicity. BKC gels were prepared using carbopol 974 at 1% concentration. BKC was dispersed in propylene glycol-water mixture and sonicated (Vibra Cell VCX 500, Sonics & Materials Ins.,) for 5 min. Required quantity of carbopol 974 was wet blended thoroughly. This was then suspended in water and allowed to swell. The polymeric dispersion was stirred in a magnetic stirrer for 60 min to which BKC was incorporated in a drop wise manner and the pH was then adjusted to 7.0 by addition of triethanolamine.

5.1 Experimental procedure using slugs[122,123,163,164]

Slugs (*Arion lusitanicus*) weighing between 3.0 to 4.0 g were used. They were collected in the local gardens during the rainy season when they are found in plenty. After collection they were stored in vented plastic box lined with paper towel moistened with phosphate buffer saline, (PBS) pH 7.4. The slugs were divided into groups and five slugs were placed in each group. Group 1 – 6 received formulations C_1BF8, C_2BF8, PBF8, C_1HF8, C_2HF8 and PHF8 respectively. Group 8 was the negative control group which did not receive any treatment. Group 7 consisted of five positive control slugs (placed on a petridish containing 50 mg of gel containing 5% benzalkonium chloride (BKC) and six series of five treated slugs placed on 50 mg of formulations. The slugs were placed individually in petridishes at 25^{o}C. After the 30 min contact period the % mucus produced was measured by weighing the petridishes containing the slugs before and after the contact period. The mucus production was expressed as % w/w of the body weight. For each slug the total mucus produced was calculated by adding up the mucus produced during each 30 min contact period and then the mean of the five slugs was calculated. The slugs

were then transferred to a fresh petridish with 1 ml PBS. After 30 min contact period the slugs were transferred to a fresh petridish containing 1 ml PBS.

The PBS was collected after 30 min with a micropipette and the slugs were placed in a fresh petridish containing 1 ml PBS for 1 h and then 1 ml PBS was added for another hour. The slugs were kept on a petridish on a moist filter paper after the treatment until the next day when it was taken up for the experiment. This procedure was repeated for five successive days. The PBS samples were analyzed for the presence of proteins, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) released form the body wall of slugs.

Analytical procedures

The protein and the enzyme determination were carried out using the Kone Lab 20 Auto Analyzer. Total protein content in the PBS samples was determined using the Biuret method. The lactate dehydrogenase activity and alkaline phosphatase activity were measured with enzyme kits and expressed as IU/l.

5.2 Rabbit nasal irritation test

White female rabbits weighing[122] between 2-2.5 kg were used for the study following the guidelines and approval of the Institutional Animal Ethics Committee (No.IAEC/XII/08/CLBMCP/2008). Three rabbits were used per group. Group 1 received formulation C_1BF8, Group 2 C_1HF8. Group 3 was the positive control and Group 4 was negative control. A total of 50 mg of the formulation per nostril was administered daily using blunt polyethylene tube. The nasal cavities were washed twice with 1 ml of phosphate buffer saline (PBS, pH 7.4) and the release of proteins and lactate dehydrogenase (LDH) was measured. For washing out the nasal cavity, the rabbits were held in supine position and upside down on their back; the back was lifted up making an angle of 45^o while the head was kept horizontally. In this way drainage of the PBS from the nasal cavity was prevented. The PBS was immediately collected from the nostrils after the washing by turning the rabbit back to its normal position. The gels were administered for 12 consecutive days. The nasal cavity was

washed out daily just before and 1 h after the administration of the gel. Rabbits which did not receive the gel were considered as the negative control group.

5.3 Histological study

At the end of each experiment the rabbits were euthanized by exsanguinations of the femoral arteries. Immediately after exsanguinations the nasal cavity was excised. The nose was then fixed in 4% neutral buffered formaldehyde solution and afterwards decalcified in 1N nitric acid solution for 72 h. Next the nasal cavities were immersed for 24 h in a 5% w/v potassium sulphate solution and then for 24 h in distilled water. The nasal cavity was transectioned and embedded in paraffin, sectioned, stained with haematoxylin eosin and evaluated with a light microscope for epithelial integrity and presence of granulocytes, mucus and blood. The examined mucosa was then scored according to a ranking system where, 0 = no damage, equal to control, 1 = less than 25% epithelial cells lost, 2 = less than 50% epithelial cells lost, 3 = less than 75% epithelial cells lost, 4 = only basal cells left, 5= all basal and epithelial cells lost.

6. RHEOLOGY

6.1 Effect of mucin on viscosity enhancement (synergy) and calculation of force of bioadhesion

It was generally accepted[165] that chain interlocking (physical entanglement), conformational change and chemical interactions (like hydrogen and Van der Waal bonds), which occurred between a mucoadhesive polymer and mucin (or mucus) were likely to produce changes in the rheological behavior of the two macromolecules species. The interaction at the functional group level often resulted in the formation of mixtures capable of exhibiting rheological synergy that is, to demonstrate gel like properties when mixed, greatly in excess of when the mucin and polymer dispersions were examined separately. Several authors[170] had suggested that rheological synergism between polymer and mucin could be used as an *in vitro* parameter to determine the mucoadhesive properties of a material. Determination of mucoadhesive bond strength was also important in the

development of mucoadhesive drug delivery systems as it could quantitatively compare different bioadhesive materials and allow for quality control testing. The rheological characterization of the polymers and the polymer/mucin mixtures were performed to determine whether exposure to mucus caused a significant alteration in polymer rheology.

To study the polymer-mucin interaction, mucin solutions were prepared in deionised water (DI). Dried mucin was gently hydrated in DI by gentle stirring for 10 min to yield a concentration of 10 % w/w solution. 100 mg of the mucin solution was mixed with 100 mg of each of the prepared gels to give a final concentration of mucin at 5 %. The rheological analysis[166] of all the prepared systems were measured using R.E.L Cone and Plate Viscometer (Research Equipment London Ltd.,). Type of cone used was 'C' at an angle of 0.5^{o} with diameter of 19.4 mm and volume of plate was 13 µL. Samples of each of the formulations were added to the plate and allowed to equilibrate for at least 2 min prior to test. The viscosity measurements were made at shear rates of 5 s^{-1} . The viscosity of the mucin solution (5%) was also measured.

6.2 Texture profile analysis

This is a technique used to characterize the mechanical properties of gels and other semi solids. The mechanical properties of three gel formulations (C_1F1, C_1HF2 and C_1HF3) were determined using a texture analyzer (QTS 25 Texture Analyser, Brookefield). Each gel sample was packed to a fixed height in a universal bottle. A stainless steel probe was compressed into the formulation at a defined rate of 4 mm/s to a depth of 1 cm. Two parameters (hardness and adhesiveness) were used to characterize the gel.

7. MUCOCILIARY CLEARANCE STUDY

The nasal mucociliary system[167] transports the mucus layer that covers the nasal epithelium towards the nasopharynx, by ciliary beating. Its function is to protect the respiratory system from damage by inhaled substances. Impairment of the mucociliary clearance can result in diseases of the upper airways. Therefore it is

important to study the effect of drugs and drug excipients on nasal mucociliary clearance. Also nasal mucociliary clearance has implications for nasal drug absorption and largely determines the absorption profile of nasal drug delivery since the residence time of drugs administered to nasal cavity is limited by mucociliary clearance[168,169].

7.1 Mucociliary transport rate (MTR) using frog palate

A modified *in vitro* technique[170] using frog palate explant was used to study mucociliary transport. Frog palate was obtained after dissecting the frog and immediately placing it in Ringer solution at room temperature. It was then immersed in reconstituted mucus solution for 5 min.

The MTR of the polymer gels across the frog palate explants was measured by placing a small quantity of the gel spiked with activated charcoal particles in the center of the explant. The movement of the charcoal particles was followed using a microscope using ×10 magnification with a 1 mm calibrated eyepiece. Control studies were performed on the explant prior to each gel MTR measurement to verify that the explant still retained normal ciliary movement. A suspension of charcoal-spiked, reconstituted mucus was used as the control and MTR measurements were made in an identical manner with the gel. After each measurement the explant was rinsed with ringer solution to remove the previous sample and the surface was replenished with reconstituted mucus. Mucociliary transport rate was calculated using the formula

% MTR decrease = (control MTR – gel MTR)/control MTR ×100

8. STABILITY STUDIES

The stability studies[171] were carried out according to ICH guidelines. The design and execution of the stability study followed the principles outlined in the ICH parent guidelines. The purpose of the study was to establish, based on testing a minimum of three batches of the product, a retest period or shelf life and label storage instructions applicable to all future batches manufactured and packaged

under similar conditions. The progesterone nasal gels were stored in clean, dry, moisture proof bottles. The samples chosen were C_1BF3 and C_1HF3. They were stored in air tight containers and placed in the humidity chamber at $40^oC \pm 2^oC/75\% \pm 5\%$ RH. The accelerated studies were carried out for six months and the testing frequency was at 0, 1, 3 and 6 months. The recommended storage condition for Climatic Zone IV was followed which was $40^oC \pm 2^oC/75\% \pm 5\%$ RH. The attributes tested were appearance, pH, assay, viscosity and *ex vivo* release study.

The following were considered as significant change

- A 5% change in assay from initial value
- Failure to meet acceptance criteria for appearance, physical attributes and functionality test.

Statistical tests

Statistical analysis of all the data obtained was performed using GraphPad Prism version 4.03 (GraphPad Software, Inc. San Diego, CA). For comparison of data one way analysis of variance ANOVA using Dunnett multiple comparisons test and independent samples t test were used. The level of statistical significant was chosen as less than 0.05 (i.e. $P < 0.05$).

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Identification of the drug: The I.R spectrum of the drug progesterone was compared with the reference spectrum given in Indian Pharmacopoeia and was found to be similar.

1.1 Compatibility studies

The IR spectra of the pure drug and mixed drug samples with excipients are shown in Fig.3-6. Results obtained from the spectra showed that under stressed conditions the polymers does not influence any interaction with the drug. All the functional groups assigned in the wave numbers (Table 10) in all the different polymer mixtures exhibited maxima which are around the same wavelength and had similar intensities to that of the reference spectrum.

DSC curves obtained for pure drug and polymers and their corresponding physical mixtures are shown in Fig.7-13. The progesterone differential scanning calorimetry thermogram presented a single characteristic endothermic melting peak at 138.84^{o}C. It could be seen that in the case of the physical mixtures with carbopol 974, carbopol 971 and polycarbophil the endothermic peak of progesterone was at 137.75 ^{o}C, 136.07 ^{o}C and 136.66 ^{o}C which is similar to that of pure drug sample.

The formulation additives used did not affect the stability and there was no chemical interaction between the drug and the polymers as per FTIR and DSC studies. This indicated the stable nature of progesterone in the solid admixtures of the drug and various excipients.

1.2 Calibration curve of progesterone

1.2.1 Linearity and Range

The values for the absorbance at different concentration are shown in Table 11 and Fig.14.The equation for the resultant calibration curve was y = 18.42x + 0.213.

The calibration curve constructed for samples covered a range of concentrations from 2 to 24 µg/ml and was found to be linear with a linear regression coefficient value of 0.9996.

1.2.2 Precision

Intra assay precision (Table 12) was determined using three different concentrations. Each concentration was determined in triplicate and the intra assay was found to be less than 2% RSD for all samples and hence method was precise.

1.4 Stability test of progesterone in nasal mucosa extracts of sheep

For the delivery of drug through the nasal mucosa it is critical that the drug remain stable physiochemically and enzymatically for a certain period of time. One of the advantages of nasal delivery is the low enzymatic activity of nasal secretions. But nasal secretions contain enzymes and the low bioavailability of certain drugs through nasal delivery is due to the enzymatic degradation.

The results of the stability test of progesterone in nasal mucosal extracts are shown in Table 13 and Fig.15. It was found from the study that progesterone was stable for 4 h in the mucosa extract. The starting concentration was 200 µg, the amount remaining at 1, 2, 3 and 4 hr were 199.5±2.3 199.4±1.6 198.2±6.0 and 197.1±4.9 µg respectively. From this it was concluded that progesterone was stable in the nasal mucosa and hence suitable for nasal administration.

1.5 Membrane activity of carbomers, polycarbophil and cyclodextrins

The relative membrane activity of different substances identified by this method provided information about their biological effects and possible mechanism of action. The two controls used were the deionized water and PBS. The readings obtained for PBS indicated the relative stability of the erythrocytes in the buffer. The effect of the different substances used on the stability of erythrocytes in PBS compared to deionized water was therefore considered to reflect their membrane activity (Table 14 and Fig.16). The haemolysis due to the carbomers and the CDs

was compared to the control PBS incubations. The carbomers showed a haemolytic activity between 2.3-3.8%. The difference in the haemolytic activity between the three carbomers was not found to be significantly different (P>0.05). The two absorption enhancers used CDs, β-CD and HPβ-CD showed a haemolysis of

8.27 % and 6.39 % respectively. Tweens were the most lytic agent among the materials used. It resulted in about maximum level of haemolysis of 35.34% and 36.65% at the concentration used.

Hence based on the % haemolysis studies it was found that the absorption enhancers chosen for this study, which was β-CD and HPβ-CD, show some level of membrane activity. The haemolytic activity of cyclodextrins[145] was probably the result of membrane disruptions, caused by the removal of membrane components such as phospholipids, proteins and cholesterol. It was observed that haemolysis by cyclodextrins was easily detectable in plasma free medium *in vitro* which was not the case when it is observed *in vivo*. Hence many studies had shown that the CDs could be used *in vivo* without adverse effects, Also it has been shown[172] that haemolytic activity is 4 to 10 times smaller in serum than in buffer. Hence from these preliminary studies it was concluded that carbomers, CDs and propylene glycol (PG) as solubilizer with low membrane perturbing activity could be safely used for nasal preparations as it is toxicological safe. Tweens were not selected as solubilizer due to high membrane activity.

2.2 Preparation yield of progesterone inclusion complex

Preparation yield was evaluated for both the inclusion complexes using β-CD and HPβ-CD and shown in Table 15. The molar ratios for the complexation of progesterone with both β-CD and HPβ-CD at 1:1 and gave preparation yields of 90.4% and 92.8% respectively. The preparation yields could be considered as good.

Table 10: Assignment of wave numbers in drug mixture samples.

Assignment	Proges-terone	Drug + C-974	Drug + C- 971	Drug + P
C_3=O (cm^{-1})	1661.9	1664.68	1662.07	1662.79
C_{20}=O (cm^{-1})	1699.1	1700.01	1698.93	1698.97

C-974 indicates carbopol 974; C-971, carbopol 971; P, polycarbophil

Table 11: Calibration curve of progesterone.

Sl.No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	2	0.098
3	4	0.199
4	6	0.309
5	8	0.417
6	10	0.528
7	12	0.639

Table 12: Intra assay precision data

Sl.No	Range (µg/ml)	Average concentration recovered (µg/ml)	S.D	% RSD
1	4	3.92	0.23	0.23
2	8	7.79	0.19	0.20
3	12	11.86	0.54	0.55

S.D: Standard deviation; %RSD: Relative standard deviation

Table 13: Stability testing of progesterone in nasal mucosa extracts of sheep

Sl.No	Time (h)	Drug Content (µg)	Drug Content (%)
1	Initial	200	100
2	1	199.5±2.3	99.75
3	2	199.4±1.6	99.7
4	3	198.2±3.0	99.1
5	4	197.1±4.9	98.6

All values are expressed as mean ± SD, n = 3

Table 14: Membrane activity of ingredients

Sl.No	Substance	Absorbance	% Haemolysis
1	Deionized water	0.532 ± 0.011	-
2	PBS	0.041 ± 0.016	-
3	Carbopol 974 solution	0.060 ± 0.036	3.57
4	Carbopol 971 solution	0.061 ± 0.056	3.76
5	Polycarbophil solution	0.053 ± 0.044	2.26
6	β-CD	0.085 ± 0.041	8.27
7	HPβ-CD	0.075 ± 0.052	6.39
8	Propylene glycol	0.119± 0.044	14.66
9	Tween 40	0.229 ± 0.65	35.34
10	Tween 80	0.236 ± 0.42	36.65

All values are expressed as mean ± SD, n = 2

Table 15: Preparation yield of progesterone inclusion complex

Sl.No	Inclusion complex	% yield
1	Progesterone-β-CD	90.4
2	Progesterone-HPβ-CD	92.8

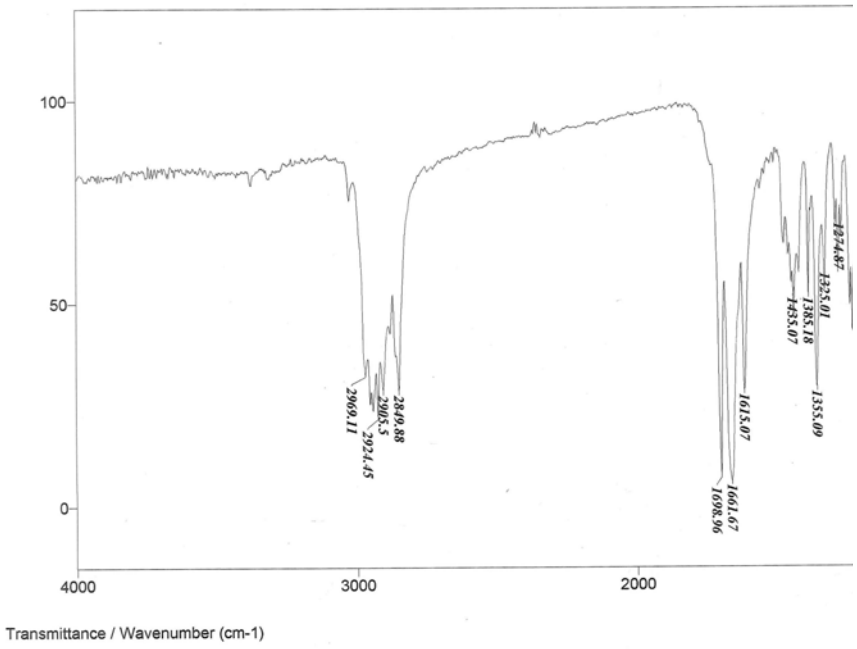

Fig.3 Fourier transform infrared spectroscopy spectra of progesterone

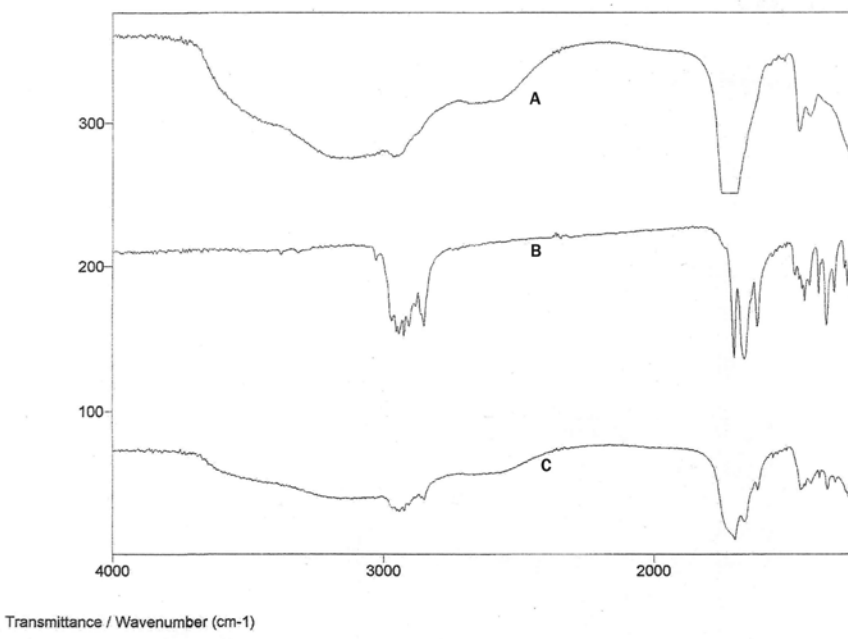

Fig.4 Fourier transform infrared spectroscopy spectra of carbopol 974 (A), progesterone (B) and c
mixture (C)

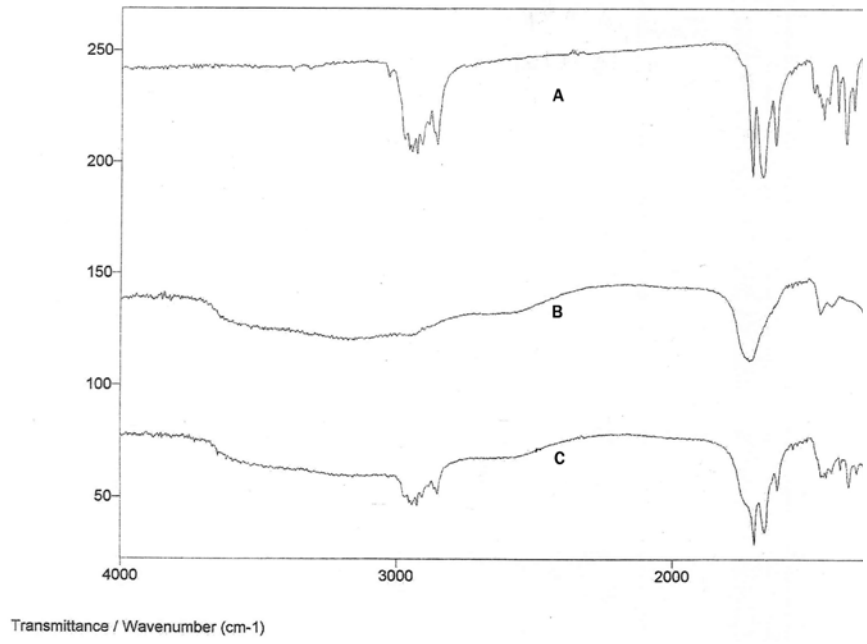

Fig.5 Fourier transform infrared spectroscopy spectra of progesterone (A), carbopol 971 (B), and c mixture (C)

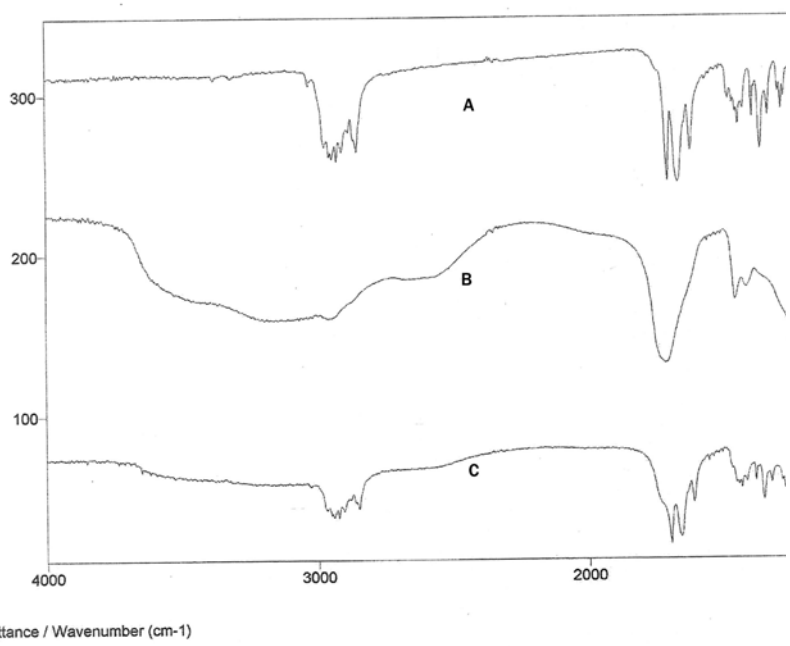

Fig.6 Fourier transform infrared spectroscopy spectra of progesterone (A), polycarbophil (B), and p mixture (C)

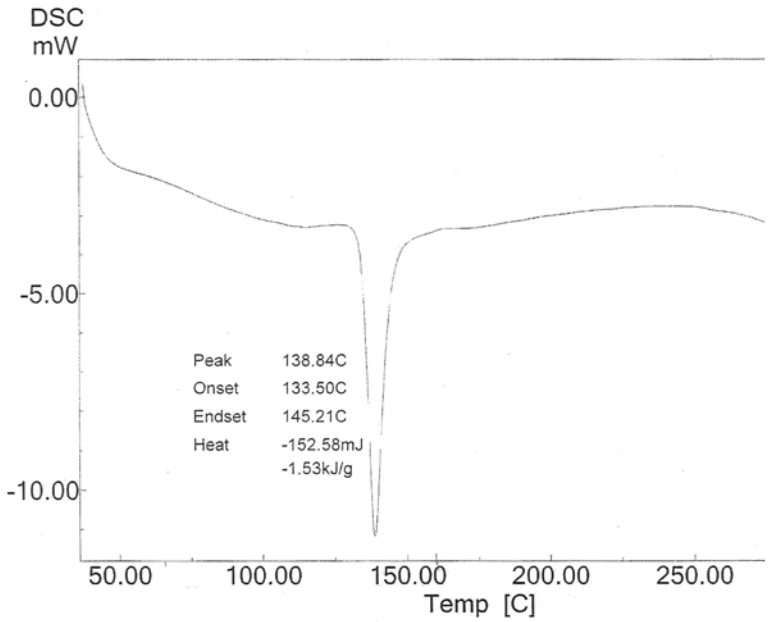

Fig.7 Differential scanning calorimetry spectra of progesterone

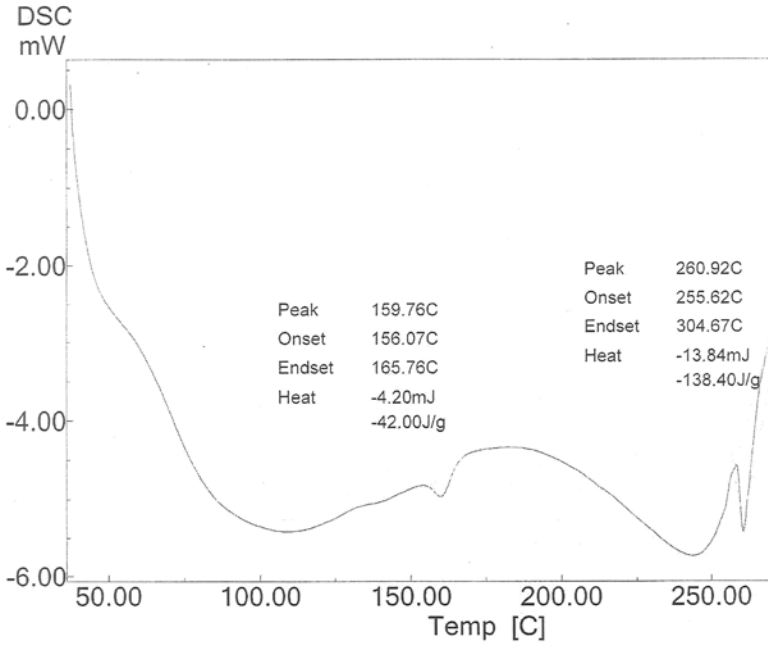

Fig.8 Differential scanning calorimetry spectra of carbopol 974.

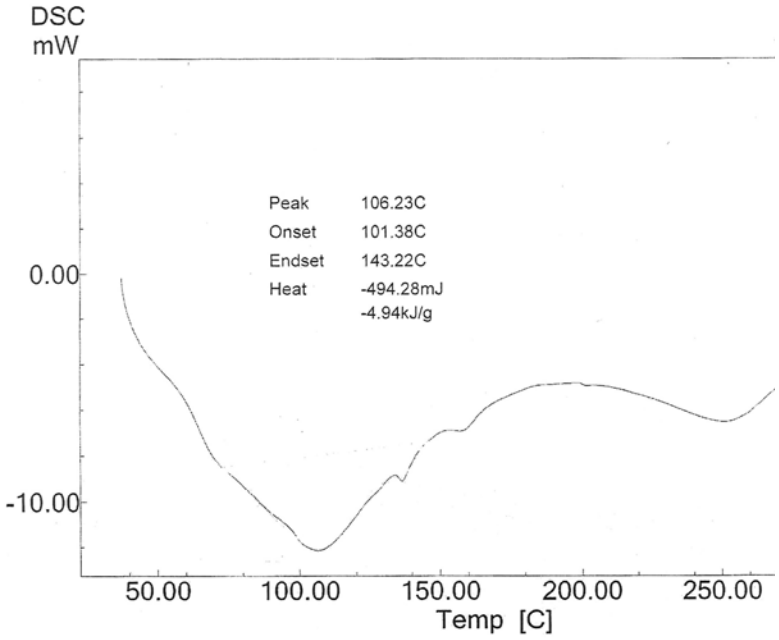

Fig.9 Differential scanning calorimetry spectra of carbopol 974 and progesteron

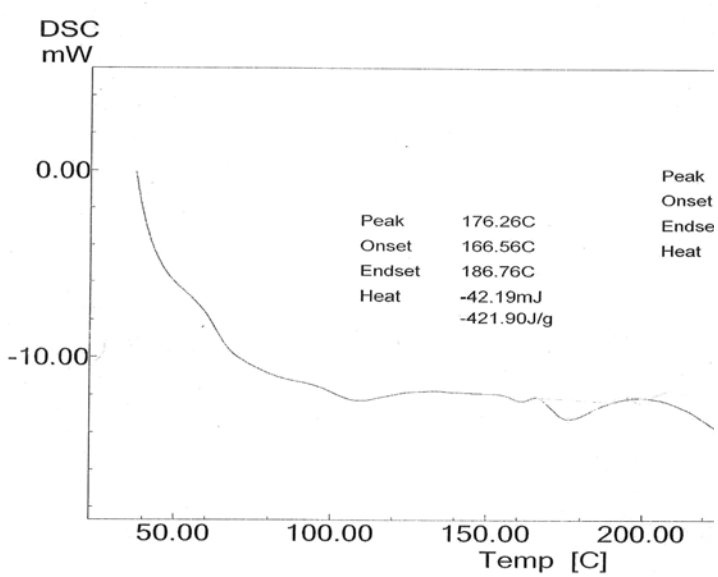

Fig.10 Differential scanning calorimetry spectra of carbopol 971

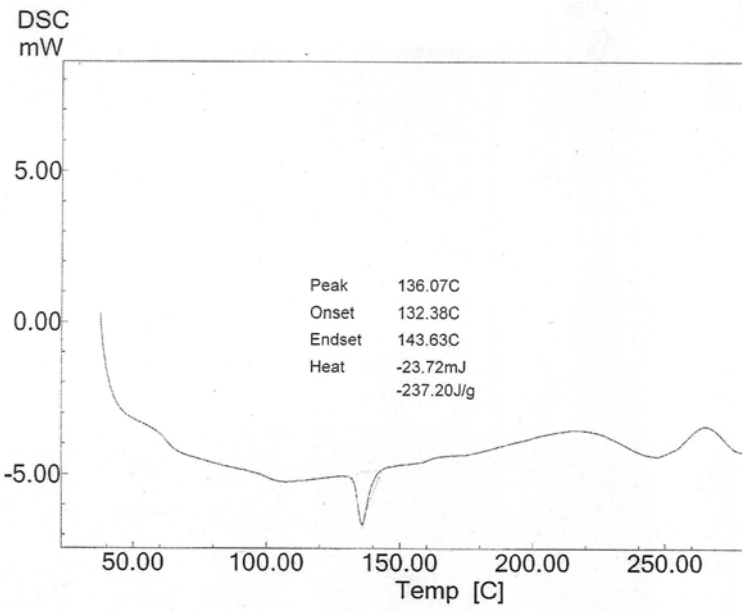

Fig.11 Differential scanning calorimetry spectra of carbopol 971 and progesteron

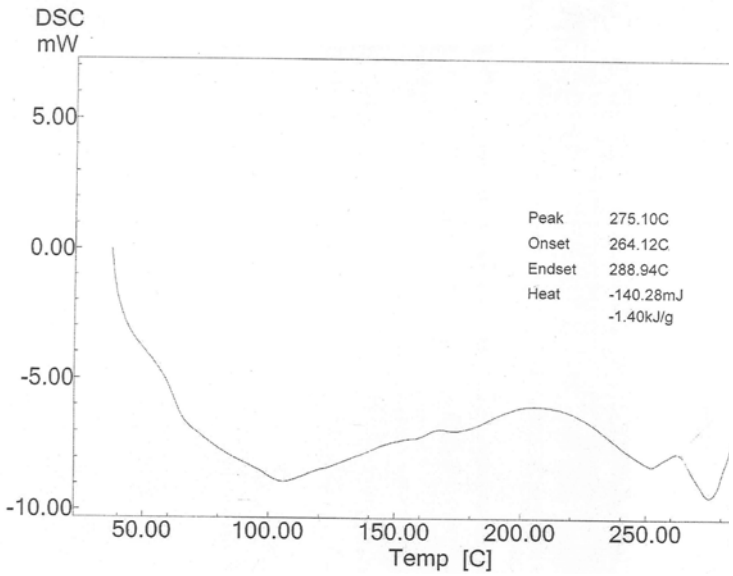

Fig.12 Differential scanning calorimetry spectra of polycarbophil

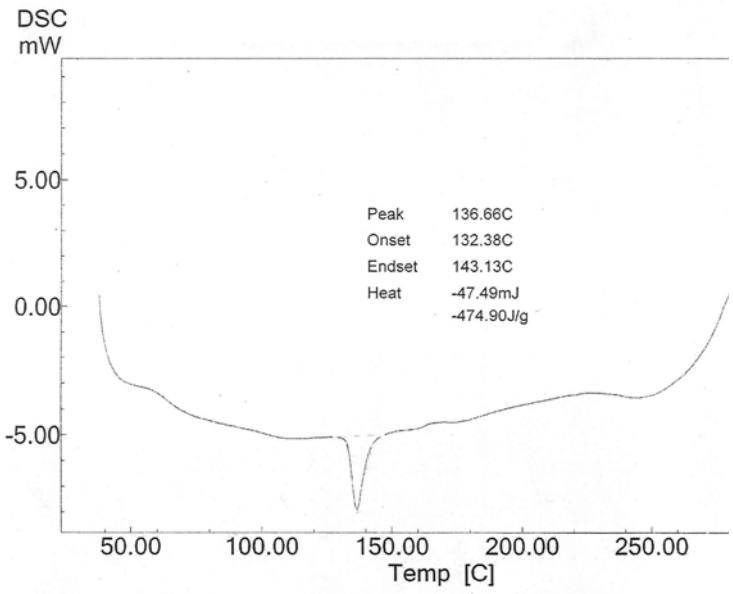

Fig.13 Differential scanning calorimetry spectra of polycarbophil and progesteror

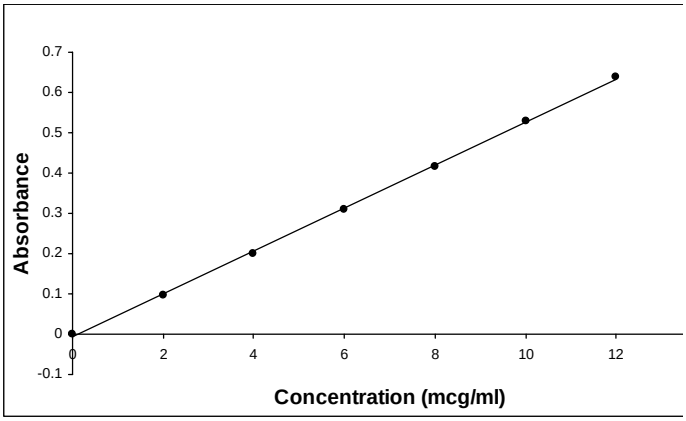

Fig. 14. Calibration curve of progesterone in phosphate buffer, pH 6.2 at 24(

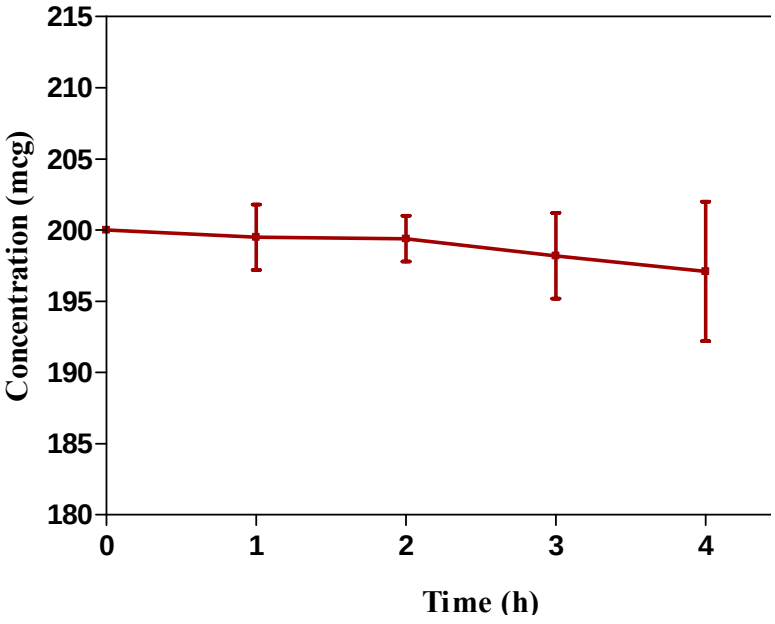

Fig. 15. Stability test of progesterone in nasal mucosa extract

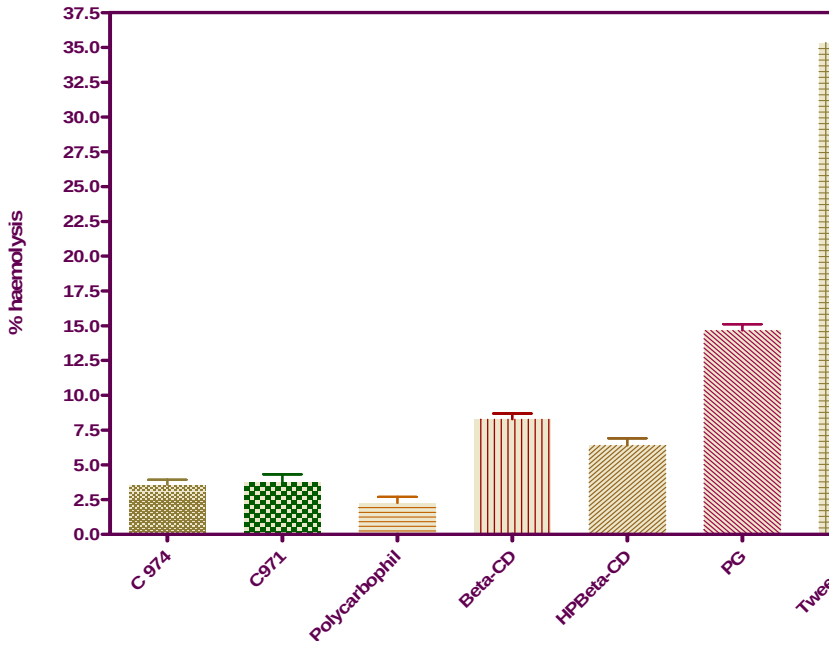

Fig. 16. Membrane activity of various ingredients

3. Characterization of the complex

3.1 Fourier transform infra red spectrophotometry (FTIR)

Fig.17 shows the IR spectra of progesterone, β-CD and progesterone-β-CD complex. Fig. 18 shows the IR spectra of progesterone-HPβ-CD complex . The IR spectra of progesterone illustrate the presence of the carbonyl stretching bands at 1661.9 cm^{-1} and 1699.1 cm^{-1}. Cerchiara *et al* [79] assigned these peaks to C_3=O in and C_{20}=O in progesterone respectively. The spectra of pure β-CD presented a broad band between 3100 cm^{-1} and 3800 cm^{-1} attributed to free OH groups, between 2800 cm^{-1} and 3100 cm^{-1}corresponding to bound OH, at 1626 cm^{-1}due to water molecules present in the cavity and a large band that displayed distinct peaks between 900 cm^{-1} and 1200 cm^{-1}, responsible for C-O vibrations. In case of HPβ-CD, IR spectra (Fig.19a) showed a band between 2800 and 3000 cm^{-1} corresponding to bound OH group, distinct peaked 1644 cm^{-1} and distinct peaks between 1000 and 1200 cm^{-1} .

The IR spectra of physical mixtures of progesterone and β-CD and HPβ-CD are shown in fig.19 and 19a. In these spectra, bands of the CDs overlap with progesterone main characteristic peak. No such overlap was observed in the case of the inclusion complex for which the peaks assigned to the stretching bands of the carbonyl group of progesterone disappeared. These could be explained by the dissociation of the intermolecular hydrogen bonds of progesterone through inclusion complexation.

3.2 Differential Scanning Calorimetry (DSC)

DSC was performed on the raw materials and on the inclusion complexes and is shown in Fig.20-21. The thermogram of progesterone showed the characteristic endothermic peak at 138.84 ^oC corresponding to the drug melting. Hence no polymorphs of progesterone could be found by DSC study. Endothermic peaks appeared at 118.1^oC in the thermograms for β-CD. This endothermic peak[34] was attributed to the loss of the water molecules inside the cavity of the cyclodextrin. In the thermogram of HPβ-CD, two endothermic peaks were observed. In the temperature range between 100^oC and 150^oC, loss of water occurred and near 330^oC the endothermic peak corresponding to HPCD fusion was observed.

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With regard to the complexes, the disappearance of the endothermic peak of progesterone was observed in all the complex thermograms. The disappearance of the thermal feature of the drug indicated that progesterone had penetrated into the CD cavity and replaced the water molecules which confirmed the inclusion complex. Also progesterone was no longer crystalline in free form and that it had converted to amorphous state. In case of physical mixtures (Fig.22), the progesterone peaks were shifted to slightly lower temperatures. The progesterone peaks were observed at 129.8 ^{o}C and 134.27 ^{o}C for β-CD and HPβ-CD respectively. These results were explained by the existence of a very weak interaction between progesterone and the cyclodextrins.

3.3 Powder X-ray diffraction (PXRD)

PXRD patterns for solid complex, pure drug and cyclodextrins are shown in Fig.23-26. The crystalline nature of progesterone was clearly demonstrated by its characteristic PXRD pattern containing well defined peaks. The drug characteristic peaks were observed at 12.86, 16.99, 22.61, 26.65 2θ values with intensities of 53.5%, 100%, 22.02% and 17.04% respectively. The β-CD exhibited well defined peaks at 12.5 and 19.2 2θ values with intensities[173]of 100% and 82% respectively. Similarly HPβ-CD also exhibited peaks at 18.95 and 19.72 2θ with intensities of 100 and 24.4 % respectively.

Even though the solid complexes displayed characteristic peaks corresponding to the crystalline drug, the peak intensities were diffused, less intense, less sharp and reduced which indicated decrease in the drug crystallinity and that new less crystalline solid phase had been obtained.

3.4 Scanning Electron Microscopy (SEM)

The particle morphology of progesterone β-CD and HPβ-CD can be seen in SEM photographs presented in Fig.27-31. Pure progesterone was characterized by the presence of crystalline particles of regular size. Pure β-CD also appeared crystalline, irregular, without any definite shape whereas HPβ-CD appeared as shrunken circular particles. The inclusion complexes with both β-CD and HPβ-CD showed a change in morphology and crystalline nature. Small size particles tended to aggregate suggesting existence of an amorphous product with the presence of a single component in the complex, thus suggesting maximum or complete complex formation.

The IR, DSC, PXRD and SEM studies collectively suggested a stronger interaction between drug and cyclodextrins in the solid complex due to formation of inclusion complex.

3.5 Drug Content of physical mixture and inclusion complex

Progesterone was complexed with two cyclodextrins, β-CD and HPβ-CD in the molar ratio 1:1 The drug content determined was found to be 191.9 mg/g and 160.3 mg/g respectively (Table16).

3.6 Solubility

Cyclodextrins are cyclic oligosaccharides with hydrophilic outer surface and a somewhat lipophilic central cavity. The central cavity provides a lipophilic microenvironment into which suitably sized drugs may enter and include. No covalent bonds are formed or broken during the drug/CD complex formation and in aqueous solutions, the complexes are readily dissociated. The solubility results are the mean and standard deviation of 3 replicates and is shown in Table 17. The apparent solubility of progesterone without cyclodextrins was 8.2 µg/ml. For the physical mixtures and inclusion complexes the solubility results were also expressed as the increment of solubility when compared with the solubility of the drug alone. From the results it could be seen that the solubility was significantly increased through the formation of inclusion complexes with β-CD and HPβ-CD. The increase in the solubility in the complexes to 34.1µg/ml and 666.2 µg/ml of

inclusion complexes with β-CD and HPβ-CD showed an enhancement of 4.16 and 81.24 times. The solubility of progesterone was significantly different in both the complexes. This was attributed to the lower aqueous solubility of β-CD at 18.5 mg/ml due to its rigid nature because of intermolecular hydrogen bonding occurring inside the cavity. Compared to that, its derivative HPβ-CD had an aqueous solubility of 400 mg/ml. The increase in solubility was attributed by Zannou *et al.*[174] to the reverse flexibility due to disruption of hydrogen bonding due to the substitution of some hydrogen bonds by hydrxypropyl groups. It was also observed by Gonzalez-Gaitano *et al.* [175] that the aggregation displayed by β-CD with partial substitution of the hydroxyl groups was much weaker hence solubility was increased. This fact could be attributed to the lower aqueous solubility of progesterone in the β-CD complex compared to the corresponding values for HPβ-CD. The enhancement seen in case of the physical mixtures were less pronounced than that of the corresponding inclusion complexes.

3.7 Dissolution parameters of progesterone inclusion complexes and physical mixtures

The results of the dissolution profile of progesterone physical mixture and inclusion complex with cyclodextrins HPβ-CD and β-CD are shown in Table 18 and Fig. 32. The dissolution parameters and mechanism of drug release are shown in Table 19.

Dissolution of progesterone/HPβ-CD complex was instantaneous. Almost the entire progesterone included in the complex was released in less than 20 min. The improvement in the dissolution with the progesterone/β-CD complex was less pronounced. The time taken for 90% drug to be released for HPβ-CD and β-CD complexes was 0.8 min and 70.9 min respectively. In case of the physical mixtures 59.2% and 22.8% of the drug was released at the end of 60 min for HPβ-CD and β-CD respectively. The time taken for 90% of the drug to be released for β-CD and HPβ-CD physical mixtures was 510 min and 146 min respectively. The dissolution rate of progesterone from complexes and physical mixtures was found to be in the following order P:HPβ-CD (complex) > P: β-CD (complex) > P:HPβ-CD (physical mixture)>P: β-CD (physical mixture).

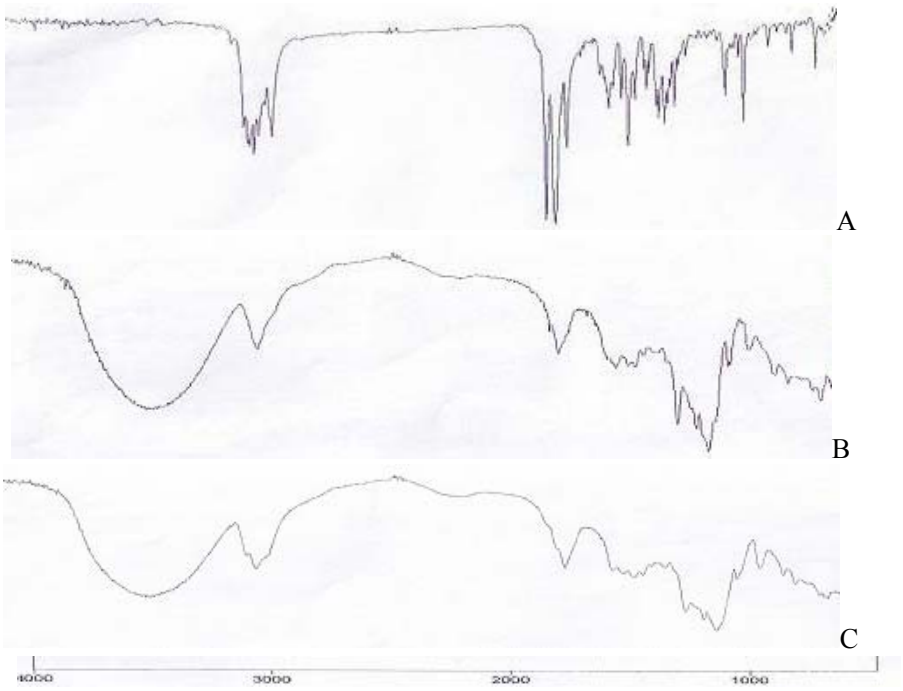


Fig.17 Fourier transform infra-red spectroscopy spectra of Progesterone (A), β-CD(B) and Progesterone-β-CD complex (C).

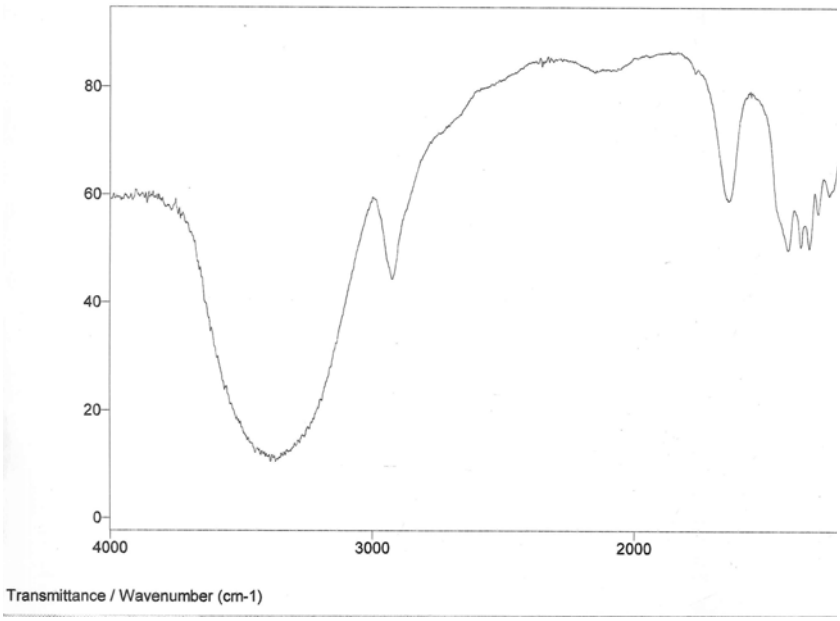


Fig.18 Fourier transform infrared spectroscopy spectra of progesterone-HPβ-CD incl

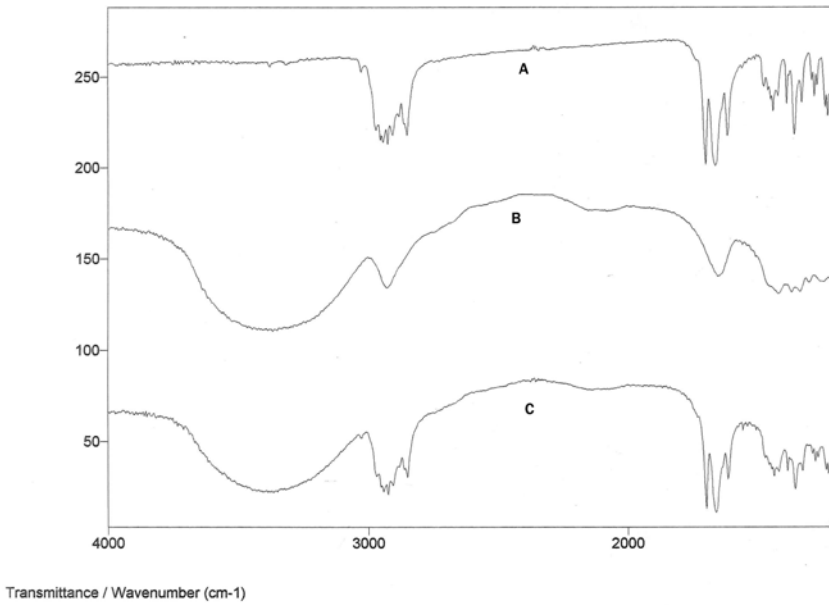


Fig.19 Fourier transform infrared spectroscopy spectra of progesterone (A) and progesterone- β-CD physical mixture (C).

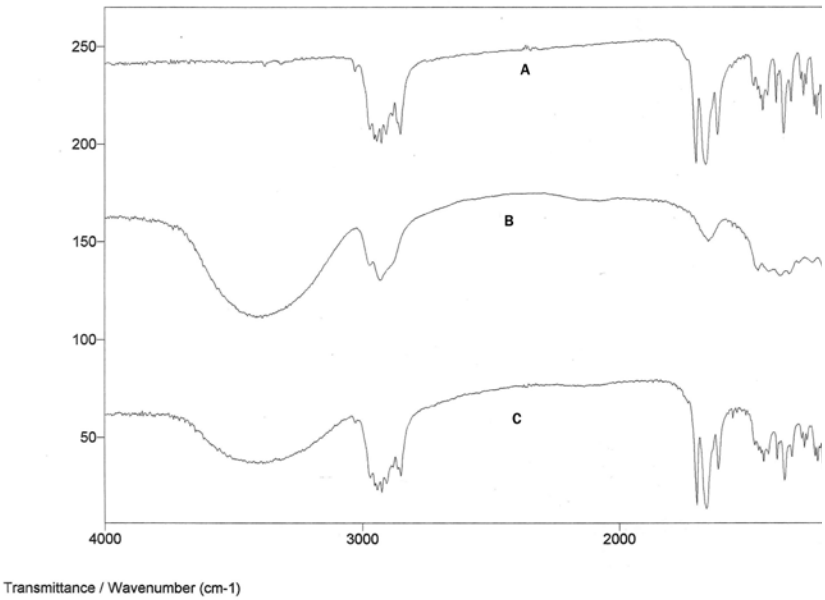


Fig.19a Fourier transform infrared spectroscopy spectra of progesterone (A), HP and progesterone-HPβ-CD physical mixture (C).

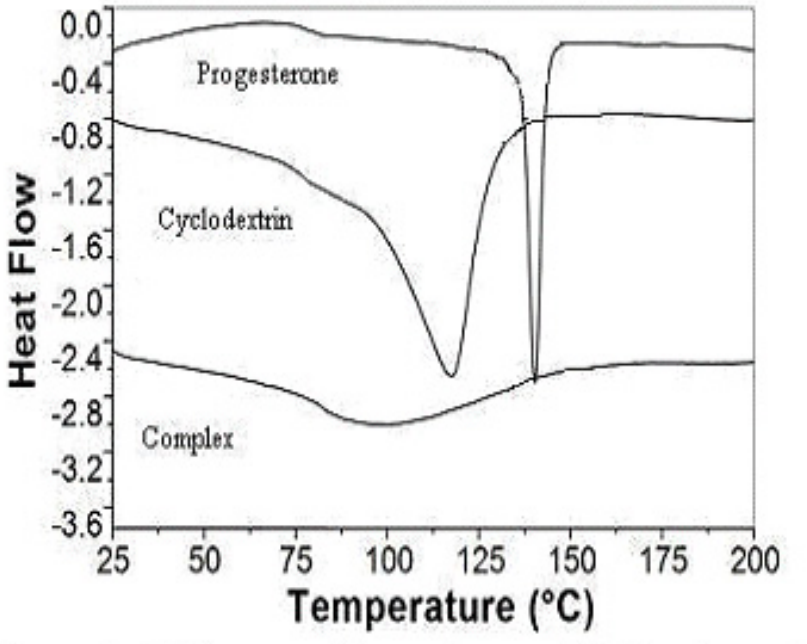
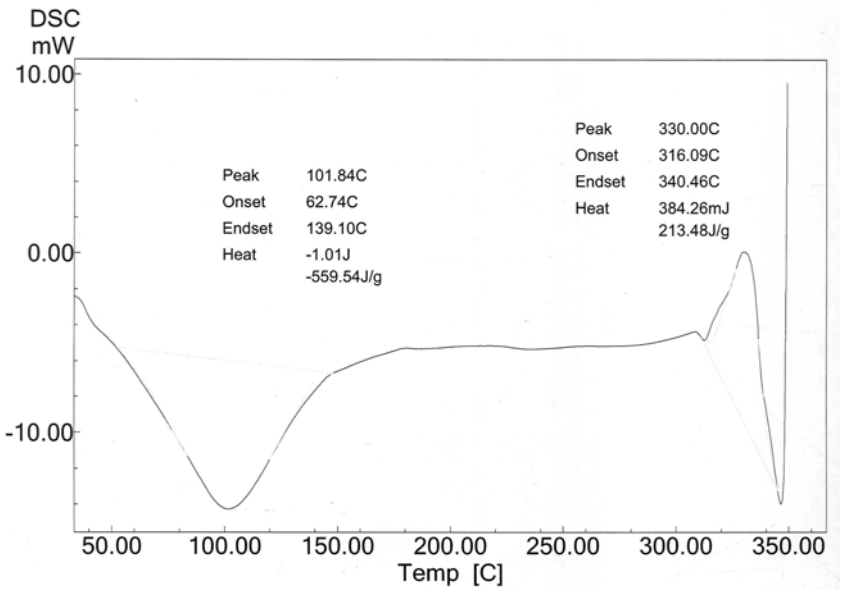
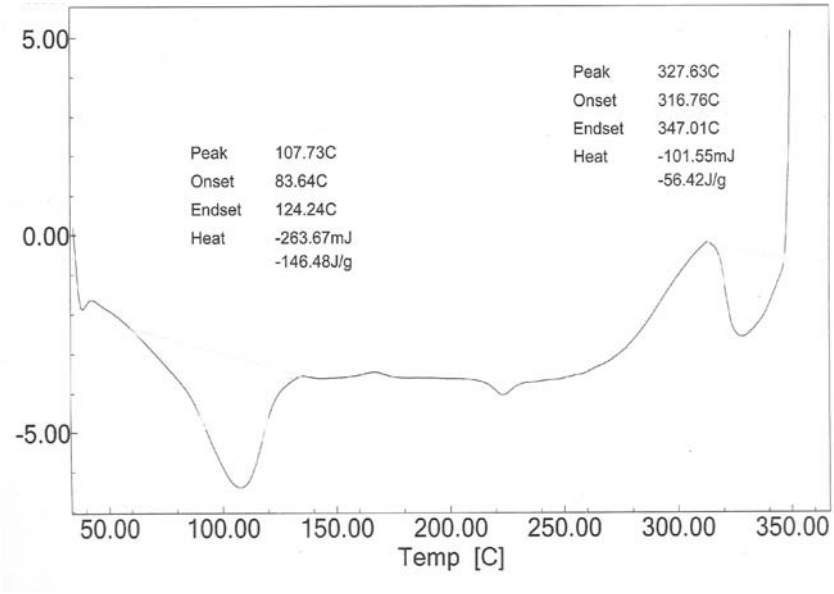


Fig.20 DSC curves of progesterone, β-CD and progesterone-β-CD complex

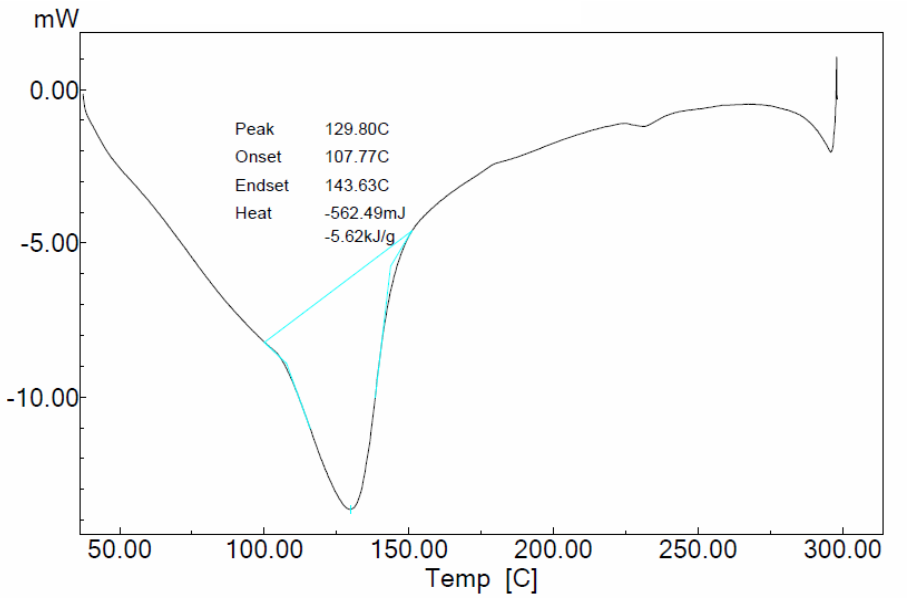


(a)

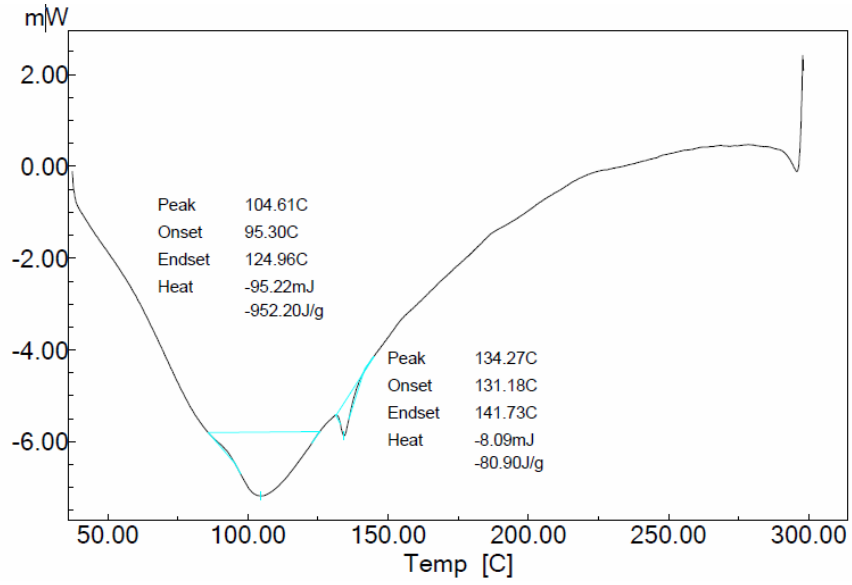


(b)

Fig.21 Differential scanning calorimetry spectra of HPβ-CD (a) and progesterone - HPβ-CD complex (b)



(a)



(b)

Fig. 22 Differential scanning calorimetry spectra of progesterone - β-CD physical mixture (a) and progesterone - HPβ-CD physical mixture (b)

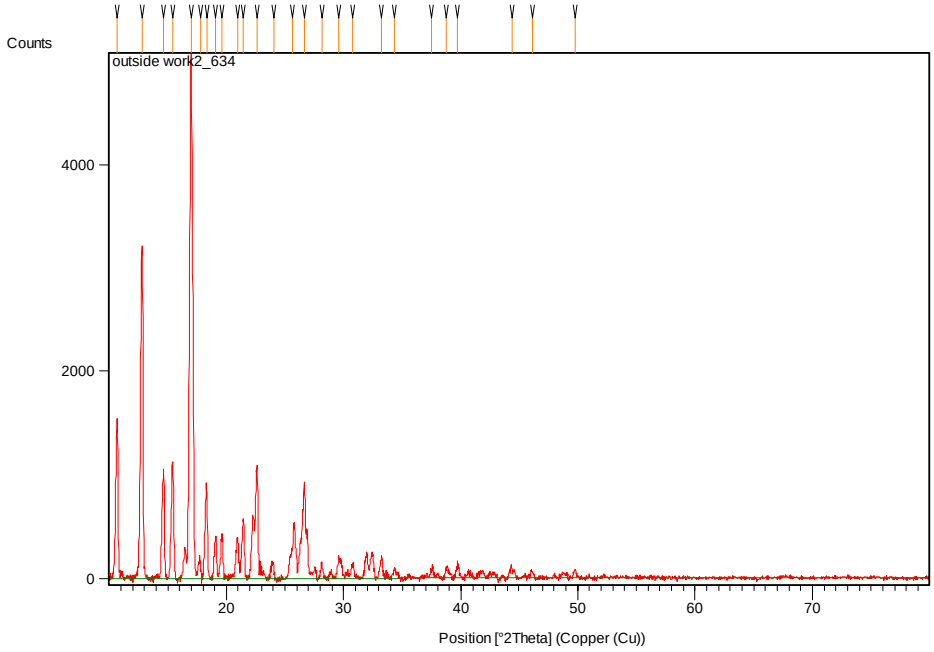


Fig.23 X-ray powder diffractogram of progesterone

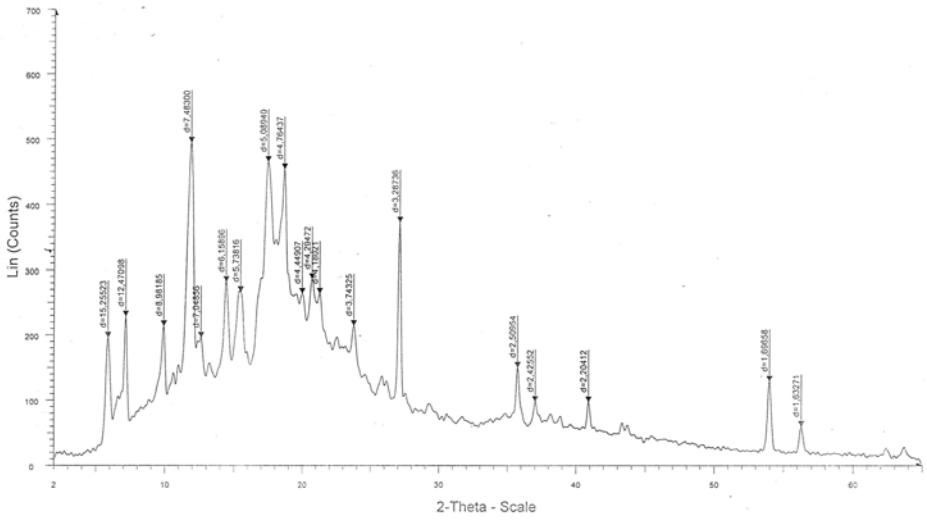


Fig.24 X-ray powder diffractogram of progesterone-β-CD complex

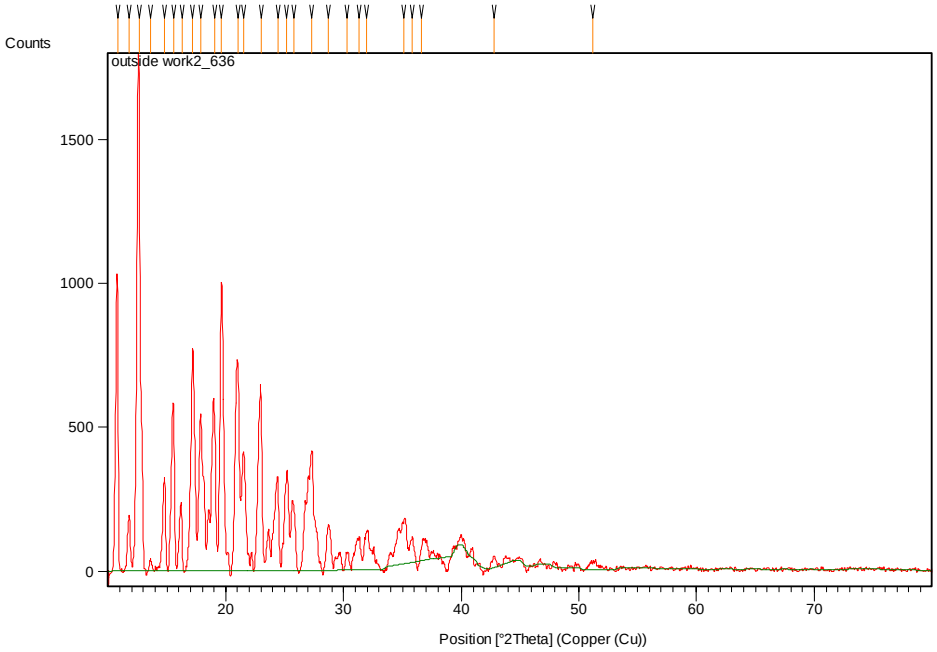


Fig.25 X-ray powder diffractogram of HPβ-CD

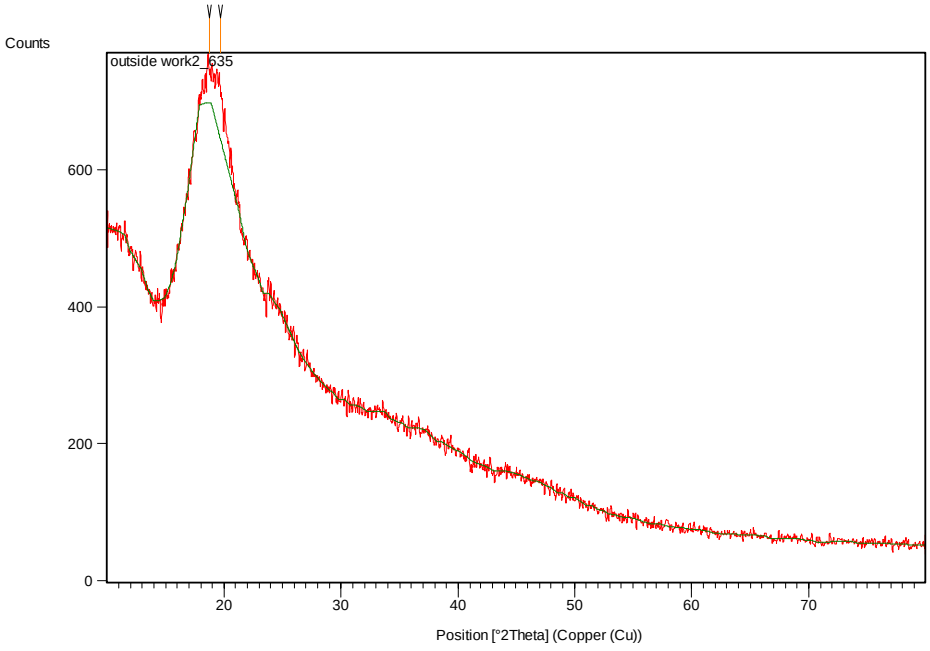


Fig.26 X-ray powder diffractogram of progesterone-HPβ-CD complex

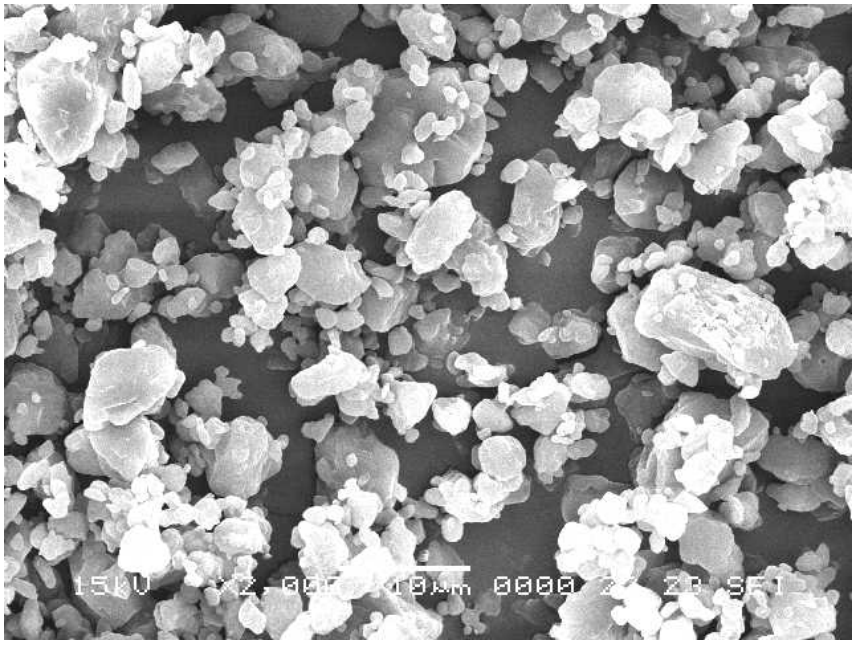


Fig.27 Scanning electron micrograph of progesterone at x 2000 magnification

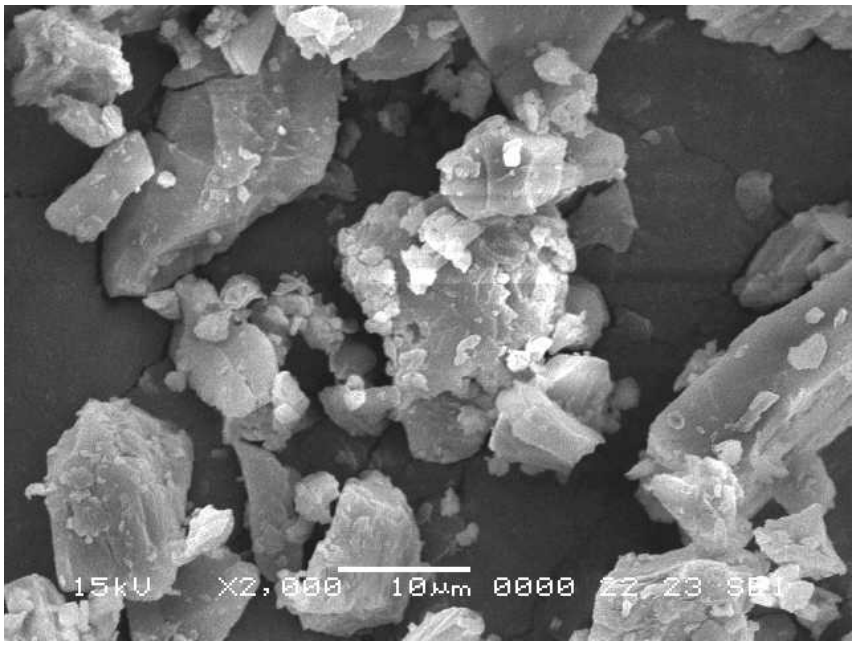


Fig.28 Scanning electron micrograph of β-CD at x 2000 magnification

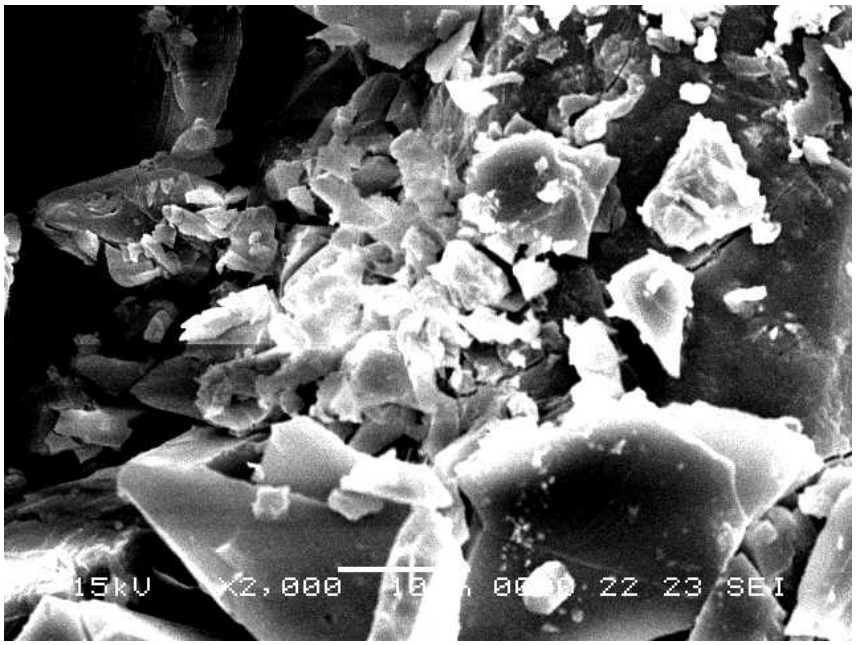


Fig.29 Scanning electron micrograph of progesterone-β-CD complex at x 2000 magnification

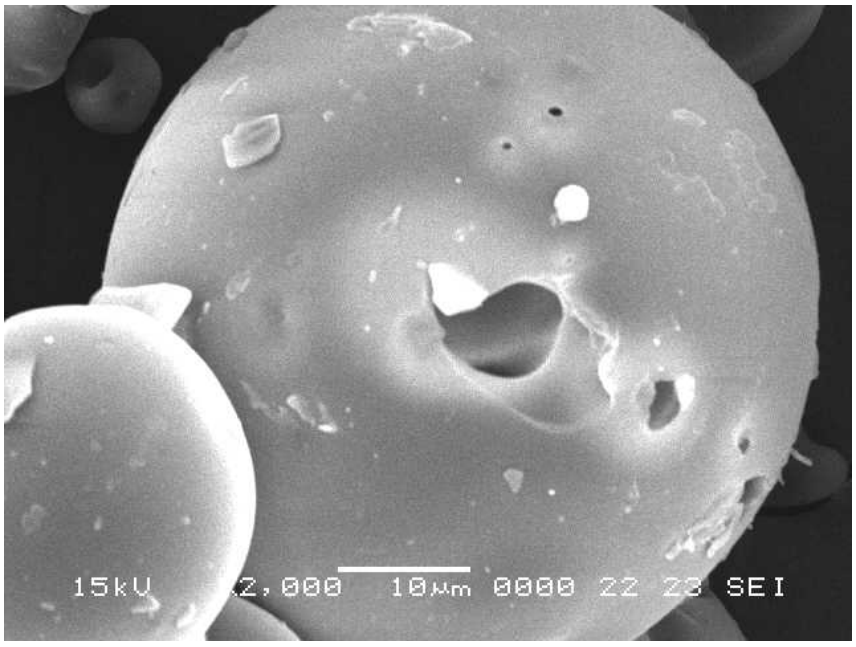


Fig.30 Scanning electron micrograph of HPβ-CD at x 2000 magnification

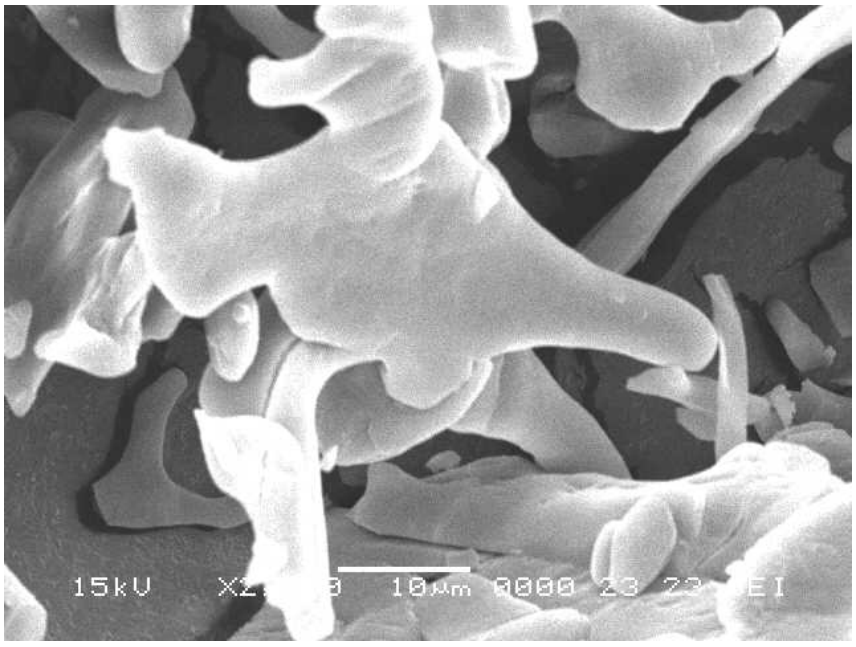


Fig.31 Scanning electron micrograph of progesterone-HPβ-CD complex at x 2000 magnification

The dissolution profiles of P:β-CD(complex), P:β-CD (PM) and P:HPβ-CD(complex) followed first order kinetics (r>0.97). The drug release from P:HPβ-CD(complex) followed Korsmeyer Peppas equation (r > 0.99). The dissolution efficiency values are shown in Table 19. The complex with HPβ-CD exhibited high percentage dissolution efficiency (%DE) of 91.12% as compared with complex with β-CD at 57.7%. The %DE of both the complexes were markedly enhanced as compared with that of the physical mixtures. Mean dissolution time (MDT) value was used to characterize drug release rate and indicated the efficiency of the product to release the drug. MDT was highest in case of β-CD (PM) at 29.4 min and was lowest in case of P:HPβ-CD (complex) at 6.2 min.

Table 16: Drug content of inclusion complexes of progesterone.

Sl.No	Inclusion complex	Progesterone content in mg/g
1	Progesterone-β-CD	191.9
2	Progesterone-HPβ-CD	160.3

Table 17: Solubility profile of progesterone with CDs

Sl.No	Compound	Solubility (µg/ml)	Solubility enhancement
1	Pure progesterone	8.2	-
2	Progestereone:β-CD (PM)	13.5	1.65
3	Progestereone:β-CD complex	34.1	4.16
4	Progestereone:HPβ-CD (PM)	30.1	3.67
5	Progestereone:HPβ-CD complex	666.2	81.24

PM: physical mixture

Table 18: Dissolution profile of progesterone inclusion complexes and physical mixtures

Sl.No.	Time (min)	P-β-CD complex	P-β-CD (PM)	P-HPβ-CD complex	P-HPβ-CD (PM)
1	10	25.4±1.06	5.9±1.5	96.8±3.6	22.1±5.3
2	20	53.5±2.23	10.1±0.24	98.6±4.1	35.2±4.6
3	30	67.9±4.1	14.6±4.5	99.3±4.4	40.9±2.3
4	40	72.1±0.14	16.9±0.8	99.5±0.9	43.9±2.0
5	50	79.03±2.6	17.5±3.6	101.8±0.8	51.8±0.9
6	60	85.6±1.2	22.8±2.5	101.1±2.5	59.2±0.9

All values are expressed as mean ± SD, n = 3

P-β-CD complex: progesterone inclusion complex with β-CD

P-β-CD (PM): progesterone physical mixture with β-CD

P-HPβ-CD complex: progesterone inclusion complex with HPβ-CD

P-HPβ-CD (PM): progesterone physical mixture with HPβ-CD

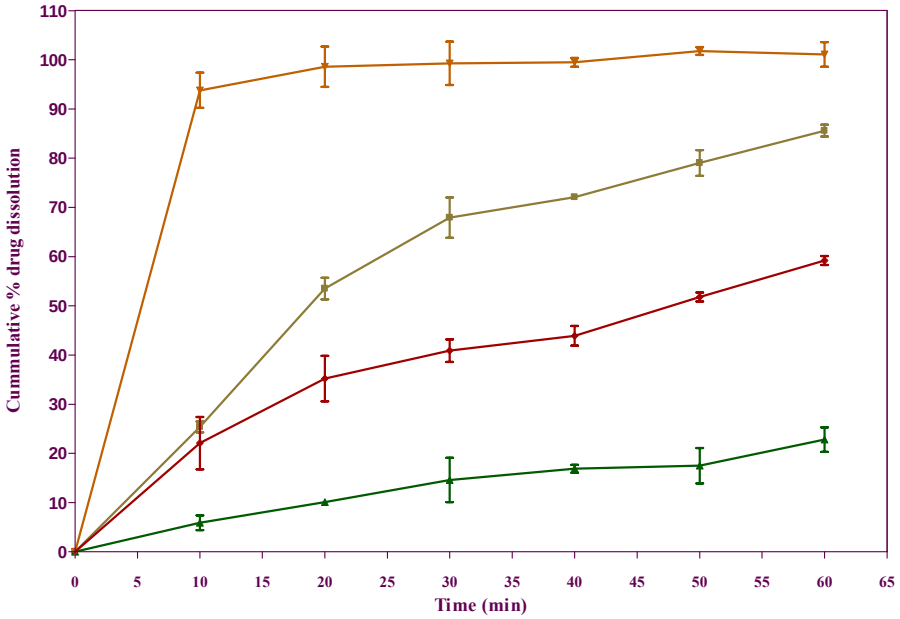


Fig.32: Dissolution profile of P-beta CD (complex) , P-beta CD (PM) P-HP beta CD (complex) and P- HP beta CD (PM)

Table 19: Dissolution parameters of progesterone from inclusion complexes and physical mixtures and mechanism of drug release

Sl.No	Sample	Best fit model	R^2	% D.E	M.D.T (min)	T (90%) (min)
1	P:β-CD (complex)	First order	0.9895	57.7	17.8	70.9
2	P:β-CD (PM)	First order	0.9769	12.55	29.44	510.0
3	P:HPβ-CD (complex)	Korsmeyer Peppas	0.9967	91.12	6.2	0.8
4	P:HPβ-CD (PM)	First order	0.9802	38.37	20.57	146.1

P-β-CD complex: progesterone inclusion complex with β-CD

P-β-CD (PM): progesterone physical mixture with β-CD

P-HPβ-CD complex: progesterone inclusion complex with HPβ-CD

P-HPβ-CD (PM): progesterone physical mixture with HPβ-CD

%D.E: % dissolution efficiency

M.D.T: mean dissolution time

T (90%): time for 90% drug release

R^2: coefficient of determination

4.2 Determination of drug content of nasal gels

The drug content of all the gels prepared were determined and shown in Tables 20-25. The drug content was found to be satisfactory and was between 97.6-100.3 %.

4.3 *In vitro* drug release measurements and determination of diffusion coefficient

The *in vitro* release of progesterone from the various gels are presented in Tables 26-28, 30-32, 34-36, 39-41, 43-45, 47-49 and represented graphically in Fig.33-50. The kinetic evaluation of drug release data and diffusion co-efficient (D) values are shown in Table 29,33,37, 38 and 42,46, 50,51 and n values are shown in Table 52 and 53 (Fig.51-53).

Effect of different poly acrylic acid polymers

The rate of release could be controlled by formulating the drug in different poly acrylic acids with either the presence or absence of CD, addition of CD as physical mixture or as an inclusion complex with the drug. The release from the carbopol 974 gels was faster as compared to carbopol 971 and polycarbophil. The D values decreased significantly ($P<0.05$) as the polymer was changed from carbopol 974 to carbopol 971 to polycarbophil. The drug release was fastest from C_1BF3 with a release of 92.4% at the end of 60 min whereas it was 82.9% with C_2BF3 and PBF3 showed a release of 80.4% at 60 min.

Effect of polymer concentration

The polymer concentration also affected the rate of release. High concentrations of the polymer resulted in slower release since there was more polymer present than the drug could electrostatically or electrophobically interact with. Though the polymer concentration affected the rate of release of the drug, polymer concentration alone could not be used as an effective tool for tailoring the release rate. This was also because the formulation should have optimum

rheological properties. In case of the nasal route the gels should be soft and also have a long residence time at the site of application. There was no significant difference in the release rate of drug from gels with varying percentages of polymer used that is 1%, 1.5% and 2% ($P>0.05$).

A brief summary of the values obtained for the diffusion coefficient (D) for the gels with various polymers in different concentrations are discussed. F1-3 formulations are those with polymer used in concentration of 1%, whereas F4-6 formulations are those with polymer concentrations at 1.5% and F7-9 contain polymer at 2%.

The diffusion coefficient value in case of formulations with carbopol 974 for C_1F1, C_1F4 and C_1F7 were 0.33, 0.31 and 0.29 cm^2/s respectively which were not significantly different ($P>0.05$). This was also observed in gels with contained CDs as the physical mixture. In case of C_1BF2, C_1BF5 and C_1BF8 the D values were 0.54, 0.51 and 0.45 cm^2/s respectively. In case of formulation with CD as inclusion complex D values were 0.59, 0.60 and 0.55 cm^2/s for C_1BF3, C_1BF6 and C_1BF9. From these values it was concluded that the concentration of the polymer played a small role in controlling the release of the drug.

This similar pattern was also observed in case of the carbopol 971 gels. The C_2BF3, C_2BF6 and C_2BF9 gels show D values of 0.57, 0.5 and 0.51 cm^2/s respectively which were not significantly different ($P>0.05$). In case of C_2BF2, C_2BF5 and C_2BF8 gels D values were found to be 0.51, 0.49 and 0.40 cm^2/s which was again not significantly different ($P>0.05$).

This was also seen in the HPβ-CD gels where the D values were 0.60, 0.55 and 0.53 cm^2/s for C_2HF3, C_2HF6 and C_2HF9 which were not significantly different ($P>0.05$).

This pattern was found to be repeated for the polycarbophil gels where the different concentration did not significantly ($P>0.05$) alter the diffusion coefficients.

Effect of addition of CD

The effect of addition of CDs to the gel is discussed below. Formulations F1, 4, and 7 were those with no added CD. F2, 5 and 8 were those with CD added as a physical mixture and in F 3, 6 and 9 drug was added to the gels as a complex with CD. The rate of release from the gels with no added CD was the slowest while that from gels with drug as inclusion complex with CD was the fastest.

In case of β-CD gels diffusion coefficient was 0.33, 0.54 and 0.59 cm^2/s for C_1F1, C_1BF2 and C_1BF3 respectively. In case of C_2F7, C_2BF8 and C_2BF9 the D values were 0.26, 0.40 and 0.51 cm^2/s respectively. In case of HPβ-CD gel with polycarbophil as the polymer the D values were 0.24, 0.42 and 0.53 cm^2/s for PF4, PHF5 and PHF6 respectively.

The D values for all the formulation were compared. It was seen that the formulations with no added CDs gave the lowest release which was significantly different from the gels which contained the CDs ($P<0.05$). In case of gels with CDs added either as a complex or physical picture there was no significant difference in the diffusion coefficient values ($P>0.05$). This pattern was observed in all the three types of polymers used.

Effect of addition of β-cyclodextrin and hydroxypropyl β-cyclodextrin

There was a difference in the rate of release of progesterone from gels which contained β-cyclodextrin or hydroxypropyl β-cyclodextrin as the absorption enhancer with the HPβ-CD gels showing a higher release rate profiles.

Release kinetics

The order and mechanism of progesterone release from the nasal gels were determined by fitting the release rate data in various kinetic equations like zero order (% release vs. t), first order (log % release vs. t) and Higuchi model (M_t/M_∞ vs. $t^{1/2}$). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation, $M_t/M_\infty=kt^n$, where M_t is the

amount of drug released at time t and M_∞ is the amount released at time ∞, thus the M_t/M_∞ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent. The zero order, first order and Higuchi equations failed to explain the drug release mechanism from polymeric system that undergo swelling and or erosion during dissolution. In such cases based on the value of n, obtained by fitting in Peppas Korsemeyer equation, the mechanism of drug release could be described. In case of the Fickian release mechanism, the rate of drug release was much less than that of polymer relaxation (erosion). So the drug release was chiefly dependent on the diffusion through the matrix. In the non Fickian (anomalous) case the rate of drug release was due to the combined effect of drug diffusion and polymer relaxation. Case II release generally referred to polymer relaxation

The kinetic parameters for all the formulations are given in Table 29, 33, 37, 42, 46, 50. Nature of release of the drug from the designed nasal gels was inferred based on the correlation coefficients obtained from the plots of the kinetic models. For most of the formulations good correlation coefficients (R values) were obtained for Higuchi-Matrix square root equation. The n values for the formulation which do not contain CD were found to be between 0.8-0.9. In case of formulations which contained CD both as a physical mixture as well as inclusion complex the n values ranged from 0.45 to 0.65. This indicated that that the release mechanism for all formulations was non-Fickian or anomalous release (0.45<n>0.89). Hence it was inferred that the release of the drug was dependent on both drug diffusion and polymer relaxation. However there was a difference in n values between gels which contained CD and those which do not. In case of the gels which do not contain CD the values of n were closer to 1 and hence it could be concluded that the drug release was more dependent on the effect of polymer relaxation. In case of formulations which contained CD the effect of diffusion on drug release was more than the effect of polymer relaxation as the values were nearer to 0.5. The poor correlation coefficients (r values) observed for kinetic parameters based on zero-order and first-order model equations were mainly due to the drug release mechanism. Because the n values were closer to 0.5 in most cases good correlation coefficients were obtained for Higuchi's square root kinetics.

The diffusion coefficients (D) for all the three polymer gels followed a similar pattern. It was seen that there was a significant difference in the D values for gels without CD and gels which contain CD ($P<0.05$). There was no significant difference in gels which contained CD as physical mixture or as an inclusion complex ($P=0.061$-0.31). The presence of CD was sufficient to increase the rate of release of the drug.

Table 20: Percentage drug content of nasal gels C_1F1- C_1BF9

Sample	C_1F1	C_1BF2	C_1BF3	C_1F4	C_1BF5	C_1BF6	C_1F7	C_1BF8	C_1BF9
% Drug Content	100.2 ±0.65	97.6 ±0.42	96.9 ±0.31	99.2 ±1.01	98.6 ±0.56	97.23 ±0.21	99.8 ±1.20	99.1 ±0.35	98.2 ±0.42

All values are expressed as mean ± SD, n = 3

Table 21: Percentage drug content of nasal gels C_2F1- C_2BF9

Sample	C_2F1	C_2BF2	C_2BF3	C_2F4	C_2BF5	C_2BF6	C_2F7	C_2BF8	C_2BF9
% Drug Content	99.96 ±0.53	98.4 ±0.63	98.4 ±0.87	99.8 ±1.11	97.6 ±0.87	97.8 ±0.89	99.9 ±0.63	98.3 ±0.58	98.1 ±1.01

All values are expressed as mean ± SD, n = 3

Table 22: Percentage drug content of nasal gels PF1- PBF9

Sample	PF1	PBF2	PBF3	PF4	PBF5	PBF6	PF7	PBF8	PBF9
% Drug Content	100.3 ±0.35	99.6 ±0.87	98.1 ±0.69	99.0 ±1.01	100.3 ±.2.31	97.7 ±0.58	99.7 ±0.55	99.6 ±0.64	98.3 ±0.44

All values are expressed as mean ± SD, n = 3

Table 23: Percentage drug content of nasal gels C_1HF2 - C_1HF9

Sample	C_1HF2	C_1HF3	C_1HF5	C_1HF6	C_1HF8	C_1HF9
% Drug Content	99.3 ±0.95	98.6 ±0.45	100.2 ±1.03	98.6 ±0.55	98.6 ±0.78	98.4 ±1.16

All values are expressed as mean ± SD, n = 3

Table 24: Percentage drug content of nasal gels C_2HF2 – C_2HF9

Sample	C_2HF2	C_2HF3	C_2HF5	C_2HF6	C_2HF8	C_2HF9
% Drug Content	98.9 ±0.22	99.1 ±0.56	99.7 ±0.48	98.9 ±0.99	99.2 ±1.12	99.1 ±0.89

All values are expressed as mean ± SD, n = 3

Table 25: Percentage drug content of nasal gels PHF2 - PHF9

Sample	PHF2	PHF3	PHF5	PHF6	PHF8	PHF9
% Drug Content	99.4 ±1.22	99.0 ±0.33	99.8 ±1.25	99.1 ±1.21	98.9 ±0.35	97.6 ±0.59

All values are expressed as mean ± SD, n = 3

Table 26: Cumulative amount of progesterone release from C_1F1, C_1BF2 and C_1BF3

Sl.No.	Time	Percentage release		
		C_1F1	C_1BF2	C_1BF3
1	0	0.0	0.0	0.0
2	5	6.97 ± 0.88	25.50 ± 0.75	33.59 ± 1.51
3	10	18.21 ± 1.20	38.00 ± 2.59	45.44 ± 0.86
4	15	28.85 ± 0.99	53.89 ± 1.47	62.81 ± 2.45
5	30	39.26 ± 0.81	64.22 ± 0.60	75.64 ± 0.46
6	45	42.81 ±2.41	75.43 ± 1.65	82.25 ± 4.76
7	60	46.28 ± 0.97	77.03 ± 0.79	92.43 ± 0.54

All values are expressed as mean ± SD, n = 3

Table 27: Cumulative amount of progesterone release from C_1F4, C_1BF5 and C_1BF6

Sl.No.	Time	Percentage release		
		C_1F4	C_1BF5	C_1BF6
1	0	0.0	0.0	0.0
2	5	5.86 ± 1.59	24.01 ± 0.48	32.36 ± 0.79
3	10	13.90 ± 0.98	42.23 ± 1.10	47.20 ± 1.34
4	15	27.97 ± 1.61	49.57 ± 0.75	60.61 ± 0.87
5	30	36.15 ± 1.37	60.99 ± 0.84	74.05 ± 0.77
6	45	40.83 ± 0.77	75.19 ± 0.69	88.00 ± 2.30
7	60	44.37 ± 0.82	76.59 ± 0.37	90.73 ± 1.30

All values are expressed as mean ± SD, n = 3

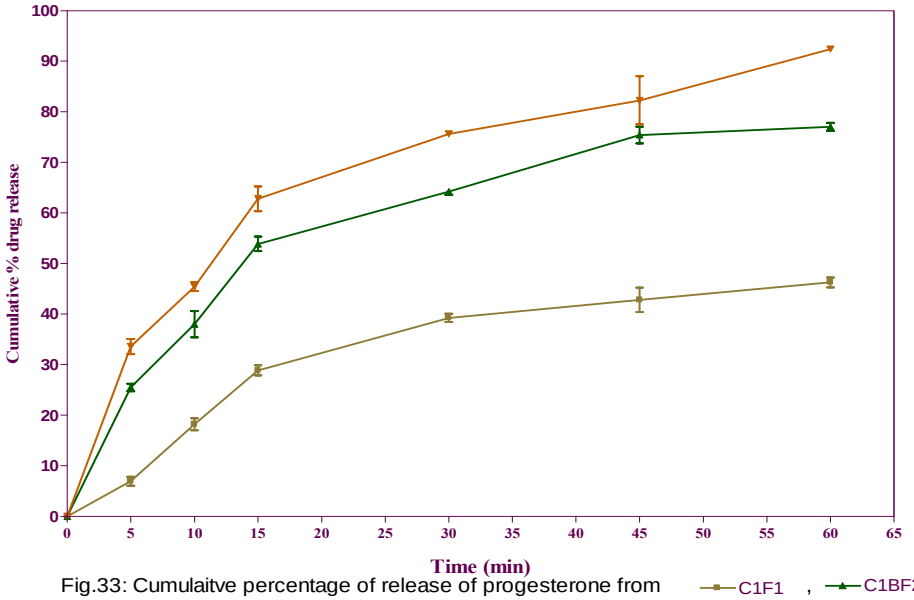


Fig.33: Cumulaitve percentage of release of progesterone from C1F1 , C1BF2 and C1BF3

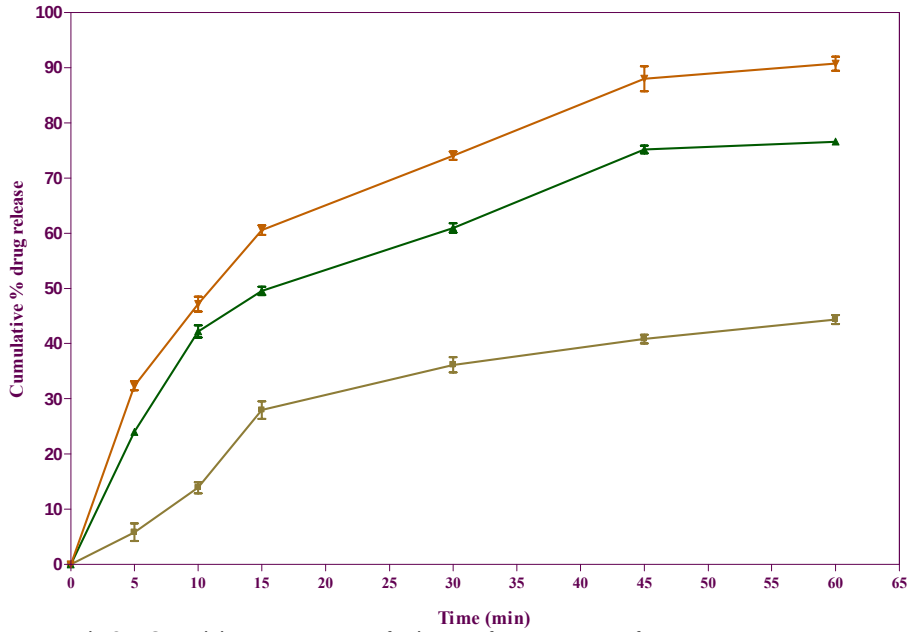


Fig.34: Cumulaitve percentage of release of progesterone from C1F4 , C1BF5 and C1BF6.

Table 28: Cumulative amount of progesterone release from C_1F7, C_1BF8 and C_1BF9

Sl.No.	Time	Percentage release		
		C_1F7	**C_1BF8**	**C_1BF9**
1	0	0.0	0.0	0.0
2	5	5.99 ± 0.37	22.76 ± 0.53	30.64 ± 0.64
3	10	15.30 ± 0.98	40.18 ± 0.67	44.20 ± 0.57
4	15	21.79 ± 1.28	52.30 ± 1.04	58.41 ± 1.07
5	30	32.67 ± 0.6	63.86 ± 1.08	70.38 ± 1.27
6	45	37.43 ± 1.42	65.42 ± 0.76	74.85 ± 1.55
7	60	41.98 ± 0.89	74.55 ± 0.72	89.02 ± 3.27

All values are expressed as mean ± SD, n = 3

Table 29: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	1st order	Higuchi Model	Hixon-Crowell model	Korsemeyer Peppas Model
1	C_1F1	0.9125	0.9575	**0.9795**	0.9513	0.9446
2	C_1BF2	0.7356	0.9272	**0.9753**	0.8801	0.9630
3	C_1BF3	0.6514	0.9490	**0.9612**	0.8853	0.9388
4	C_1F4	0.9273	0.9608	**0.9759**	0.9511	0.9630
5	C_1BF5	0.7598	0.9434	**0.9845**	0.8998	0.9755
6	C_1BF6	0.6680	0.9579	**0.9786**	0.9008	0.9741
7	C_1F7	0.9407	0.9670	**0.9745**	0.9594	0.9659
8	C_1BF8	0.7765	0.9434	**0.9872**	0.9024	0.9775
9	C_1BF9	0.7212	0.9647	**0.9804**	0.9192	0.9776

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty=kt^n$

Table 30: Cumulative amount of progesterone release from C_2F1, C_2BF2 and C_2BF3

Sl.No.	Time	Percentage release		
		C_2F1	C_2BF2	C_2BF3
1	0	0.0	0.0	0.0
2	5	5.39 ± 1.39	22.76 ± 5.72	29.09 ± 0.63
3	10	14.22 ± 1.69	33.56 ± 1.16	39.22 ± 0.74
4	15	21.29 ± 1.59	47.59 ± 1.35	54.18 ± 1.05
5	30	29.75 ± 1.60	58.87 ± 3.55	70.16 ± 0.80
6	45	39.52 ± 1.24	66.84 ± 1.72	77.08 ± 1.89
7	60	44.82 ± 1.47	73.41 ± 2.44	82.89 ± 4.32

All values are expressed as mean ± SD, n = 3

Table 31: Cumulative amount of progesterone release from C_2F4, C_2BF5 and C_2BF6

Sl.No.	Time	Percentage release		
		C_2F4	C_2BF5	C_2BF6
1	0	0.0	0.0	0.0
2	5	4.66 ± 1.44	19.10 ± 1.20	23.96 ± 0.83
3	10	9.37 ± 1.13	26.12 ± 1.32	34.89 ± 1.05
4	15	15.68 ± 3.09	34.85 ± 0.75	46.70 ± 1.39
5	30	23.82 ± 1.75	54.23 ± 0.88	66.37 ± 1.00
6	45	35.42 ± 1.53	63.53 ± 0.75	76.73 ± 0.39
7	60	42.06 ± 1.47	72.23 ± 0.90	82.63 ± 1.16

All values are expressed as mean ± SD, n = 3

Table 32: Cumulative amount of progesterone release from C_2F7, C_2BF8 and C_2BF9

Sl.No.	Time	Percentage release		
		C_2F7	C_2BF8	C_2BF9
1	0	0.0	0.0	0.0
2	5	4.49 ± 0.22	16.30 ± 1.29	22.60 ± 1.13
3	10	10.65 ± 0.41	32.40 ± 1.25	39.60 ± 0.90
4	15	16.26 ± 0.22	39.70 ± 1.50	49.80 ± 1.86
5	30	22.16 ± 0.38	48.21 ± 2.76	58.20 ± 1.18
6	45	28.29 ± 0.41	58.06 ± 1.84	70.82 ± 1.85
7	60	37.39 ± 0.48	67.81 ± 1.36	83.85 ± 1.50

All values are expressed as mean ± SD, n = 3

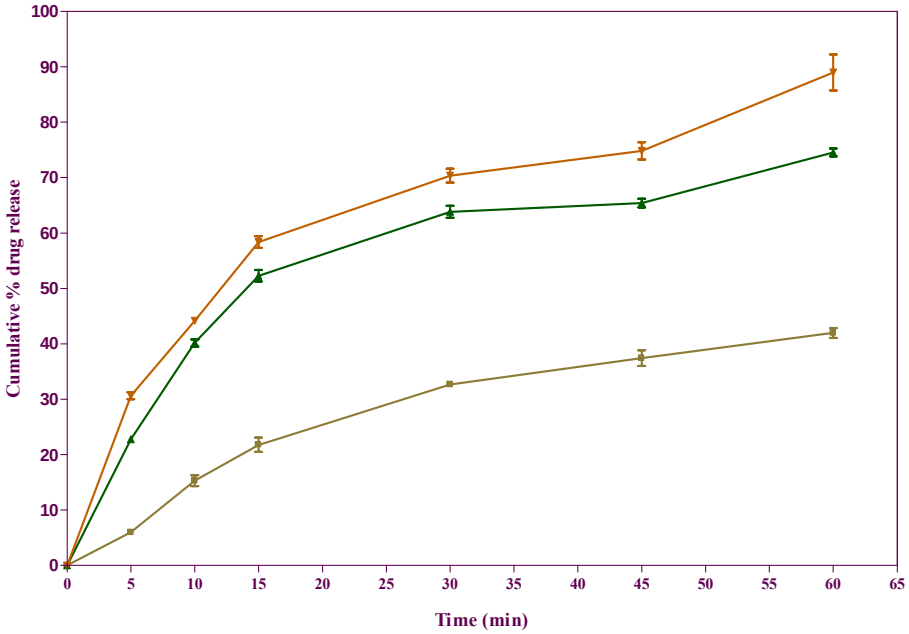


Fig.35: Cumulative percentage of release of progesterone from C1F7 , C1BF8 and C1BF9 .

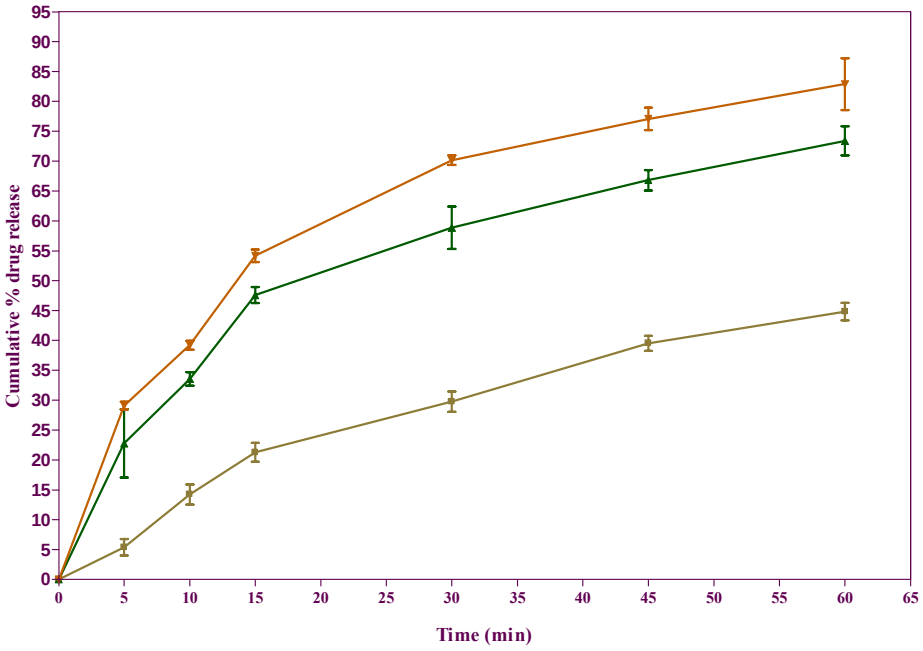


Fig.36: Cumulative percentage of release of progesterone from C2F1 , C2BF2 and C2BF3 .

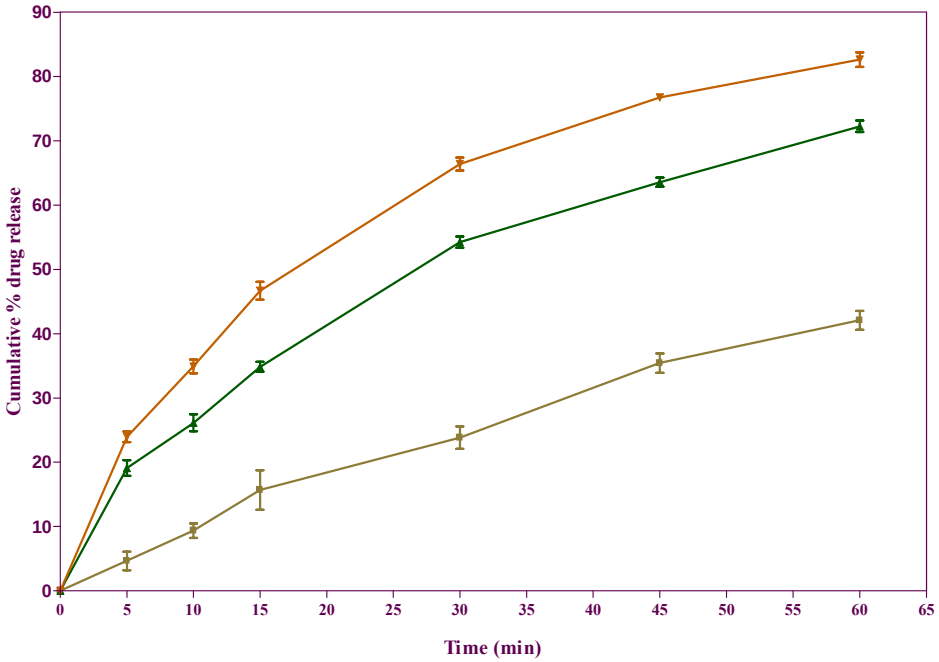


Fig.37: Cumulative percentage of release of progesterone from C2F4 , C2BF5 and C2BF6 .

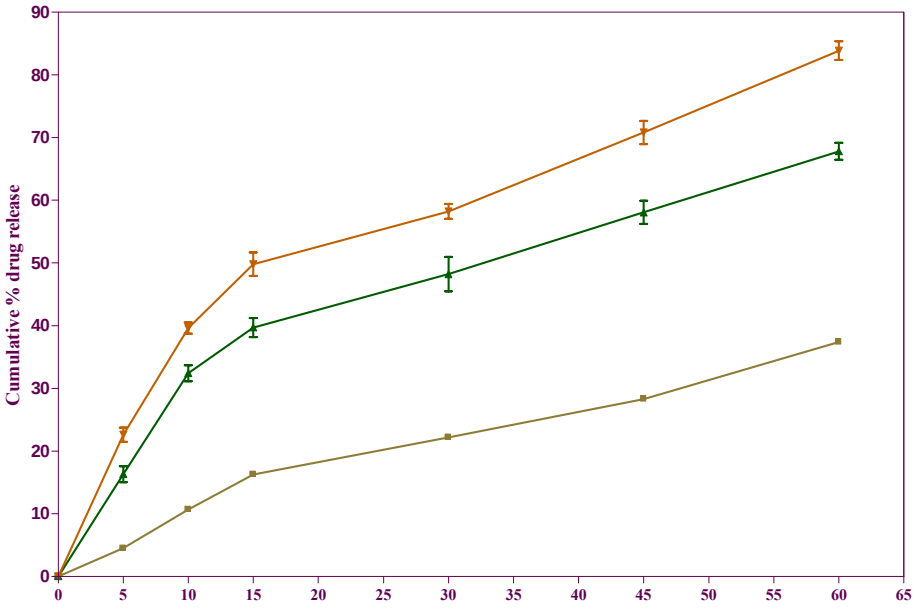


Fig.38: Cumulative percentage of release of progesterone from C2F7 , C2BF8 and C2BF9 .

Table 33: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	1st order	Higuchi Model	Hixon-Crowell model	Korsemeyer Peppas Model
1	C_2F1	0.9497	0.9781	**0.9788**	0.9710	0.9724
2	C_2BF2	0.7210	0.9186	**0.9784**	0.8687	0.9703
3	C_2BF3	0.7422	0.9431	**0.9819**	0.8960	0.9803
4	C_2F4	0.9870	0.9904	0.9916	**0.9918**	0.9862
5	C_2BF5	0.8990	0.9843	**0.9965**	0.9652	0.9908
6	C_2BF6	0.8370	0.9809	**0.9946**	0.9507	0.9934
7	C_2F7	0.9665	0.9838	**0.9839**	0.9792	0.9808
8	C_2BF8	0.8360	0.9506	**0.9904**	0.9220	0.9809
9	C_2BF9	0.8080	0.9661	**0.9898**	0.9346	0.9756

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model: Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty = kt^n$

Table 34: Cumulative amount of progesterone release from PF1, PBF2 and PBF3

Sl.No.	Time	Percentage release		
		PF1	PBF2	PBF3
1	0	0.0	0.0	0.0
2	5	4.19 ± 0.48	22.21 ± 4.97	27.10 ± 0.45
3	10	13.03 ± 1.66	30.89 ± 2.35	38.10 ± 0.89
4	15	16.87 ± 1.11	41.59 ± 4.38	50.50 ± 0.24
5	30	27.03 ± 0.41	51.62 ± 5.55	69.95 ± 1.11
6	45	32.35 ± 0.49	62.11 ± 4.44	75.62 ± 0.54
7	60	39.13 ± 1.02	67.12 ± 4.79	80.43 ± 1.49

All values are expressed as mean ± SD, n = 3

Table 35: Cumulative amount of progesterone release from PF4, PBF5 and PBF6

Sl.No.	Time	Percentage release		
		PF4	PBF5	PBF6
1	0	0.0	0.0	0.0
2	5	4.53 ± 0.74	25.57 ± 0.05	27.64 ± 0.57
3	10	13.59 ± 0.50	35.81 ± 0.99	42.30 ± 0.51
4	15	16.25 ± 0.49	48.12 ± 0.24	54.21 ± 0.37
5	30	22.09 ± 0.91	64.19 ± 1.70	69.80 ± 0.93
6	45	31.31 ± 0.61	71.69 ± 1.75	75.60 ± 0.33
7	60	39.40 ± 0.66	75.79 ± 0.35	84.10 ± 0.70

All values are expressed as mean ± SD, n = 3

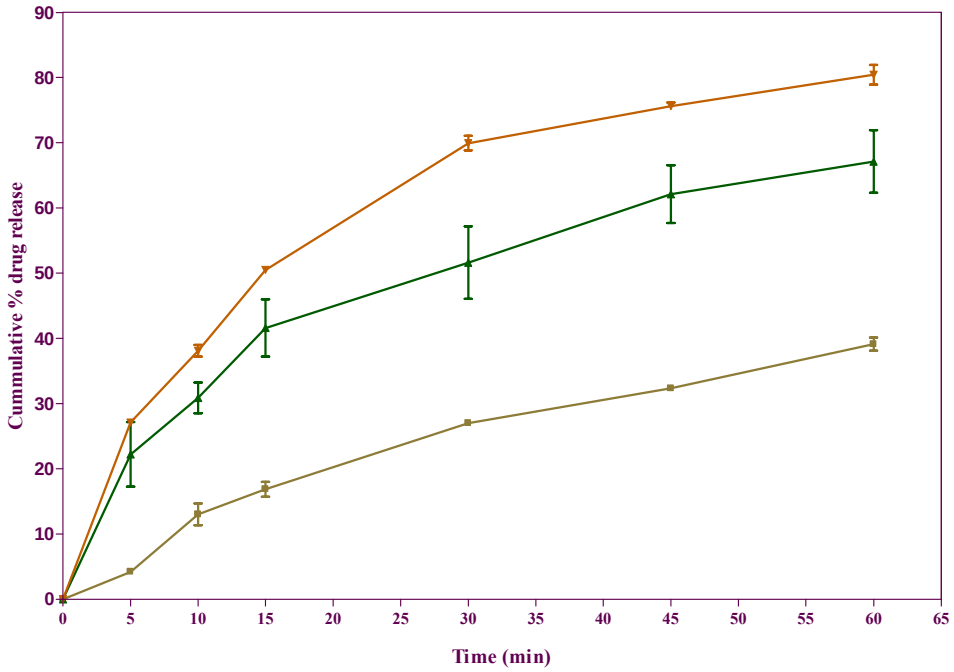


Fig.39: Cummlative percentage of release of progesterone from PF1 , PBF2 and PBF3 .

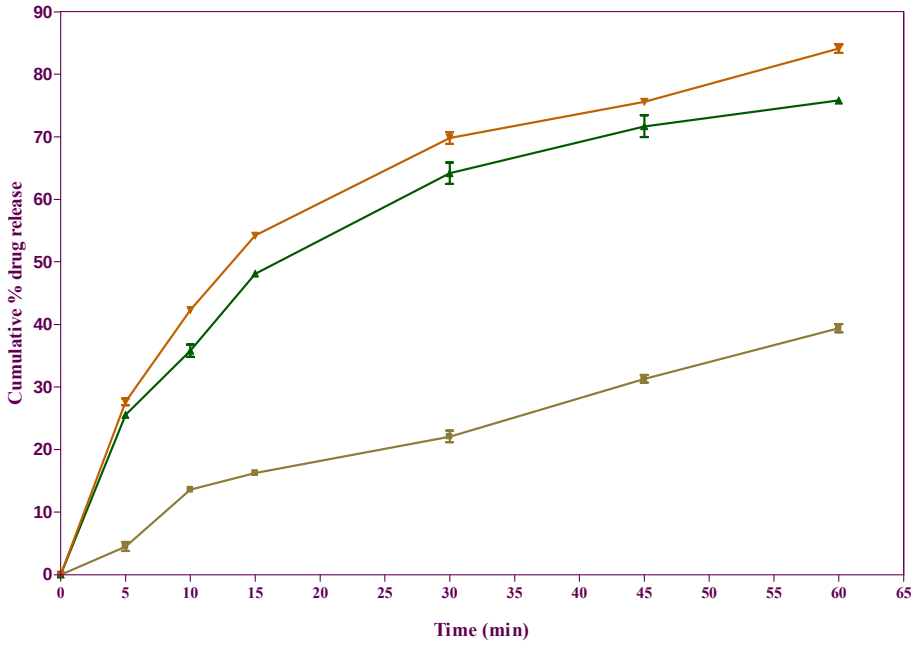


Fig.40: Cumulative percentage of release of progesterone from PF4 , PBF5 and PBF6 .

Table 36: Cumulative amount of progesterone release from PF7, PBF8 and PBF9

Sl.No.	Time	Percentage release		
		PF7	PBF8	PBF9
1	0	0.0	0.0	0.0
2	5	4.60 ± 0.35	5.51 ± 0.43	9.99 ± 0.43
3	10	10.44 ± 0.59	12.70 ± 0.12	19.05 ± 0.71
4	15	14.90 ± 0.70	17.54 ± 0.39	27.32 ± 1.48
5	30	20.33 ± 0.40	23.61 ± 0.47	38.70 ± 0.90
6	45	29.15 ± 0.89	32.31 ± 0.70	48.72 ± 0.91
7	60	37.53 ± 0.70	41.16 ± 0.88	59.33 ± 0.68

All values are expressed as mean ± SD, n = 3

Table 37: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	1st order	Higuchi Model	Hixon-Crowell model	Korsemeyer Peppas Model
1	PF1	0.9623	0.9723	**0.9836**	0.9770	0.9666
2	PBF2	0.7752	0.9401	**0.9828**	0.9000	0.9762
3	PBF3	0.7727	0.9381	**0.9817**	0.8974	0.9740
4	PF4	0.9514	0.9760	**0.9772**	0.9689	0.9650
5	PBF5	0.7249	0.8931	**0.9728**	0.8483	0.9590
6	PBF6	0.7564	0.9401	**0.9789**	0.8967	0.9733
7	PF7	0.9743	0.9816	0.9657	**0.9843**	0.9679
8	PBF8	0.9472	0.9742	**0.9787**	0.9669	0.9638
9	PBF9	0.9253	0.9749	**0.9761**	0.9616	0.9595

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty = kt^n$

Table 38: Diffusion coefficient D (cm^2/s) values for nasal gels with β-CD

Sl.No	Sample	D(cm^2/s)	Sample	D(cm^2/s)	Sample	D(cm^2/s)
1	C_1F1	0.33	C_2F1	0.31	PF1	0.25
2	C_1BF2	0.54	C_2BF2	0.51	PBF2	0.45
3	C_1BF3	0.59	C_2BF3	0.57	PBF3	0.54
4	C_1F4	0.31	C_2F4	0.27	PF4	0.24
5	C_1BF5	0.51	C_2BF5	0.49	PBF5	0.41
6	C_1BF6	0.60	C_2BF6	0.50	PBF6	0.50
7	C_1F7	0.29	C_2F7	0.26	PF7	0.24
8	C_1BF8	0.45	C_2BF8	0.40	PBF8	0.26
9	C_1BF9	0.55	C_2BF9	0.51	PBF9	0.43

Table 39: Cumulative amount of progesterone release from C_1F1, C_1HF2 and C_1HF3

Sl.No.	Time	Percentage release		
		C_1F1	C_1HF2	C_1HF3
1	0	0.0	0.0	0.0
2	5	6.97 ± 0.88	27.79 ± 0.89	32.49 ± 3.36
3	10	18.21 ± 1.20	43.39 ± 2.20	44.43 ± 1.39
4	15	28.85 ± 0.99	57.91 ± 0.25	60.82 ± 2.35
5	30	39.26 ± 0.81	67.41 ± 0.47	75.46 ± 0.61
6	45	42.81 ±2.41	74.63 ± 1.26	88.20 ± 1.01
7	60	46.28 ± 0.97	90.26 ± 0.46	98.59 ± 0.94

All values are expressed as mean ± SD, n = 3

Table 40: Cumulative amount of progesterone release from C_1H4, C_1HF5 and C_1HF6

Sl.No.	Time	Percentage release		
		C_1F4	C_1HF5	C_1HF6
1	0	0.0	0.0	0.0
2	5	5.86 ± 1.59	6.42 ± 0.30	23.02 ± 0.49
3	10	13.90 ± 0.98	14.87 ± 0.19	39.40 ± 0.88
4	15	27.97 ± 1.61	31.05 ± 0.59	51.30 ± 0.43
5	30	36.15 ± 1.37	39.16 ± 0.62	64.17 ± 1.22
6	45	40.83 ± 0.77	43.47 ± 0.46	72.34 ± 0.28
7	60	44.37 ± 0.82	48.08 ± 0.31	78.64 ± 0.63

All values are expressed as mean ± SD, n = 3

Table 41: Cumulative amount of progesterone release from C_1F7, C_1HF8 and C_1HF9

Sl.No.	Time	Percentage release		
		C_1F7	C_1HF8	C_1HF9
1	0	0.0	0.0	0.0
2	5	5.99 ± 0.37	25.74 ± 0.26	31.16 ± 0.74
3	10	15.30 ± 0.98	40.37 ± 0.49	40.93 ± 0.91
4	15	21.79 ± 1.28	53.77 ± 1.27	60.43 ± 0.42
5	30	32.67 ± 0.6	65.22 ± 1.62	73.31 ± 1.33
6	45	37.43 ± 1.42	68.03 ± 1.11	82.46 ± 4.65
7	60	41.98 ± 0.89	78.66 ± 0.83	89.57 ± 0.85

All values are expressed as mean ± SD, n = 3

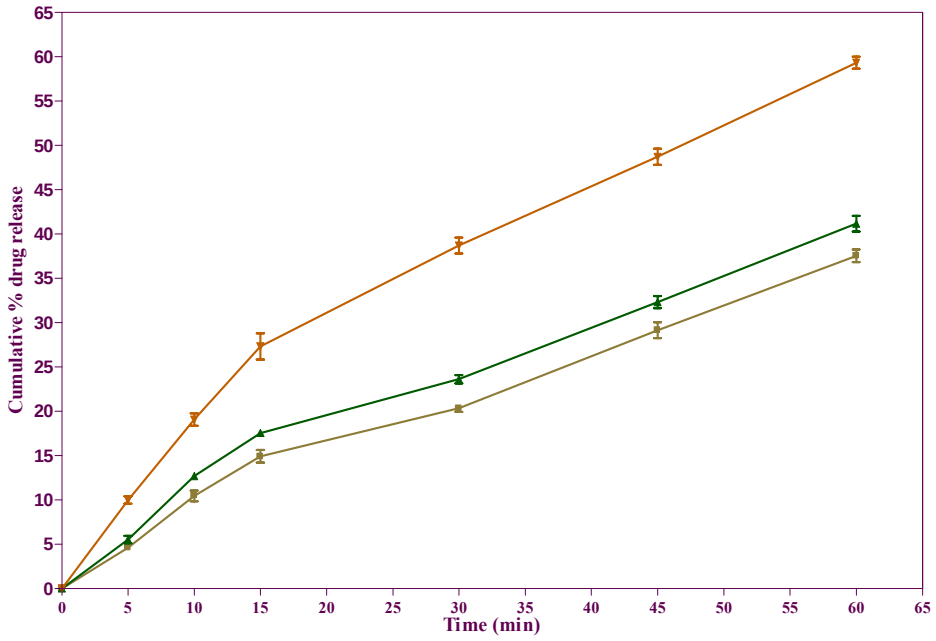


Fig.41: Cumulative percentage of release of progesterone from PF7 , PBF8 and PBF9 .

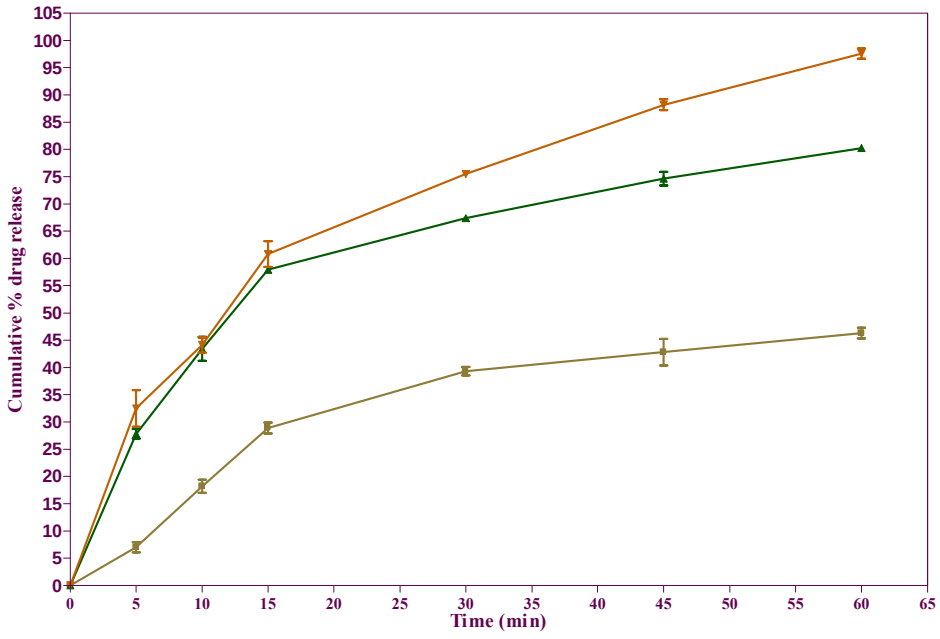


Fig.42: Cumulative percentage of release of progesterone from C1F1 , C1HF2 and C1HF3 .

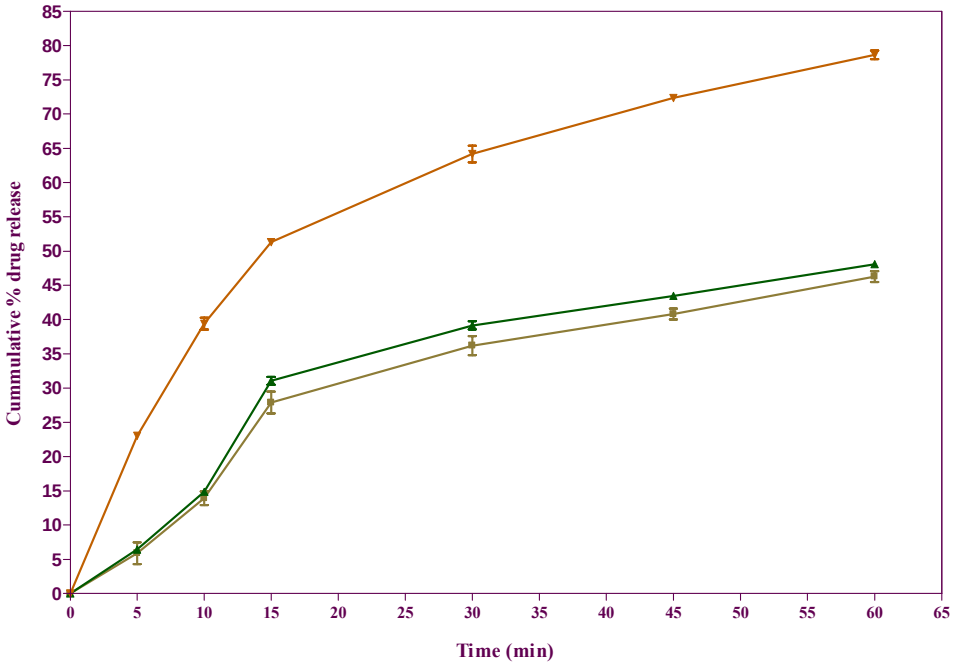


Fig.43: Cumulative percentage of release of progesterone from C1F4 , C1HF5 and C1HF6 .

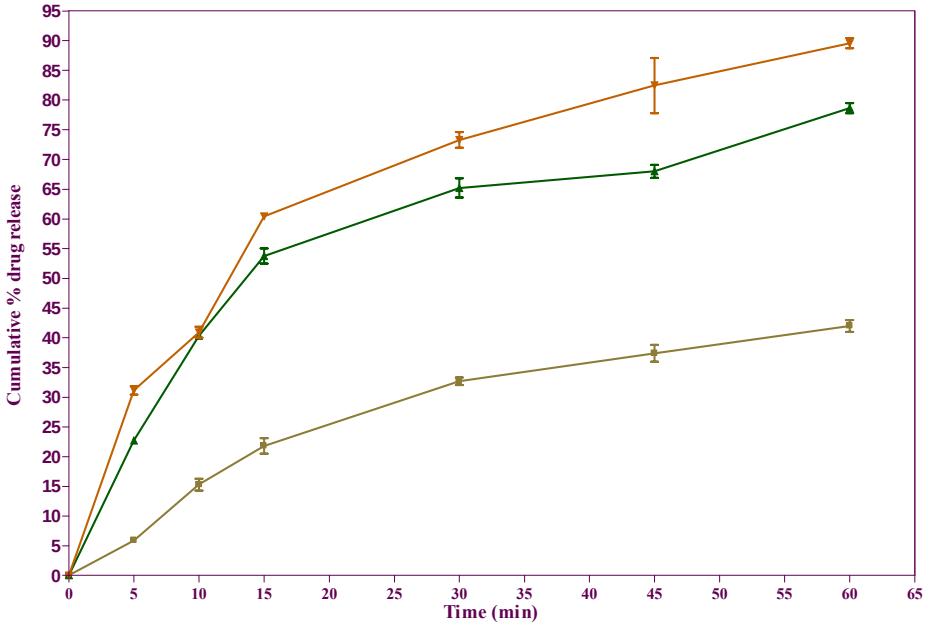


Fig.44: Cumulative percentage of release of progesterone from C1F7 , C1HF8 and C1HF9 .

Table 42: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	1st order	Higuchi Model	Hixon-Crowell model	Korsemeyer Peppas Model
1	C_1F1	0.9125	0.9575	**0.9795**	0.9513	0.9446
2	C_1HF2	0.6463	0.9087	**0.9691**	0.8446	0.9668
3	C_1HF3	0.7739	0.9770	**0.9894**	0.9755	0.9884
4	C_1F4	0.9273	0.9608	**0.9759**	0.9511	0.9630
5	C_1HF5	0.7523	0.9508	**0.9825**	0.9059	0.9695
6	C_1HF6	0.7403	0.9782	**0.9827**	0.9368	0.9825
7	C_1F7	0.9407	0.9670	**0.9745**	0.9594	0.9659
8	C_1HF8	0.7099	0.9173	**0.9714**	0.8659	0.9573
9	C_1HF9	0.7309	0.9707	**0.9899**	0.9247	0.9800

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty=kt^n$

Table 43: Cumulative amount of progesterone release from C_2F1, C_2HF2 and C_2HF3

Sl.No.	Time	Percentage release		
		C_2F1	C_2HF2	C_2HF3
1	0	0.0	0.0	0.0
2	5	5.39 ± 1.39	20.72 ± 0.37	41.43 ± 2.78
3	10	14.22 ± 1.69	39.83 ± 0.71	48.24 ± 0.96
4	15	21.29 ± 1.59	48.40 ± 0.81	55.25 ± 1.22
5	30	29.75 ± 1.60	62.13 ± 0.43	72.45 ± 1.05
6	45	39.52 ± 1.24	69.61 ± 1.45	80.54 ± 2.11
7	60	44.82 ± 1.47	77.46 ± 0.23	97.52 ± 3.69

All values are expressed as mean ± SD, n = 3

Table 44: Cumulative amount of progesterone release from C_2F4, C_2HF5 and C_2HF6

Sl.No.	Time	Percentage release		
		C_2F4	C_2HF5	C_2HF6
1	0	0.0	0.0	0.0
2	5	4.66 ± 1.44	21.53 ± 0.76	23.87 ± 0.76
3	10	9.37 ± 1.13	28.04 ± 0.17	31.36 ± 0.64
4	15	15.68 ± 3.09	37.49 ± 0.27	47.21 ± 1.41
5	30	23.82 ± 1.75	57.24 ± 0.34	78.07 ± 1.33
6	45	35.42 ± 1.53	68.07 ± 0.79	78.07 ± 5.57
7	60	42.06 ± 1.47	77.37 ± 0.62	82.00 ± 0.99

All values are expressed as mean ± SD, n = 3

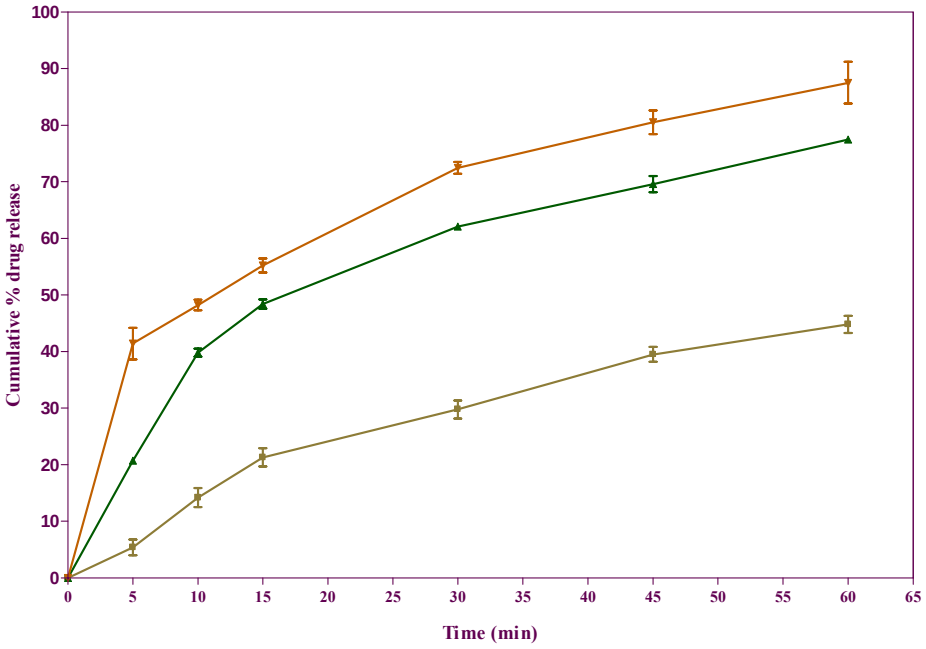


Fig.45: Cumulative percentage of release of progesterone from C2F1 , C2HF2 and C2HF3 .

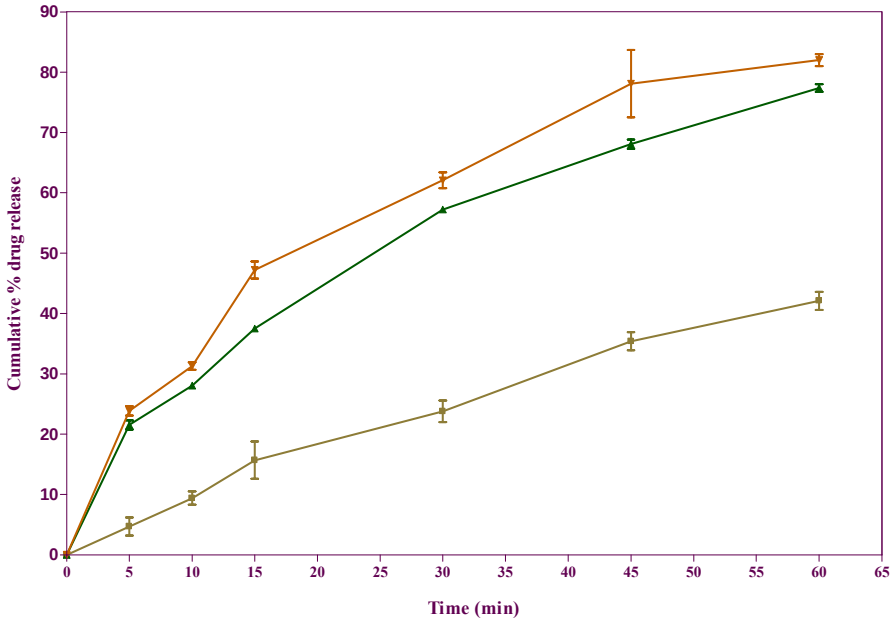


Fig.46: Cumulative percentage of release of progesterone from C2F4 , C2HF5 and C2HF6 .

Table 45: Cumulative amount of progesterone release from C_2F7, C_2HF8 and C_2HF9

Sl.No.	Time	Percentage release		
		C_2F7	C_2HF8	C_2HF9
1	0	0.0	0.0	0.0
2	5	4.49 ± 0.22	21.21 ± 0.89	23.64 ± 0.54
3	10	10.65 ± 0.41	27.49 ± 0.43	34.53 ± 0.31
4	15	16.26 ± 0.22	42.43 ± 0.43	45.32 ± 2.25
5	30	22.16 ± 0.38	53.31 ± 0.51	57.44 ± 0.83
6	45	28.29 ± 0.41	60.16 ± 2.17	76.48 ± 1.39
7	60	37.39 ± 0.48	71.89 ± 0.22	81.09 ± 5.08

All values are expressed as mean ± SD, n = 3

Table 46: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	1st order	Higuchi Model	Hixon-Crowell model	Korsemeyer Peppas Model
1	C_2F1	0.9497	0.9781	**0.9788**	0.9710	0.9724
2	C_2HF2	0.7701	0.9462	**0.9843**	0.9032	0.9663
3	C_2HF3	0.8042	0.9045	**0.9311**	0.8395	0.8584
4	C_2F4	0.9870	0.9904	**0.9918**	0.9916	0.9862
5	C_2HF5	0.8957	0.9897	**0.9975**	0.9708	0.9961
6	C_2HF6	0.8797	0.9859	**0.9963**	0.9756	0.9946
7	C_2F7	0.9665	0.9838	**0.9839**	0.9792	0.9808
8	C_2HF8	0.8341	0.9575	**0.9922**	0.9281	0.9849
9	C_2HF9	0.8737	0.9849	**0.9966**	0.9701	0.9953

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty=kt^n$

Table 47: Cumulative amount of progesterone release from PF1, PHF2 and PHF3

Sl.No.	Time	Percentage release		
		PF1	PHF2	PHF3
1	0	0.0	0.0	0.0
2	5	4.19 ± 0.48	26.17 ± 0.79	27.89 ± 0.89
3	10	13.03 ± 1.66	32.33 ± 0.39	31.51 ± 1.73
4	15	16.87 ± 1.11	51.39 ± 1.97	55.66 ± 0.83
5	30	27.03 ± 0.41	66.93 ± 2.31	71.53 ± 1.27
6	45	32.35 ± 0.49	72.89 ± 1.08	81.94 ± 5.22
7	60	39.13 ± 1.02	77.80 ± 0.47	83.85 ± 4.60

All values are expressed as mean ± SD, n = 3

Table 48: Cumulative amount of progesterone release from PF4, PHF5 and PHF6

Sl.No.	Time	Percentage release		
		PF4	PHF5	PHF6
1	0	0.0	0.0	0.0
2	5	4.53 ± 0.74	21.36 ± 2.36	24.99 ± 3.54
3	10	13.59 ± 0.50	33.60 ± 2.90	39.70 ± 1.26
4	15	16.25 ± 0.49	41.20 ± 4.03	51.52 ± 0.97
5	30	22.09 ± 0.91	51.11 ± 2.88	66.79 ± 0.79
6	45	31.31 ± 0.61	59.43 ± 0.96	73.61 ± 1.04
7	60	39.40 ± 0.66	67.23 ± 0.49	78.80 ± 0.74

All values are expressed as mean ± SD, n = 3

Table 49: Cumulative amount of progesterone release from PF7, PHF8 and PHF9

Sl.No.	Time	Percentage release		
		PF7	PHF8	PHF9
1	0	0.0	0.0	0.0
2	5	4.60 ± 0.35	6.85 ± 0.95	8.19 ± 1.01
3	10	10.44 ± 0.59	12.60 ± 1.43	19.06 ± 1.56
4	15	14.90 ± 0.70	22.93 ± 2.37	33.01 ± 1.77
5	30	20.33 ± 0.40	28.40 ± 2.52	44.93 ± 1.95
6	45	29.15 ± 0.89	35.22 ± 3.04	55.20 ± 1.93
7	60	37.53 ± 0.70	43.09 ± 2.72	62.28 ± 1.80

All values are expressed as mean ± SD, n = 3

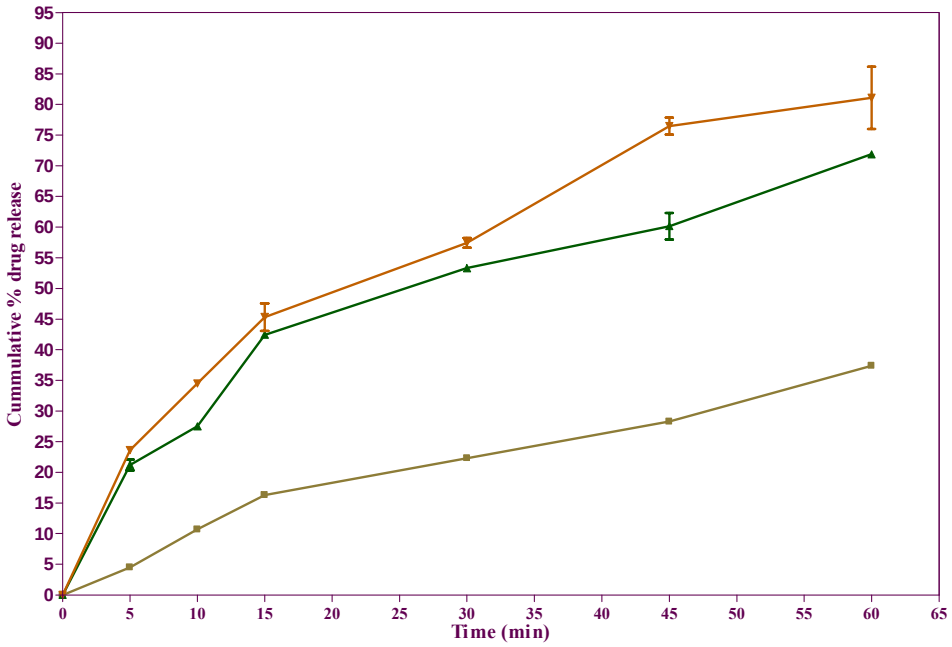


Fig.47: Cumulative percentage of release of progesterone from C2F7 , C2HF8 and C2HF9 .

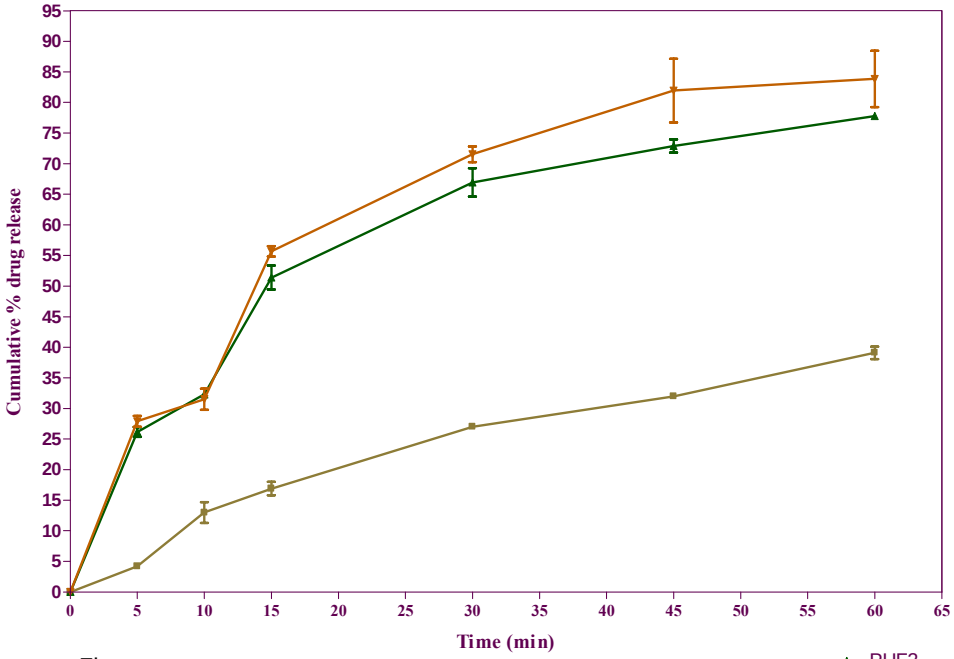


Fig. .48: Cumulative percentage of release of progesterone from PF1 , PHF2 and PHF3 .

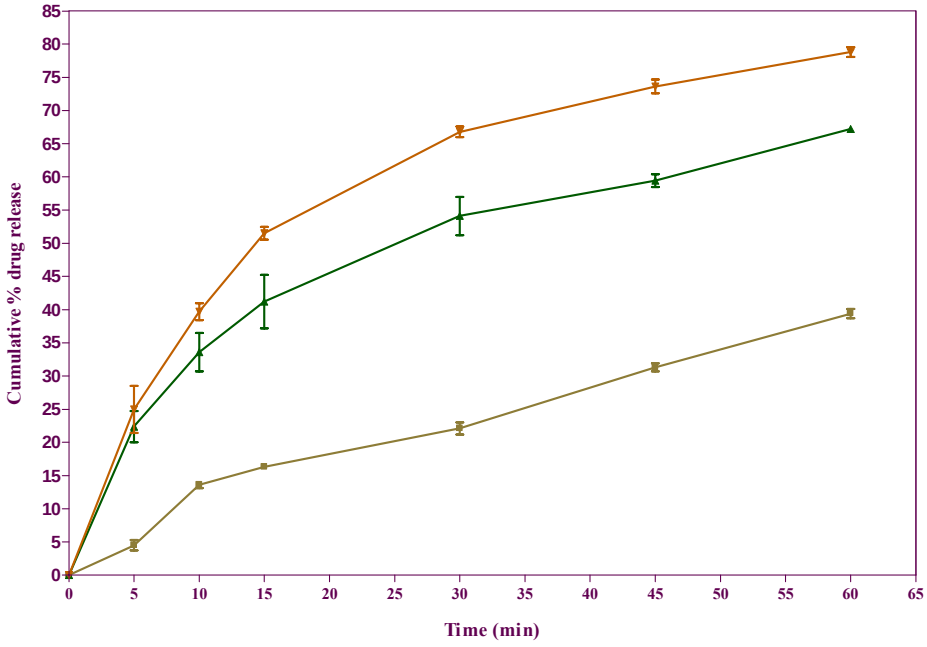


Fig.49: Cumulative percentage release of progesterone from PF4, PHF5 and PHF6 .

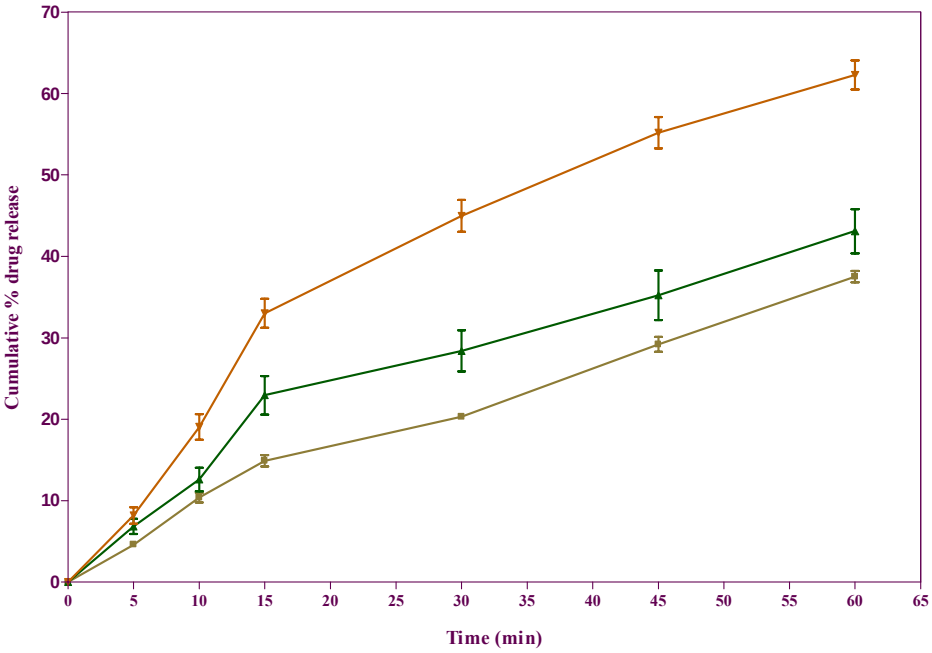


Fig.50: Cumulative percentage of release of progesterone from PF7 , PHF8 and PHF9 .

Table 50: Kinetic evaluation of drug release data for nasal gels

Sl.No.	Sample	Regression coefficient for selected release kinetic models				
		Zero order	**1st order**	**Higuchi Model**	**Hixon-Crowell model**	**Korsemeyer Peppas Model**
1	PF1	0.9623	0.9723	**0.9836**	0.9770	0.9666
2	PHF2	0.7641	0.9366	**0.9803**	0.8946	0.9732
3	PHF3	0.7735	0.9438	**0.9790**	0.9108	0.9720
4	PF4	0.9514	0.9760	**0.9772**	0.9689	0.9650
5	PHF5	0.6445	0.8614	**0.9880**	0.8045	0.9832
6	PHF6	0.6655	0.9130	**0.9578**	0.8544	0.9147
7	PF7	0.9743	0.9816	0.9657	**0.9843**	0.9679
8	PHF8	0.9150	0.9534	**0.9774**	0.9427	0.9588
9	PHF9	0.9299	0.9703	**0.9788**	0.9673	0.9691

Equations considered for the regression:

Zero order model: % drug released = $K_o.t$

First order model Log 10(% drug released) = $K_t/2.303t$

Higuchi model: % drug released = $K_H.t^{1/2}$

Hixon-Crowell model: $[(\text{total drug released})^{1/3} - (\text{drug release at time t})^{1/3}] = K_S.t$

Korsemeyer Peppas model**:** $M_t/M_\infty=kt^n$

Table 51: Diffusion coefficient D (cm^2/s) values for nasal gels with HPβ-CD

Sl.No	Sample	D(cm^2/s)	Sample	D(cm^2/s)	Sample	D(cm^2/s)
1	C_1F1	0.33	C_2F1	0.31	PF1	0.25
2	C_1HF2	0.56	C_2HF2	0.53	PHF2	0.51
3	C_1HF3	0.64	C_2F3	0.60	PHF3	0.56
4	C_1F4	0.31	C_2F4	0.27	PF4	0.24
5	C_1HF5	0.55	C_2HF5	0.52	PHF5	0.42
6	C_1HF6	0.63	C_2HF6	0.55	PHF6	0.53
7	C_1F7	0.29	C_2F7	0.26	PF7	0.24
8	C_1HF8	0.48	C_2HF8	0.46	PHF8	0.28
9	C_1HF9	0.51	C_2HF9	0.53	PHF9	0.46

Table 52: Release exponent 'n' for nasal gels with β-CD

Sl.No.	Sample	n value	Sample	n value	Sample	n value
1	C_1F1	0.77	C_2F1	0.84	PF1	0.77
2	C_1BF2	0.48	C_2BF2	0.50	PBF2	0.48
3	C_1BF3	0.45	C_2BF3	0.47	PBF3	0.47
4	C_1F4	0.82	C_2F4	0.91	PF4	0.83
5	C_1BF5	0.47	C_2BF5	0.59	PBF5	0.45
6	C_1BF6	0.44	C_2BF6	0.50	PBF6	0.48
7	C_1F7	0.82	C_2F7	0.82	PF7	0.89
8	C_1BF8	0.48	C_2BF8	0.56	PBF8	0.63
9	C_1BF9	0.44	C_2BF9	0.46	PBF9	0.61

Table 53: Release exponent 'n' for nasal gels with HPβ-CD

Sl.No.	Sample	n value	Sample	n value	Sample	n value
1	C_1F1	0.77	C_2F1	0.84	PF1	0.77
2	C_1HF2	0.45	C_2HF2	0.50	PHF2	0.48
3	C_1HF3	0.44	C_2HF3	0.48	PHF3	0.48
4	C_1F4	0.82	C_2F4	0.91	PF4	0.83
5	C_1HF5	0.46	C_2HF5	0.54	PHF5	0.48
6	C_1HF6	0.43	C_2HF6	0.55	PHF6	0.44
7	C_1F7	0.82	C_2F7	0.82	PF7	0.89
8	C_1HF8	0.46	C_2HF8	0.52	PHF8	0.64
9	C_1HF9	0.43	C_2HF9	0.50	PHF9	0.62

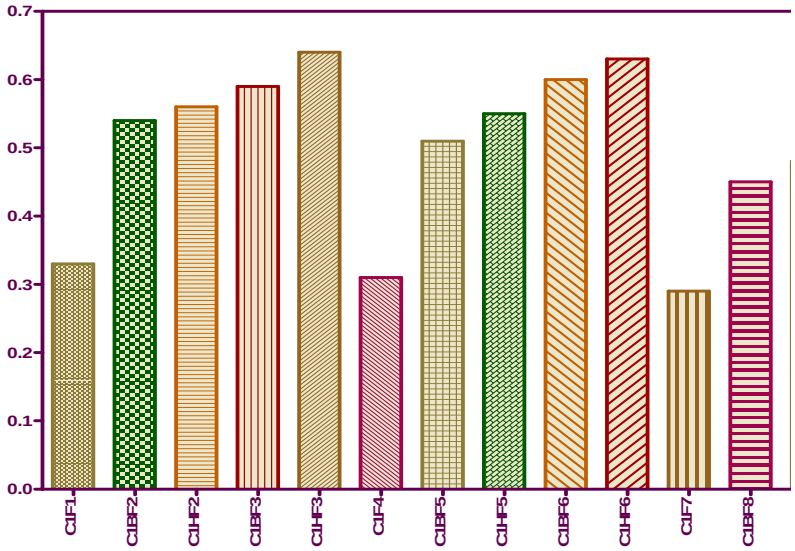


Fig. 51: Diffusion coefficient of various progesterone nasal gels

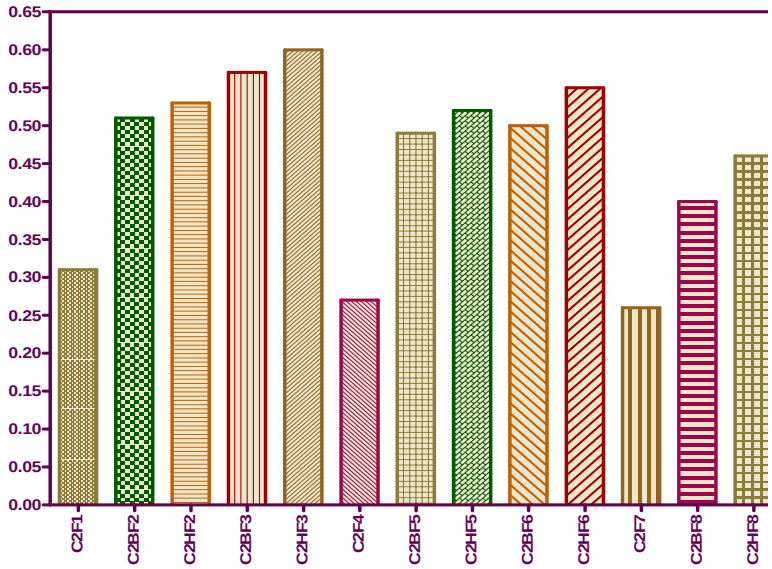


Fig.52: Diffusion coefficient of various progesterone nasal gels

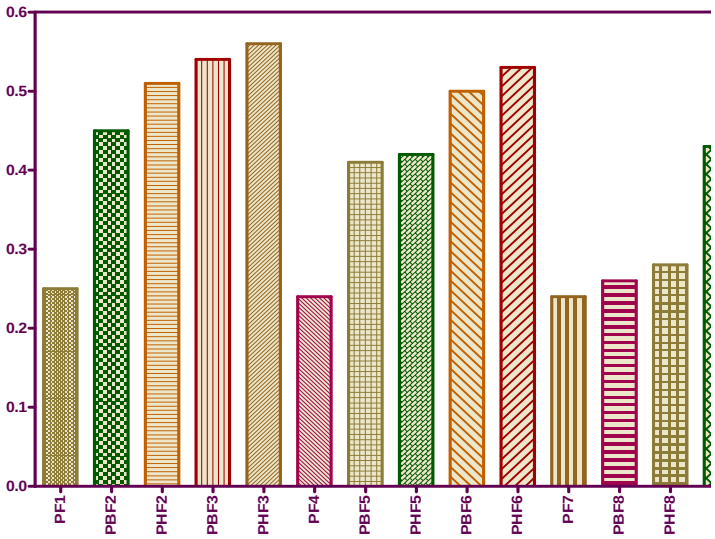


Fig. 53: Diffusion coefficient of various progesterone nasal gels

4.4 *Ex vivo* permeation studies with excised sheep nasal mucosa- as a tool to predict *in vivo* absorption

The permeation profiles of the various formulations are shown in Tables 54-62 and 66-74 and represented graphically in Fig.54-71. The permeation flux and permeability coefficient determination for each of the various nasal gel formulations are given in Tables 63-65 and 75-77 (Fig.72-74).

Effect of various PAA polymers on drug permeation

There was a significant difference between the different PAA polymer gels on the amount of drug permeated per unit area of the sheep nasal mucosa ($P<0.05$). In correlation to the dissolution study, the release from carbopol 974 gels was higher than that from carbopol 971 and polycarbophil gels. The permeability coefficient was 7.56×10^{-5} cm/s for C_1BF3 , 6.60×10^{-5} cm/s for C_2BF3 and 5.66×10^{-5} cm/s for PBF3. In case of HPβ-CD gels the permeability coefficient for C_1HF3, C2HF3 and PHF3 were 7.83×10^{-5} cm/s, 6.83×10^{-5} cm/s and 5.93×10^{-5} cm/s respectively. The release was in the order carbopol 974 > carbopol 971 > polycarbophil.

Effect of CD on the permeation kinetics

There was no significant difference in the gels with β-CD as a physical mixture with drug or as an inclusion complex ($P=0.0681-0.5474$). This was also seen in case of the HPβ-CD gels where there was no significant difference in the gels with different methods of addition of absorption enhancer HPβ-CD ($P=0.1194-0.5333$). The maximum effective permeability of 7.83×10^{-5} cm/s was obtained for formulation C_1HF3. It was 3.17×10^{-5} cm/s for the C_1F1 formulation and 6.56×10^{-5} cm/s for C_1HF2 gel. In case of beta cyclodextrin gels C_1BF3 had a permeability coefficient of 7.56×10^{-5} cm/s while it was 6.16×10^{-5} cm/s for C_1BF2 which is significantly different from C_1F1 with a value of 3.17×10^{-5} cm/s. Addition of CD had dramatically increased the permeation coefficient.

Cyclodextrins have proven to be excellent solubilizers and absorption enhancers in nasal drug delivery. Cyclodextrins are used to improve the nasal absorption of drugs by increasing their aqueous solubility and/or by enhancing their nasal absorption. Cyclodextrins have the advantage of being potent in low concentration and inert from a pharmacological-toxicological point. The penetration of progesterone through nasal mucosa was found to be low in gels which did not contain CD. The presence of CD significantly enhanced the penetration of active substance ($P< 0.05$). The penetration enhancing activity of CD can be explained with its membrane effects on nasal mucosa and this is consistent with previous reports[139]. Cyclodextrins act as absorption enhancers by various effects including disaggregation of protein aggregates or interaction with lipids and divalent cations on membrane surface or a direct effect on the paracellular pathway by a transient effect on tight junctions[139].

Effect of β-CD and HPβ-CD

It was evident from the results that the effective permeability coefficient for progesterone was significantly lower for C_1BF3 as compared with that of formulation C_1HF3 ($P<0.05$). Both these formulations are the same except that in the case of the former, HPβ-CD is used as the absorption enhancer whereas in case of the latter β-CD is the enhancer. From comparison of all the formulations, it was inferred that the presence of HPβ-CD increased the drug permeation through the sheep nasal mucosa with a higher rate as compared with that of β-CD gels.

Table 54: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F1	**C_1BF2**	**C_1BF3**
1	0	0.0	0.0	0.0
2	10	3.50 ± 0.28	4.42 ± 2.03	17.99 ± 1.03
3	20	9.40 ± 0.34	11.72 ± 0.42	32.09± 1.21
4	30	17.54 ± 1.70	21.13 ± 0.24	42.61 ± 1.04
5	45	21.42 ± 1.29	30.62 ± 2.05	52.37 ± 1.28
6	60	24.95 ±2.31	37.52 ± 3.12	60.31 ± 2.05
7	90	28.42 ± 1.43	43.91 ± 0.67	69.01 ± 1.25
8	120	31.89 ± 2.63	54.89 ± 2.01	76.76 ± 3.05
9	150	35.58 ± 1.35	60.73 ± 1.36	85.01 ± 0.80
10	180	39.43 ± 0.48	67.14 ± 1.25	93.40 ± 1.01

All values are expressed as mean ± SD, n = 3

Table 55: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F4	**C_1BF5**	**C_1BF6**
1	0	0.0	0.0	0.0
2	10	4.42 ± 2.10	5.15 ± 2.30	14.18 ± 0.77
3	20	9.46 ± 1.59	12.58 ± 1.60	28.32± 0.98
4	30	13.714 ± 2.36	20.42 ± 1.87	37.45 ± 4.54
5	45	18.542 ± 1.45	25.24 ± 2.10	44.32 ± 3.60
6	60	22.33 ±0.98	30.11 ± 0.13	54.57 ± 4.02
7	90	26.10 ± 1.43	40.86 ± 1.05	63.13 ± 2.40
8	120	30.08 ± 2.30	49.64 ± 0.13	69.94 ± 2.36
9	150	34.39 ± 3.04	56.38 ± 1.48	75.25 ± 3.60
10	180	38.36 ± 3.10	63.92 ± 3.25	85.05 ± 1.50

All values are expressed as mean ± SD, n = 3

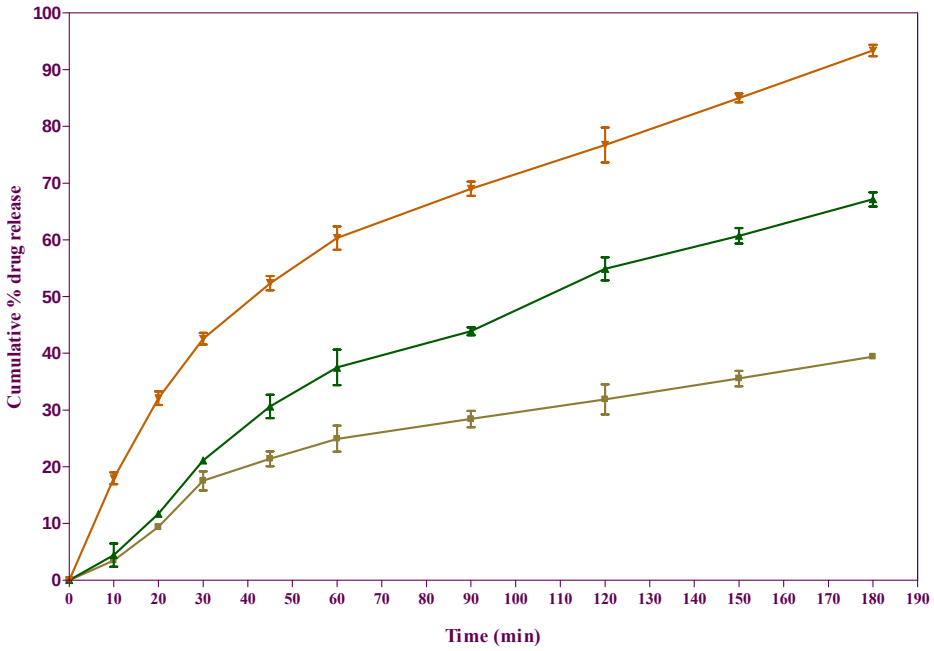


Fig.54: Permeation profile of progesterone from C1F1 , C1BF2 and C1BF3 across sheep nasal mucosa .

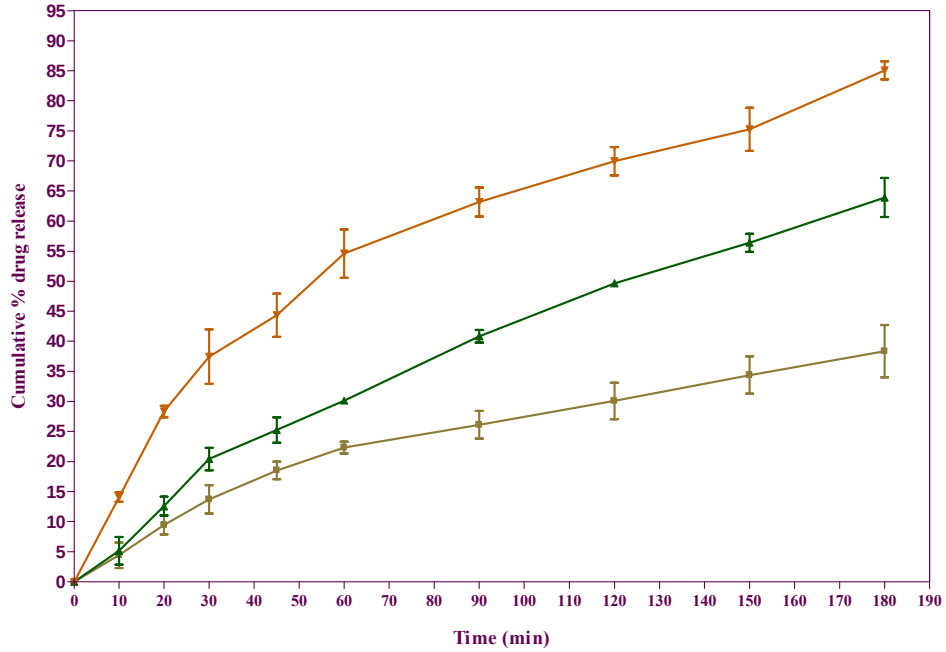


Fig.55: Permeation profile of progesterone from C1F4 , C1BF5 and C1BF6 across sheep nasal mucosa.

Table 56: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F7	**C_1BF8**	**C_1BF9**
1	0	0.0	0.0	0.0
2	10	2.57± 2.36	5.11 ± 3.26	13.51 ± 0.28
3	20	7.45 ± 3.01	13.37 ± 2.58	27.00± 2.46
4	30	11.894 ± 4.25	21.09 ± 4.01	36.41 ± 5.62
5	45	17.82 ± 0.67	25.63 ± 2.65	43.82 ± 7.62
6	60	21.56 ±1.60	30.54 ± 0.91	53.97 ± 2.10
7	90	24.76 ± 0.75	41.30 ± 1.90	62.62 ± 0.90
8	120	27.76 ± 1.21	47.30 ± 3.30	71.01 ± 3.70
9	150	31.16 ± 1.45	55.63 ± 2.69	75.06 ± 1.80
10	180	35.39 ± 1.55	62.11 ± 4.00	81.77 ± 3.10

All values are expressed as mean ± SD, n = 3

Table 57: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F1	**C_2BF2**	**C_2BF3**
1	0	0.0	0.0	0.0
2	10	5.33 ± 2.11	3.86 ± 0.61	16.26 ± 1.65
3	20	11.39 ± 1.95	10.09 ± 1.24	27.44± 2.44
4	30	15.54 ± 2.28	18.72 ± 2.07	33.96 ± 2.43
5	45	18.88 ± 2.65	28.19 ± 2.85	44.85 ± 4.58
6	60	22.24 ±2.86	34.49 ± 2.45	51.11 ± 2.40
7	90	25.62 ± 2.53	41.20 ± 2.37	58.93 ± 5.41
8	120	28.87 ± 2.81	47.49 ± 1.43	66.60 ± 5.01
9	150	32.10 ± 3.01	53.19 ± 1.66	73.59 ± 4.99
10	180	36.30 ± 4.13	61.34 ± 3.76	81.35 ± 5.03

All values are expressed as mean ± SD, n = 3

Table 58*: Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F4	C_2BF5	C_2BF6
1	0	0.0	0.0	0.0
2	10	3.48 ± 0.57	4.28 ± 1.21	11.15 ± 2.56
3	20	7.14 ± 0.81	10.29 ± 1.72	21.03± 3.45
4	30	11.48 ± 0.26	17.28 ± 4.50	26.37 ± 6.42
5	45	13.18 ± 4.92	21.22 ± 2.01	33.73 ± 2.58
6	60	19.75 ±1.31	26.44 ± 3.00	40.49 ± 3.10
7	90	22.93 ± 1.26	35.19 ± 1.91	48.43 ± 5.10
8	120	26.59 ± 1.52	42.78 ± 2.28	55.61 ± 1.50
9	150	30.44 ± 1.84	48.85 ± 1.91	63.23 ± 2.15
10	180	34.08 ± 1.92	54.61 ± 1.97	72.54 ± 3.20

All values are expressed as mean ± SD, n = 3

Table 59: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F7	C_2BF8	C_2BF9
1	0	0.0	0.0	0.0
2	10	1.87 ± 0.43	4.00 ± 0.97	11.03 ± 1.13
3	20	6.88 ± 1.29	10.26 ± 1.32	18.54± 6.12
4	30	10.26 ± 2.96	17.72 ± 3.07	25.39 ± 7.11
5	45	14.81 ± 3.20	21.91 ± 1.76	32.59 ± 6.55
6	60	19.89 ±3.91	26.70 ± 2.32	38.92 ± 5.46
7	90	23.24 ± 3.35	35.19 ± 4.91	45.77 ± 4.62
8	120	26.33 ± 2.07	41.89 ± 3.57	51.79 ± 4.53
9	150	29.98 ± 2.04	47.30 ± 3.88	57.74 ± 4.85
10	180	33.20 ± 1.92	54.21 ± 5.72	65.77 ± 3.77

All values are expressed as mean ± SD, n = 3

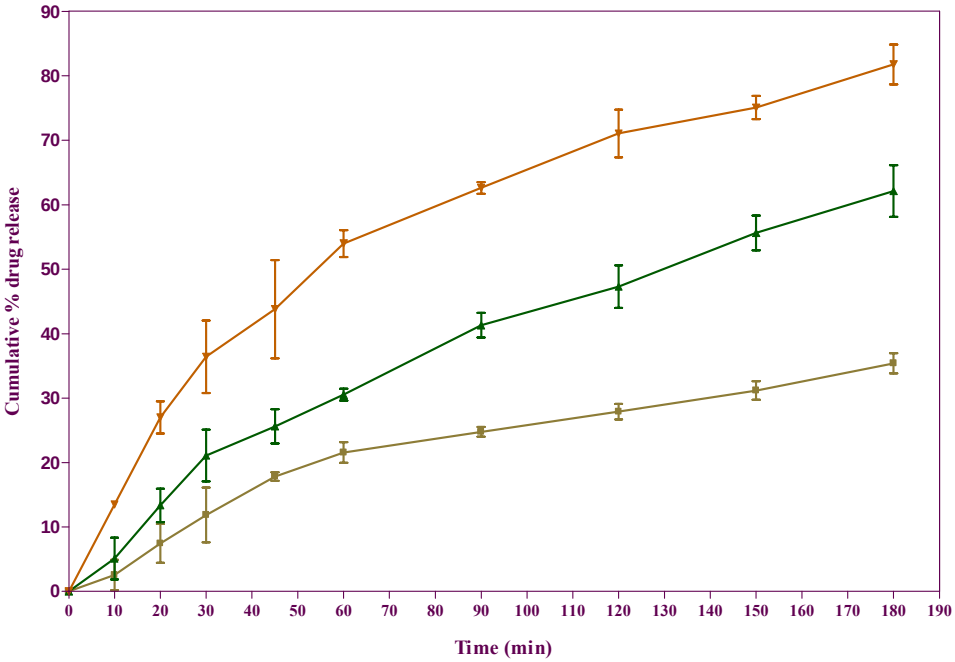


Fig.56: Permeation profile of progesterone from C1F7 , C1BF8 and C1BF9 across sheep nasal mucosa.

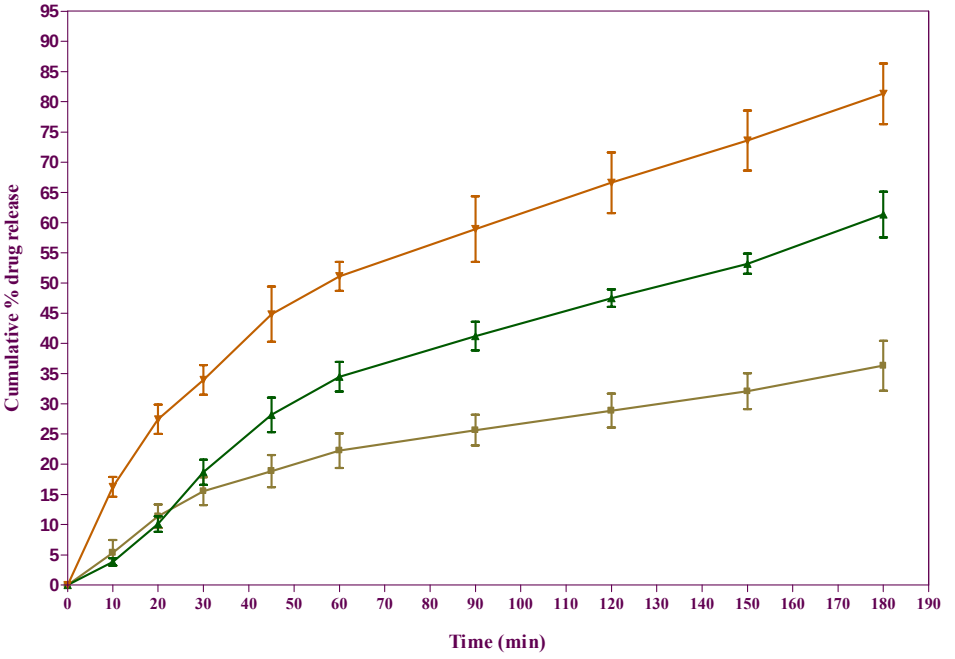


Fig.57: Permeation profile of progesterone from C2F1 , C2BF2 and C2BF3 across sheep nasal mucosa.

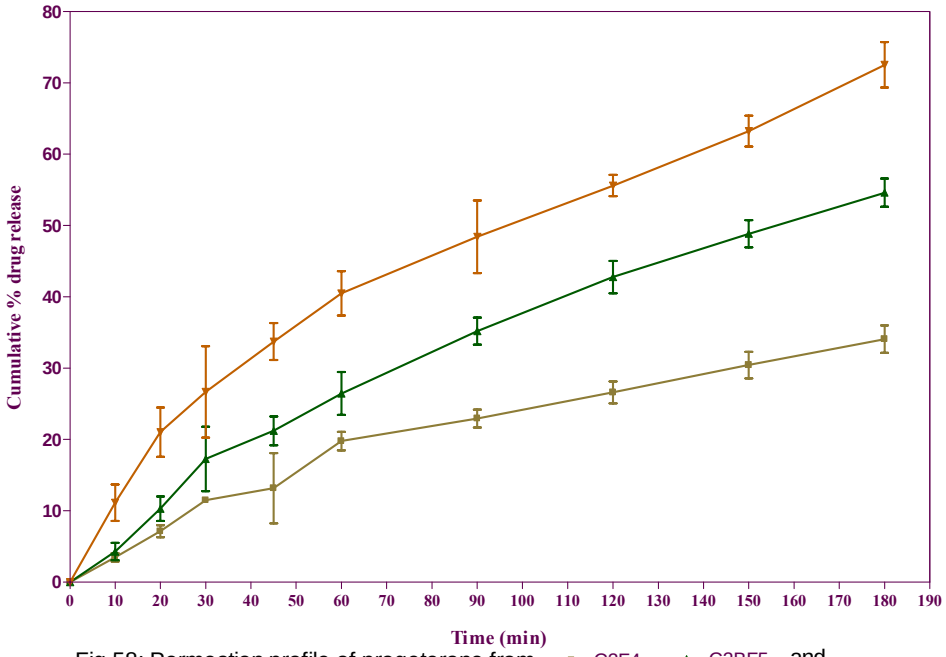


Fig.58: Permeation profile of progeterone from C2F4 , C2BF5 and C2BF6 across sheep nasal mucosa.

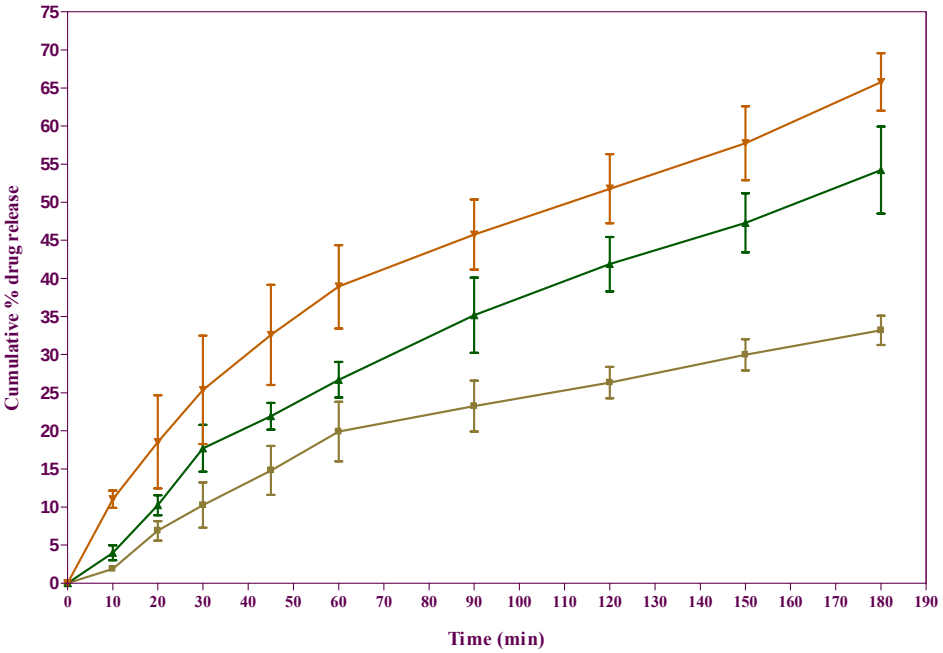


Fig.59: Permeation profile of progesterone from C2F7 , C2BF8 and C2BF9 across sheep nasal mucosa.

Table 60*: Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF1	PBF2	PBF2
1	0	0.0	0.0	0.0
2	10	4.47 ± 2.50	3.15 ± 1.13	13.45 ± 4.35
3	20	9.65 ± 4.35	7.87 ± 3.90	22.36± 5.47
4	30	12.974 ± 5.40	15.70 ± 4.56	28.16 ± 3.40
5	45	17.38 ± 4.69	23.28 ± 2.30	34.60 ± 2.30
6	60	20.76 ±4.64	29.39 ± 3.20	40.96 ± 1.50
7	90	24.18 ± 4.55	36.27 ± 1.50	46.52 ± 5.59
8	120	27.48 ± 4.27	42.16 ± 3.01	54.85 ± 3.60
9	150	30.80 ± 4.77	47.48 ± 1.10	62.78 ± 1.20
10	180	34.75 ± 5.23	54.30 ± 0.80	69.86 ± 3.40

All values are expressed as mean ± SD, n = 3

Table 61: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF4	PBF5	PBF6
1	0	0.0	0.0	0.0
2	10	2.90 ± 4.36	2.88 ± 1.15	9.34 ± 4.56
3	20	6.22 ± 5.47	7.99 ± 3.99	17.58± 1.97
4	30	9.66 ± 2.01	14.11 ± 4.1	23.19 ± 3.35
5	45	13.62 ± 5.48	18.21 ± 4.12	29.15 ± 1.70
6	60	17.83 ±3.12	21.58 ± 4.35	35.32 ± 3.03
7	90	21.36 ± 4.02	29.09 ± 4.02	43.77 ± 2.55
8	120	25.05 ± 2.52	37.00 ± 5.51	49.78 ± 3.50
9	150	28.91 ± 1.78	43.51 ± 4.39	56.35 ± 5.45
10	180	32.67 ± 3.22	49.29 ± 4.87	62.25 ± 5.20

All values are expressed as mean ± SD, n = 3

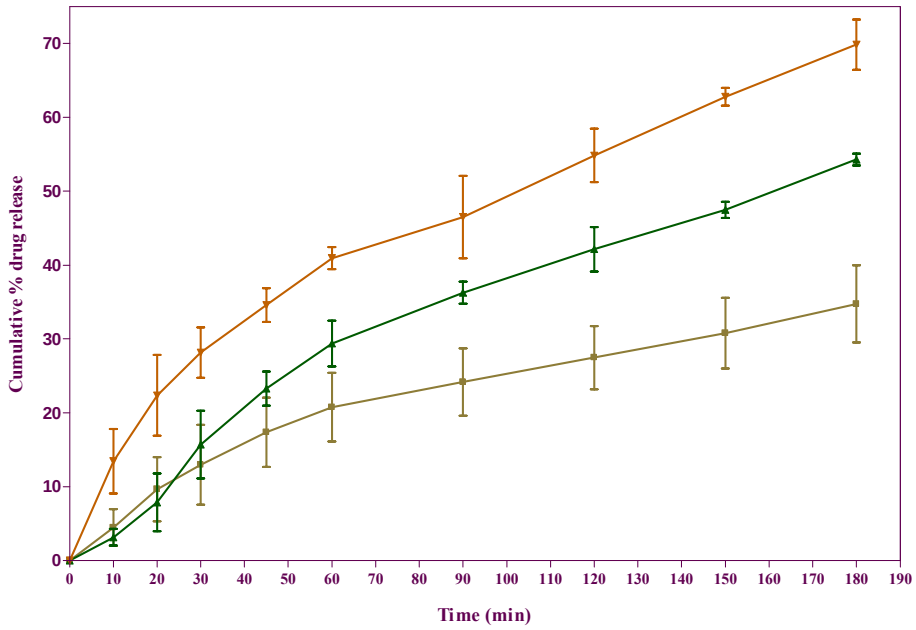


Fig.60: Permeation profile of progesterone from PF1 , PBF2 and PBF3 across sheep nasal mucosa.

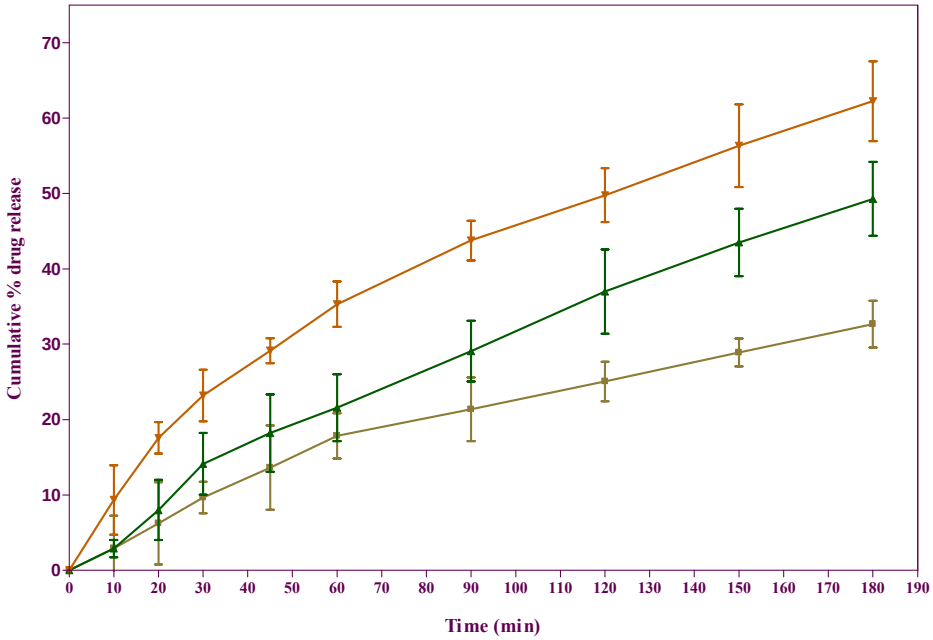


Fig.61: Permeation profile of progesterone from PF4 , PBF5 and PBF6 across sheep nasal mucosa.

Table 62: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF7	PBF8	PBF9
1	0	0.0	0.0	0.0
2	10	1.33 ± 0.78	2.87 ± 1.29	8.41± 2.70
3	20	4.89 ± 1.63	6.84 ± 2.71	14.62± 2.58
4	30	8.72 ± 3.20	13.97 ± 2.87	18.81 ± 3.08
5	45	13.01 ± 3.25	17.76 ± 2.68	25.96 ± 5.10
6	60	17.39 ±4.81	22.27 ± 2.35	32.09 ± 4.87
7	90	21.21 ± 4.48	28.59 ± 2.63	37.30 ± 5.31
8	120	24.09 ± 3.50	35.19 ± 2.18	44.12 ± 3.91
9	150	27.77 ± 4.11	40.68 ± 2.80	49.67 ± 4.45
10	180	30.26 ± 2.97	47.09 ± 3.29	55.86 ± 4.20

All values are expressed as mean ± SD, n = 3

Table 63: Flux and permeability coefficient of gels C_1F1- C_1BF9

Sl.No.	Sample	J_{ss} (mg/ cm^2.min)	K_p (cm/s)
1	C_1F1	0.95×10^{-2}	3.17×10^{-5}
2	C_1BF2	1.85×10^{-2}	6.16×10^{-5}
3	C_1BF3	2.27×10^{-2}	7.56×10^{-5}
4	C_1F4	0.95×10^{-2}	3.16×10^{-5}
5	C_1BF5	1.74×10^{-2}	5.80×10^{-5}
6	C_1BF6	2.09×10^{-2}	6.96×10^{-5}
7	C_1F7	0.94×10^{-2}	3.15×10^{-5}
8	C_1BF8	1.71×10^{-2}	5.70×10^{-5}
9	C_1BF9	2.08×10^{-2}	6.93×10^{-5}

J_{ss} is the steady state flux in mg/cm^2min

K_p is the apparent nasal permeability co-efficient in cm/s

Table 64: Flux and permeability coefficient of gels C_2F1- C_2BF9

Sl.No.	Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
1	C_2F1	0.89×10^{-2}	2.99×10^{-5}
2	C_2BF2	1.64×10^{-2}	5.46×10^{-5}
3	C_2BF3	1.98×10^{-2}	6.60×10^{-5}
4	C_2F4	$0..64 \times 10^{-2}$	2.13×10^{-5}
5	C_2BF5	1.49×10^{-2}	4.97×10^{-5}
6	C_2BF6	1.71×10^{-2}	5.71×10^{-5}
7	C_2F7	0.74×10^{-2}	2.45×10^{-5}
8	C_2BF8	1.38×10^{-2}	4.61×10^{-5}
9	C_2BF9	1.53×10^{-2}	5.11×10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min

K_p is the apparent nasal permeability co-efficient in cm/s

Table 65: Flux and permeability coefficient of gels PF1- PBF9

Sl.No.	Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
1	PF1	0.88×10^{-2}	2.95×10^{-5}
2	PBF2	1.46×10^{-2}	4.87×10^{-5}
3	PBF3	1.70×10^{-2}	5.66×10^{-5}
4	PF4	0.73×10^{-2}	2.43×10^{-5}
5	PBF5	1.35×10^{-2}	4.50×10^{-5}
6	PBF6	1.55×10^{-2}	5.16×10^{-5}
7	PF7	0.53×10^{-2}	1.77×10^{-5}
8	PBF8	1.24×10^{-2}	4.13×10^{-5}
9	PBF9	1.35×10^{-2}	4.51×10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min

K_p is the apparent nasal permeability co-efficient in cm/s

Table 66: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F1	C_1HF2	C_1HF3
1	0	0.0	0.0	0.0
2	10	3.50 ± 0.28	7.60 ± 0.33	19.45 ± 1.35
3	20	9.40 ± 0.34	11.62 ± 2.76	31.08± 1.90
4	30	17.54 ± 1.70	19.56 ± 4.37	40.10 ± 3.53
5	45	21.42 ± 1.29	26.73 ± 3.20	50.40 ± 2.93
6	60	24.95 ±2.31	35.58 ± 4.28	59.75 ± 2.52
7	90	28.42 ± 1.43	43.99 ± 0.12	67.91 ± 3.93
8	120	31.89 ± 2.63	52.98 ± 1.83	73.01 ± 3.23
9	150	35.58 ± 1.35	65.81 ± 1.32	81.43 ± 5.48
10	180	39.43 ± 0.48	69.48 ± 2.10	92.70 ± 5.33

All values are expressed as mean ± SD, n = 3

Table 67: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F4	C_1HF5	C_1HF6
1	0	0.0	0.0	0.0
2	10	4.42 ± 2.10	6.69 ± 1.57	17.00 ± 1.66
3	20	9.46 ± 1.59	13.01 ± 3.40	30.28± 2.71
4	30	13.714 ± 2.36	21.14 ± 4.68	39.39 ± 3.90
5	45	18.542 ± 1.45	26.05 ± 4.35	45.54 ± 3.50
6	60	22.33 ±0.98	30.84 ± 0.46	57.02 ± 5.40
7	90	26.10 ± 1.43	41.52 ± 0.50	64.38 ± 4.11
8	120	30.08 ± 2.30	50.83 ± 1.51	72.77 ± 5.01
9	150	34.39 ± 3.04	57.69 ± 1.61	75.44 ± 6.12
10	180	38.36 ± 3.10	65.35 ± 3.73	86.21 ± 2.30

All values are expressed as mean ± SD, n = 3

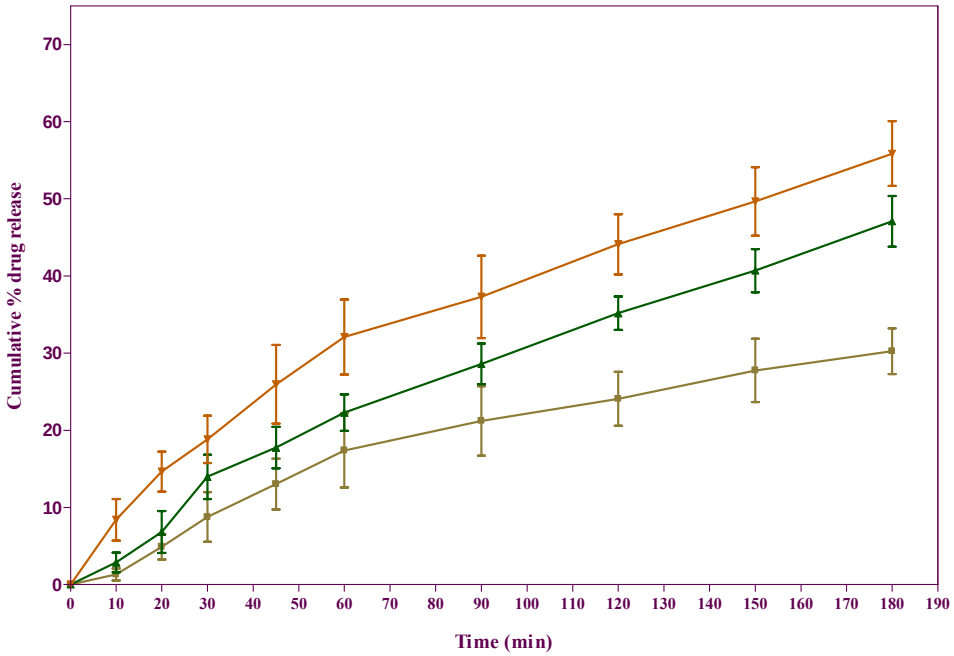


Fig.62: Permeation profile of progesterone from PF7 , PBF8 and PBF9 across sheep nasal mucosa.

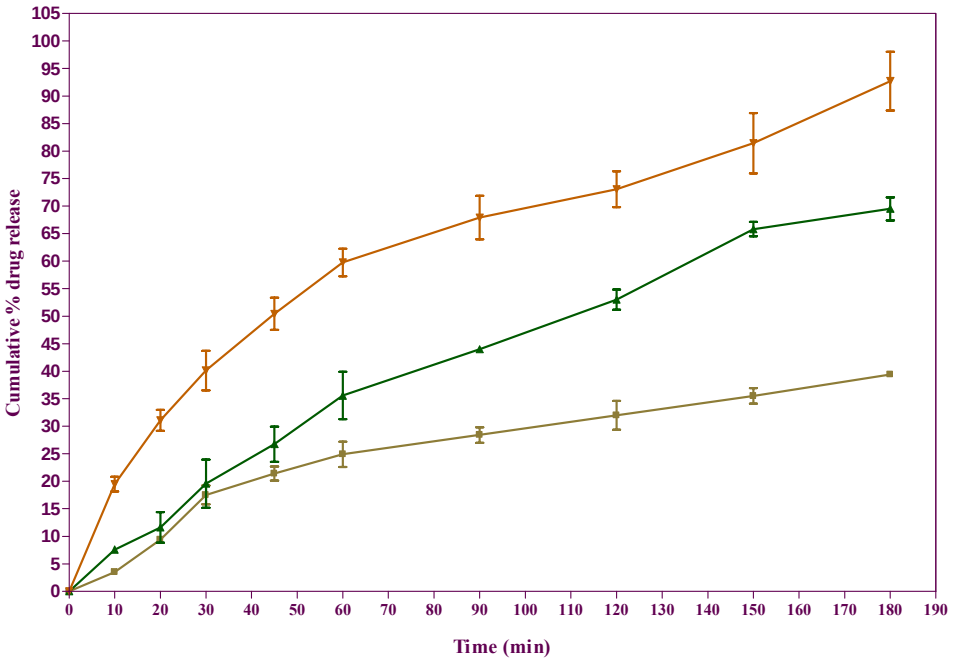


Fig.63: Permeation profile of progesterone from C1F1 , C1HF2 and C1HF3 across sheep nasal mucosa.

Table 68: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_1F7	C_1HF8	C_1HF9
1	0	0.0	0.0	0.0
2	10	2.57± 2.36	5.91 ± 0.75	15.98 ± 0.33
3	20	7.45 ± 3.01	14.49 ± 1.48	28.73± 0.88
4	30	11.894 ± 4.25	22.61 ± 2.27	37.65 ± 4.73
5	45	17.82 ± 0.67	28.33 ± 3.55	41.61 ± 3.00
6	60	21.56 ±1.60	33.98 ± 5.01	55.93 ± 2.89
7	90	24.76 ± 0.75	42.92 ± 2.26	63.22 ± 4.60
8	120	27.76 ± 1.21	51.33 ± 4.40	73.08 ± 5.60
9	150	31.16 ± 1.45	60.07 ± 4.94	75.32 ± 6.01
10	180	35.39 ± 1.55	63.21 ± 3.24	85.28 ± 2.11

All values are expressed as mean ± SD, n = 3

Table 69: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F1	C_2HF2	C_2HF3
1	0	0.0	0.0	0.0
2	10	5.33 ± 2.11	5.41 ± 2.81	17.87± 4.11
3	20	11.39 ± 1.95	12.18 ± 3.65	28.30± 2.51
4	30	15.54 ± 2.28	20.29 ± 2.97	34.16 ± 2.50
5	45	18.88 ± 2.65	29.28 ± 3.67	43.96 ± 4.39
6	60	22.24 ±2.86	35.97 ± 3.40	52.35 ± 3.56
7	90	25.62 ± 2.53	42.04 ± 3.53	61.01 ± 4.01
8	120	28.87 ± 2.81	49.31 ± 3.78	70.18 ± 2.56
9	150	32.10 ± 3.01	56.11 ± 3.47	76.05 ± 1.87
10	180	36.30 ± 4.13	64.71 ± 1.94	83.02 ± 6.53

All values are expressed as mean ± SD, n = 3

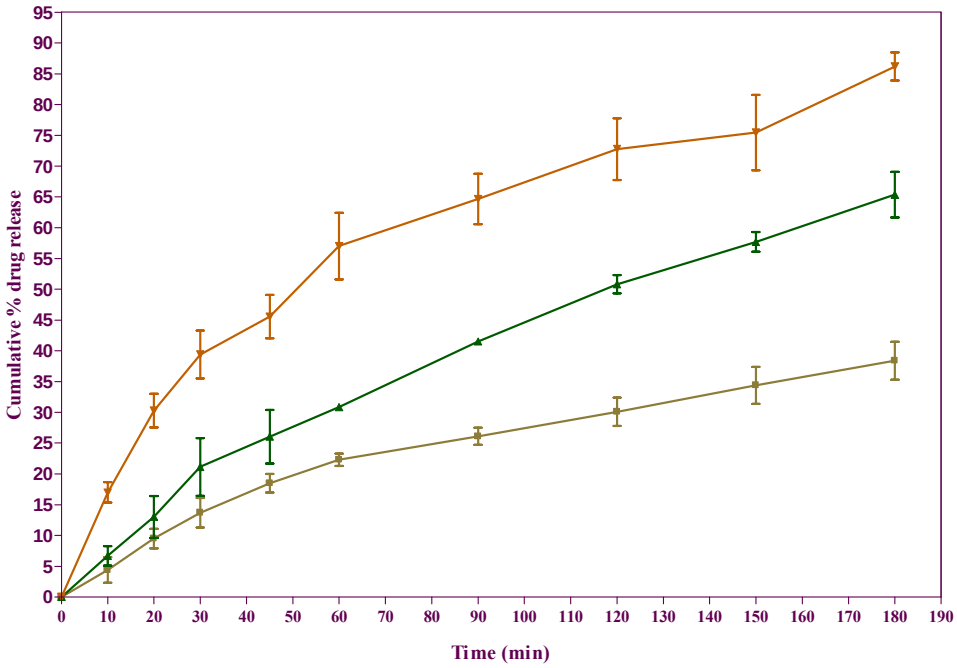


Fig.64: Permeation profile of progesterone from C1F4 , C1HF5 and C1HF6 across sheep nasal mucosa.

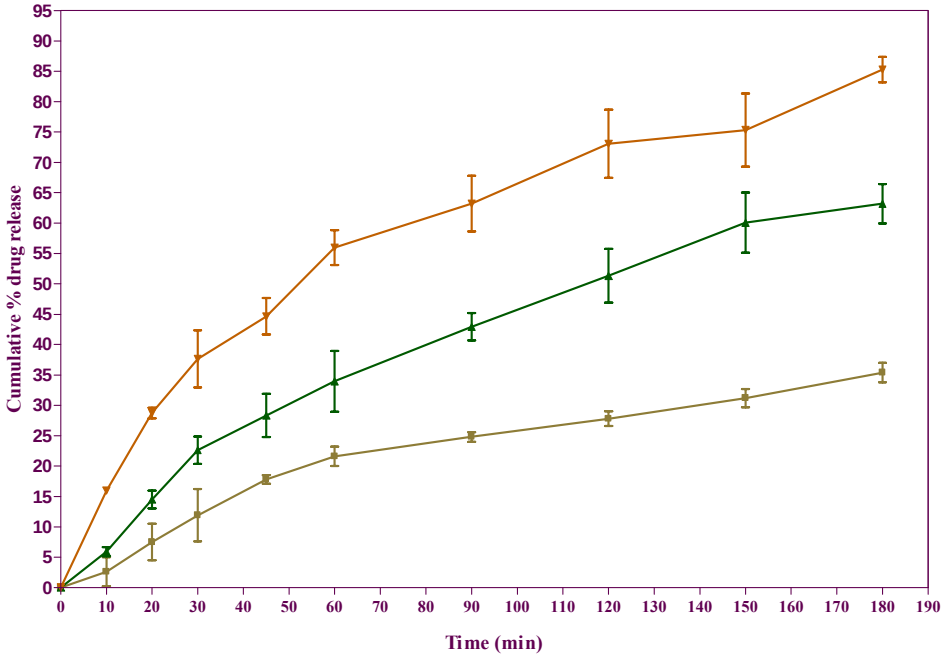


Fig.65: Permeation profile of progeterone from C1F7 , C1HF8 and C1HF9 across sheep nasal mucosa.

Table 70: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F4	C_2HF5	C_2HF6
1	0	0.0	0.0	0.0
2	10	3.48 ± 0.57	6.29 ± 1.78	13.18 ± 1.02
3	20	7.14 ± 0.81	11.43 ± 1.28	19.85± 4.88
4	30	11.48 ± 0.26	18.59 ± 2.95	26.33 ± 6.50
5	45	13.18 ± 4.92	23.39 ± 2.62	34.33 ± 7.22
6	60	19.75 ±1.31	28.29 ± 2.59	39.95 ± 5.18
7	90	22.93 ± 1.26	36.31 ± 1.89	46.99 ± 4.02
8	120	26.59 ± 1.52	43.01 ± 3.75	52.81 ± 4.27
9	150	30.44 ± 1.84	51.30 ± 3.72	58.76 ± 4.59
10	180	34.08 ± 1.92	58.74 ± 4.07	68.42 ± 2.37

All values are expressed as mean ± SD, n = 3

Table 71: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		C_2F7	C_2HF8	C_2HF9
1	0	0.0	0.0	0.0
2	10	1.87 ± 0.43	6.29 ± 1.78	13.19 ± 1.01
3	20	6.88 ± 1.29	11.43 ± 1.28	19.82± 4.88
4	30	10.26 ± 2.96	18.69 ± 2.92	26.32 ± 6.50
5	45	14.81 ± 3.20	23.39 ± 2.62	34.33 ± 7.22
6	60	19.89 ±3.91	28.29 ± 2.59	39.95 ± 5.18
7	90	23.24 ± 3.35	36.31 ± 4.89	46.99 ± 4.02
8	120	26.33 ± 2.07	43.01 ± 3.75	52.81 ± 4.27
9	150	29.98 ± 2.04	51.30 ± 3.72	58.76 ± 4.59
10	180	33.20 ± 1.92	58.73 ± 4.07	68.43 ± 3.77

All values are expressed as mean ± SD, n = 3

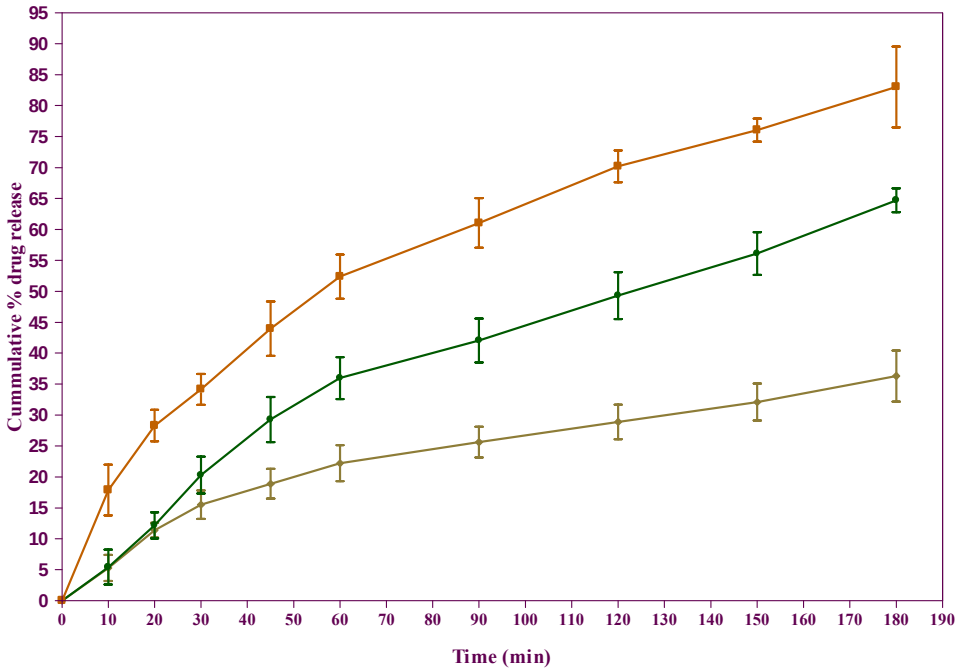


Fig.66: Permeation profile of progesterone from C2F1 C2HF2 and C2HF3 from sheep nasal mucosa.

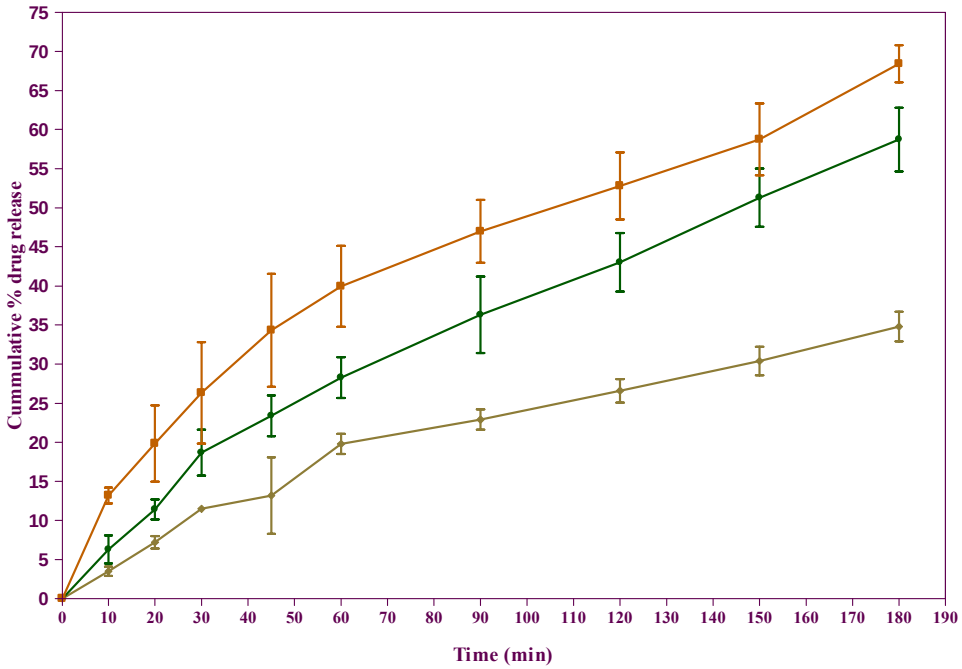


Fig.67: Permeation profile of progesterone from C2F4 C2HF5 and C2HF6 from sheep nasal mucosa.

Table 72: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF1	PHF2	PHF2
1	0	0.0	0.0	0.0
2	10	4.47 ± 2.50	4.41 ± 2.27	16.55 ± 1.24
3	20	9.65 ± 4.35	9.45± 5.03	24.44± 2.31
4	30	12.974 ± 5.40	18.40 ± 3.60	30.31 ± 2.81
5	45	17.38 ± 4.69	24.88 ± 4.21	36.17 ± 3.32
6	60	20.76 ±4.64	31.14 ± 2.30	42.63 ± 2.61
7	90	24.18 ± 4.55	37.63 ± 3.64	49.59 ± 3.17
8	120	27.48 ± 4.27	41.42 ± 1.80	57.46 ± 1.92
9	150	30.80 ± 4.77	49.25 ± 2.60	65.53 ± 2.02
10	180	34.75 ± 5.23	57.72 ± 3.91	73.82 ± 2.81

All values are expressed as mean ± SD, n = 3

Table 73: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF4	PHF5	PHF6
1	0	0.0	0.0	0.0
2	10	2.90 ± 4.36	5.56 ± 0.95	11.00 ± 0.95
3	20	6.22 ± 5.47	10.59± 1.04	19.11± 1.04
4	30	9.66 ± 2.01	16.34 ± 3.03	24.67 ± 3.03
5	45	13.62 ± 5.48	19.79 ± 2.66	31.05 ± 2.66
6	60	17.83 ±3.12	23.39 ± 2.50	36.60 ± 2.50
7	90	21.36 ± 4.02	30.68 ± 1.73	41.96 ± 1.73
8	120	25.05 ± 2.52	38.46 ± 3.99	50.71 ± 3.99
9	150	28.91 ± 1.78	44.73 ± 2.98	57.42 ± 2.92
10	180	32.67 ± 3.22	51.64 ± 1.88	64.18 ± 1.88

All values are expressed as mean ± SD, n = 3

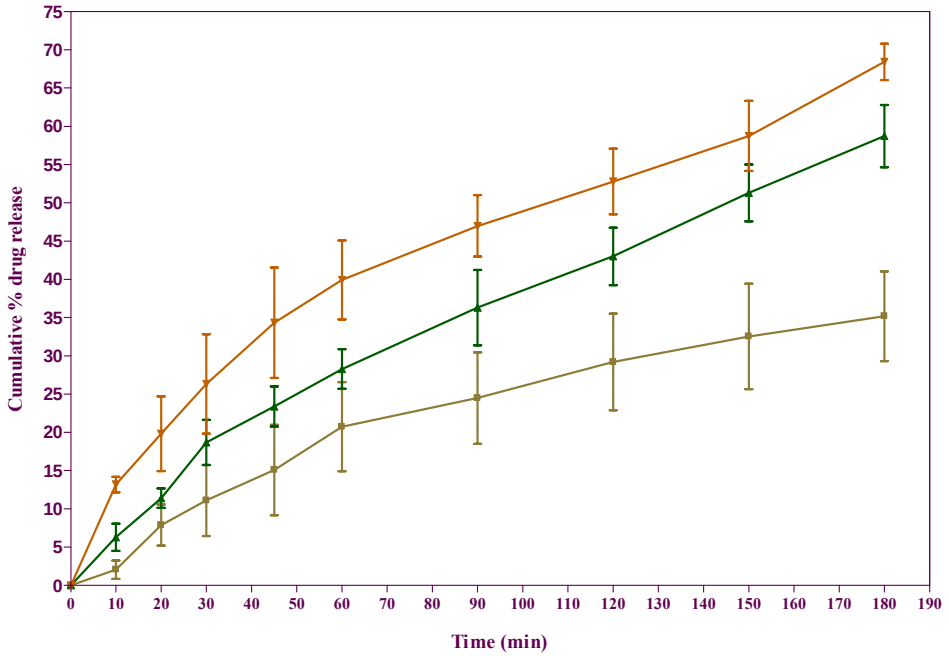


Fig.68: Permeation profile of progesterone from C2F7 , C2HF8 and C2HF9 across sheep nasal mucosa.

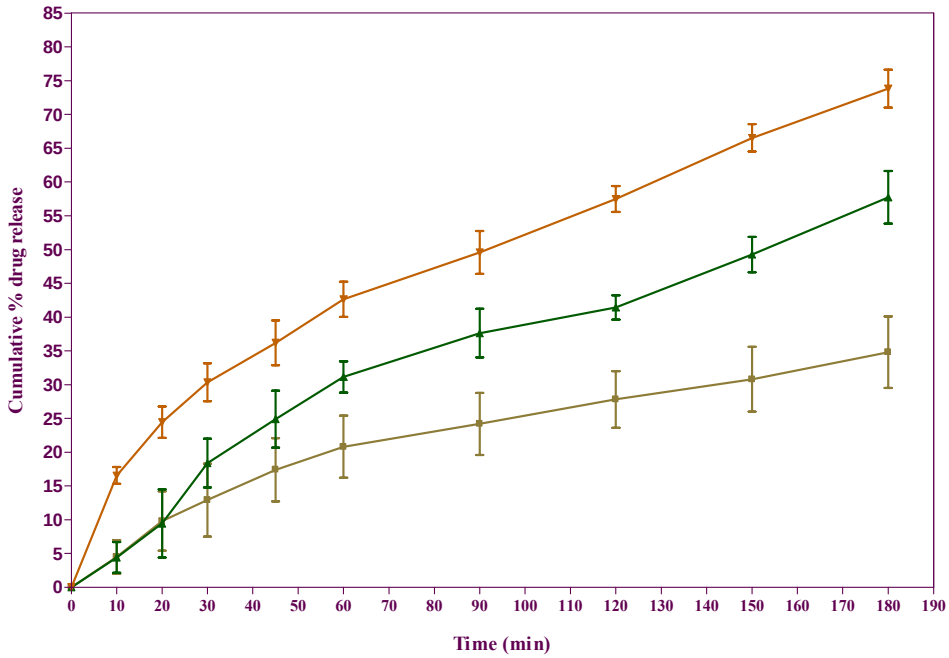


Fig.69: Permeation profile of progesterone from PF1, PHF2 and PHF3 across sheep nasal mucosa.

Table 74: *Ex vivo* permeation profile of progesterone across sheep nasal mucosa

Sl.No.	Time	Percentage release		
		PF7	PHF8	PHF9
1	0	0.0	0.0	0.0
2	10	1.33 ± 0.78	3.82 ± 0.61	10.52± 1.00
3	20	4.89 ± 1.63	7.81± 1.38	15.99± 3.92
4	30	8.72 ± 3.20	15.19 ± 1.34	20.23 ± 4.47
5	45	13.01 ± 3.25	18.74 ± 1.89	27.45 ± 5.25
6	60	17.39 ±4.81	23.06 ± 1.61	33.43 ± 5.01
7	90	21.21 ± 4.48	27.16 ± 3.01	38.74 ± 5.41
8	120	24.09 ± 3.50	36.39 ± 0.82	44.96 ± 3.76
9	150	27.77 ± 4.11	42.02± 0.72	50.77± 4.19
10	180	30.26 ± 2.97	48.32 ± 0.85	57.29 ± 3.70

All values are expressed as mean ± SD, n = 3

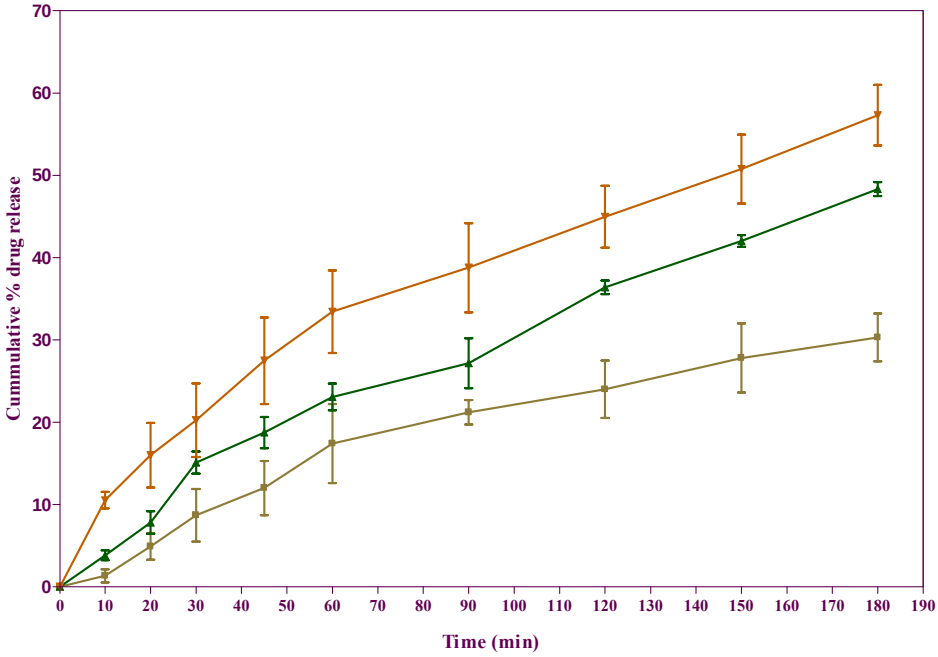


Fig.70: Permeation profile of progesterone from PF4 , PHF5 and PHF6 across sheep nasal mucosa.

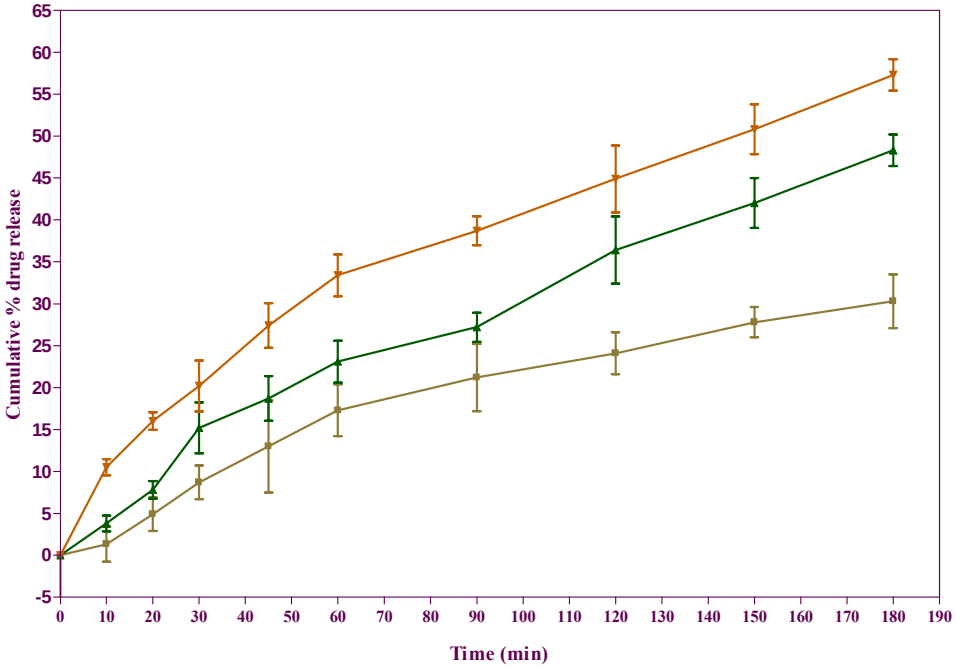


Fig.71: Permeation profile of progesterone from PF7 , PHF8 and PHF9 across sheep nasal mucosa.

Table 75: Flux and permeability coefficient of gels C_1F1- C_1HF9

Sl.No.	Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
1	C_1F1	0.95×10^{-2}	3.17×10^{-5}
2	C_1HF2	1.97×10^{-2}	6.56×10^{-5}
3	C_1HF3	2.20×10^{-2}	7.83×10^{-5}
4	C_1F4	0.95×10^{-2}	3.16×10^{-5}
5	C_1HF5	1.89×10^{-2}	6.30×10^{-5}
6	C_1HF6	2.07×10^{-2}	6.90×10^{-5}
7	C_1F7	0.94×10^{-2}	3.15×10^{-5}
8	C_1HF8	1.86×10^{-2}	6.20×10^{-5}
9	C_1HF9	2.09×10^{-2}	6.96×10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min

K_p is the apparent nasal permeability co-efficient in cm/s

Table 76: **Flux and permeability coefficient of gels C_2F1- C_2HF9**

Sl.No.	Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
1	C_2F1	0.89×10^{-2}	2.99×10^{-5}
2	C_2HF2	1.72×10^{-2}	5.73×10^{-5}
3	C_2HF3	2.05×10^{-2}	6.83×10^{-5}
4	C_2F4	0.64×10^{-2}	2.13×10^{-5}
5	C_2HF5	1.51×10^{-2}	5.03×10^{-5}
6	C_2HF6	1.86×10^{-2}	6.20×10^{-5}
7	C_2F7	0.91×10^{-2}	2.45×10^{-5}
8	C_2HF8	1.55×10^{-2}	5.16×10^{-5}
9	C_2HF9	1.66×10^{-2}	5.53×10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min

K_p is the apparent nasal permeability co-efficient in cm/s

Table 77: Flux and permeability coefficient of gels PF1- PHF9

Sl.No.	Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
1	PF1	0.88×10^{-2}	2.95×10^{-5}
2	PHF2	1.52×10^{-2}	5.11×10^{-5}
3	PHF3	1.77×10^{-2}	5.93×10^{-5}
4	PF4	0.73×10^{-2}	2.43×10^{-5}
5	PHF5	1.37×10^{-2}	4.57×10^{-5}
6	PHF6	1.64×10^{-2}	5.46×10^{-5}
7	PF7	0.53×10^{-2}	1.77×10^{-5}
8	PHF8	1.29×10^{-2}	4.33×10^{-5}
9	PHF9	1.43×10^{-2}	4.78×10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min

K_p is the apparent nasal permeability co-efficient in cm/s

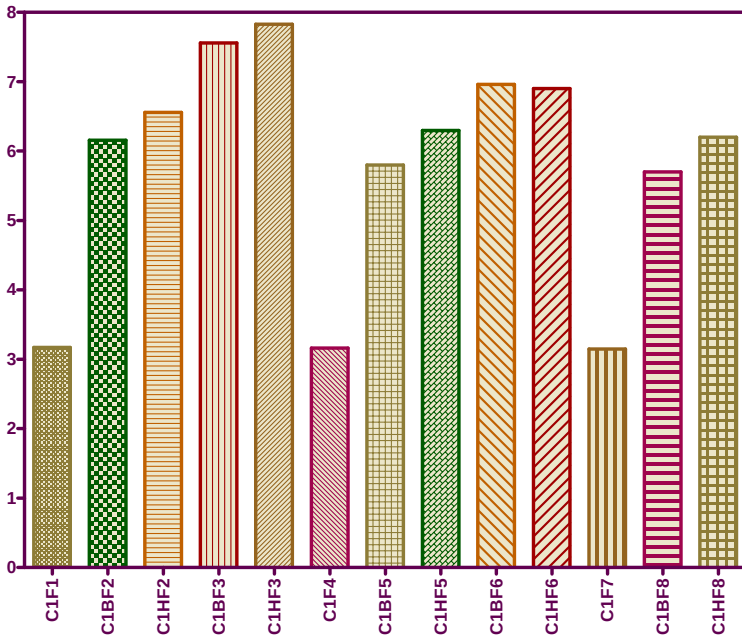


Fig. 72. Permeability coefficient Kp (X10^{-5}cm/s)

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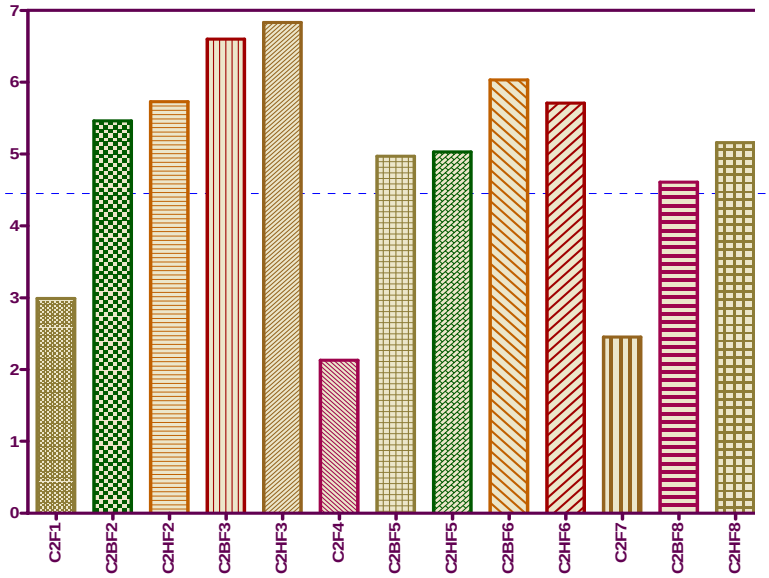

Fig. 73. Permeability coefficient Kp (X10^{-5}cm/s)

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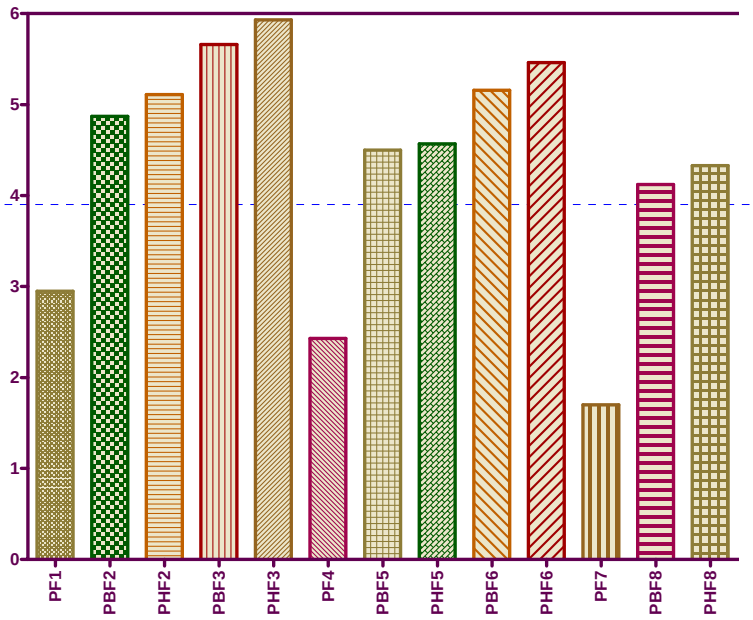

Fig. 74. Permeability coefficient Kp (X10^{-5}cm/s)

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4.5 Histopathological evaluation of the mucosa

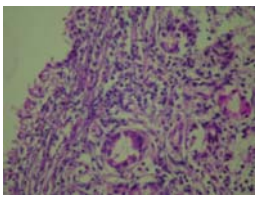
Histological evaluation of the sheep nasal mucosa was performed 3 hr after the removal of the tissue from the *ex vivo* diffusion studies and were compared with freshly excised specimen (fig.75). The results indicated that the specimen examined after 3 hr incubation in the diffusion chambers did not show extensive negative effects on the epithelium. Mainly swelling and inflammation effects were seen and no epithelial cell loss was noticed in all the samples.

4.6 Bioavailability studies

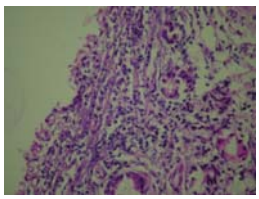
The changes in plasma progesterone levels in female rabbits[142] following administration of nasal gels are summarized in Table 78 and 80. The absolute bioavailability profile is shown in Table 79 and 81. Nasal bioavailabilities were calculated using AUC values calculated from mean plasma concentration data. The AUCs thus calculated were corrected for basal levels of progesterone by subtraction of the mean AUC. The basal level of progesterone fluctuated between 5-8 ng/ml.

In Fig 76 serum progesterone level after nasal administration of two categories of gels was compared with intravenous administration of progesterone solution at the same dose. The two categories of gels chosen were the carbopol 974 gels which contained β-CD and HPβ-CD as the absorption enhancer. These gels were chosen based on the *ex vivo* permeation profiles. The HPβ-CD gels of carbopol 974 showed the maximum *in vitro* release characteristics. The β-CD gels were also selected for bioavailability studies to compare with that of the HPβ-CD gels.

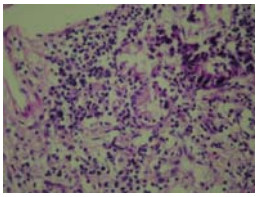
Nasal administration showed remarkable absorption rapidity: the peak was achieved within 15 min, the serum concentration decreased in 60 min. Absolute bioavailability of the best formulation C1HF3 was 83.89%. The formulation with carbopol 974 (1.5% and 2%) gels without any CD showed lowest bioavailability of 40-43%. The nasal gels C_1HF3 was characterized by a peak plasma concentration of 59.12 ng/ml at 15 min and relevant progesterone concentrations were maintained up



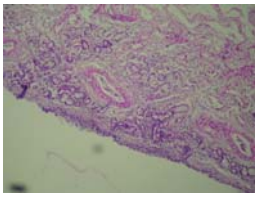
Sample C_2HF8



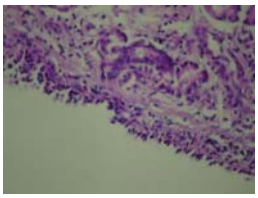
Sample C_1HF8



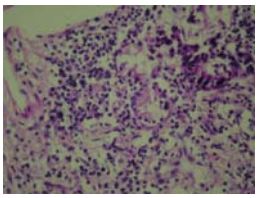
Sample PHF8



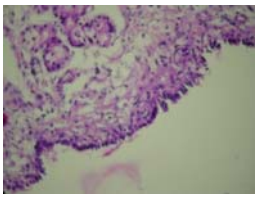
Sample C_2BF8



Sample C_1BF8



Sample PBF8



Normal sheep nasal mucosa

Fig.75. Histological evaluations of sections of sheep nasal mucosal membrane Mucosal layer after incubation in diffusion chamber with various nasal progesterone gel formulations

to 60 min. It was observed that the CD gels provided a relevant increase in bioavailability. The formulations without any CD also showed F % value between 40-43%. This was due to carbopol also possessing absorption enhancing effects[75]. Carbopols function as absorption enhancers in two ways. First, they adhere to the nasal mucosal surface, thereby increasing the contact time of the drug with the mucosa and secondly they induce a transient opening of epithelial cell tight junctions.

Serum concentration after i.v. administration declined rapidly, with basal levels reaching after 30 min with an initial half life of 6.8 min. In case of the gels especially C_1BF3, C_1HF3 and C_1HF2 administered nasally, resulted in elevated progesterone serum levels for over 30 min up to 60 min with higher bioavailability values of 78.53%, 83.89% and 76.36% respectively. In all these cases maximum concentrations were seen at 15 min. The peak concentration, however were much higher and persisted for a longer duration of time in case of gels with the added CD as compared to gels without CD. The C_{max} in case of nasal gels C_1HF3 and C_1HF2 were 59.12 and 53.69 ng/ml whereas it was 30.24 ng/ml in case of C_1F1 and higher plasma concentration were maintained up to 30 min in C_1F1 while it was 60 min in case of C_1HF3 and C_1HF2. The nasal bioavailability of progesterone was markedly enhanced by the addition of CDs. The increase in bioavailability was up to 84%. In case of nasal suspension (NS) the C_{max} was 19.86 ng/ml at 15 min with bioavailability of 29.5 % The bioavailability after nasal administration of HPβ-CD gels was significantly higher than that after β-CD gels ($P<0.05$). The reported oral bioavailability of progesterone[92] was less than 10 % and hence nasal route is 8 times higher than that by oral route.

The increased solubility of progesterone by the formation of inclusion complexes with both β-CD and HPβ-CD was an important factor in the mechanism of absorption enhancement[101]. Cyclodextrins increased the nasal delivery of drugs through multiple routes. One route would be to increase the solubility of poorly water soluble drugs like progesterone. Solubilization of drugs dramatically improves their systemic delivery via nasal administration by presenting higher overall concentrations to the nasal epithelium. A second route would be to alter nasal

permeability. However evidence is available that cyclodextrins extract lipids form the gastrointestinal mucosa, possibly leading to facilitated oral absorption[54]. This might be applicable with nasal drug transport. The use of gels afforded a greater contact time between drug or the cyclodextrin mixture with the drug or the cyclodextrin complex of the drug and the nasal mucosal surface which allowed for greater absorption.

Table78: Mean serum concentration of progesterone following intravenous and nasal administration in rabbits

Group	Sample	Plasma concentration (ng/ml)					
		05 min	15 min	30 min	60 min	120 min	240 min
1	IV	80.18±12.1	41.10±13.1	19.97±20.6	10.12±22.3	9.56±20.1	9.04±23.1
2	C_1F1	15.20±13.6	30.24±11.5	20.36±10.3	11.20±21.3	7.50±15.8	6.36±6.2
3	C_1BF2	25.27±14.2	52.45±12.6	33.10±15.6	24.11±11.5	8.40±16.2	6.41±8.9
4	C_1BF3	34.18±22.1	55.48±14.6	33.51±11.6	27.16±19.2	8.61±14.2	8.21±10.2
5	C_1F4	16.41±12.6	29.20±21.3	19.45±10.9	10.08±11.2	8.12±8.6	7.87±8.6
6	C_1BF5	28.22±14.6	48.70±8.6	34.30±9.8	20.14± 4.6	7.80±8.7	6.84±9.5
7	C_1BF6	33.83±8.9	50.10±9.7	33.14±15.6	25.05±20.1	7.13±22.3	6.58±7.6
8	C_1F7	13.21±16.1	30.63±10.6	25.19±20.5	11.31±18.3	6.74±21.4	6.03±6.6
9	C_1BF8	24.14±9.6	47.28±12.3	31.40±8.7	22.56± 6.8	8.21±11.2	6.45±10.2
10	C_1BF9	22.02±11.3	46.77±13.5	28.81±7.4	23.30± 8.9	7.91±9.6	6.41±16.5
11	NS	10.3±6.5	19.86±5.69	11.57±10.2	10.2±7.8	8.63±3.6	7.23±2.1

IV: intravenous dose

NS: nasal suspension

All values are expressed as mean ± SD, n = 5

Table 79: Pharmacodynamic parameters of nasal gels

Sample	$AUC_{(0-t)}$ ng.min/ml	C_{max} ng/ml	T_{max} min	F (%)
IV	4986	80.18	05	-
C_1F1	2578	30.24	15	41.70
C_1BF2	3820	52.45	15	71.80
C_1BF3	4099	55.48	15	78.52
C_1F4	2609	29.20	15	42.42
C_1BF5	3616	48.70	15	66.80
C_1BF6	3789	50.10	15	71.02
C_1F7	2620	30.63	15	42.70
C_1BF8	3624	47.28	15	67.02
C_1BF9	3647	46.77	15	67.58
NS	2074	19.86	15	29.49
Blank	856	-	-	-

In rabbits (n=5), $AUC_{(0-t)}$= area under plasma concentration-time (0-240 min) curve, C_{max}= peak plasma level, T_{max}= time to peak level, F= absolute bioavailability.
Blank: basal level of progesterone

Table 80: Mean serum concentration of progesterone following intravenous and nasal administration in rabbits

Group	Sample	Plasma concentration (ng/ml)					
		05 min	**15 min**	**30 min**	**60 min**	**120 min**	**240 min**
1	IV	80.18±12.1	41.10±13.1	21.44±20.6	10.12±22.3	9.56±20.1	9.04±23.1
12	C_1HF2	33.9±18.2	53.69±16.9	34.10±13.1	28.70±21.5	7.60±12.2	7.10±4.9
13	C_1HF3	37.89±17.1	59.12±17.1	36.85±21.3	27.32±11.8	9.02±10.2	7.15±18.2
14	C_1HF5	27.23±8.91	50.12±21.54	28.12±20.12	25.64±11.56	9.75±5.89	7.19±4.78
15	C_1HF6	29.4±16.5	52.71±10.25	32.6±6.98	21.4±4.58	9.84±12.45	7.12±14.89
16	C_1HF8	29.36±13.6	51.41±18.0	33.40±21.9	22.95±11.6	7.5±16.7	6.6±19.5
17	C_1HF9	33.21±5.2	52.10±17.7	34.60±11.6	23.40±12.4	8.49±2.8	7.20±8.6

IV: intravenous dose

All values are expressed as mean ± SD, n = 5

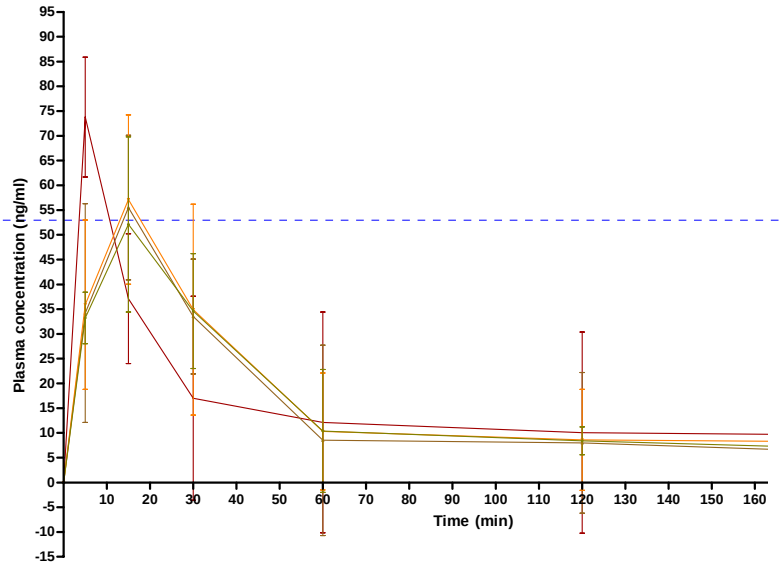


Fig.76: Mean plasma concentration of progesterone in rabbits following intravenous administration iv and nasal adminstration of C1BF3 , C1HF3 and C1HF6 gels.

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Table 81: Pharmacokinetic parameters of nasal gels

Sample	$AUC_{(0-t)}$ ng.min/ml	C_{max} ng/ml	T_{max} min	F (%)
IV	4986	80.18	10	-
C_1HF2	4009	53.69	15	76.36
C_1HF3	4321	59.12	15	83.89
C_1HF5	3872	50.12	15	73.02
C_1HF6	3989	52.71	15	75.85
C_1HF8	3837	51.41	15	72.22
C_1HF9	4029	52.10	15	76.82

In rabbits (n=5), $AUC_{(0-t)}$= area under plasma concentration-time (0-240 min) curve, C_{max}= peak plasma level, T_{max}= time to peak level, F= absolute bioavailability.

4.7 Optimization Results

In the present work, a two factor three level (3^2) factorial design was employed to investigate the effect of factors such as different concentrations of polyacrylic acid polymers and method of addition of cyclodextrin on diffusion coefficient (D), permeability coefficient (K_p) and bioavailability (F). The independent variables selected were concentrations of polyacrylic acid polymers and method of addition of cyclodextrin. The response variable studied were Y_1, diffusion coefficient (D), Y_2, permeability coefficient (K_p) and Y_3, bioavailability (F). The mathematical relationships containing only significant factors influencing each response were generated using multiple linear regression and analyzed on software PCP-RSM and Design Expert 7.1 trial (Statease Statistical Software Package). ANOVA was performed on the response parameters to identify the statistically significant effects and generate a predictor equation comprising only the significant main and interaction effects. All the polynomial equations were found to be highly statistically significant ($P< 0.05$).

Carbopol 974 gels with β-CD.

$$Y_1 = 0.5 - 0.028X_1 + 0.135X_2 - 0.055\ X_2\ X_2 \quad \text{- - - - (1)}$$

$$Y_2 = 5.89 - 0.185X_1 + 1.99X_2 - 0.0732\ X_2\ X_2 \quad \text{- - - - (2)}$$

$$Y_3 = 68.54 + 15.05X_2 - 11.22\ X_2\ X_2 \quad \text{- - - - (3)}$$

Carbopol 974 gels with HPβ-CD.

$$Y_1 = 0.53 - 0.0417X_1 + 0.142X_2 - 0.078\ X_2\ X_2 \quad \text{- - - - (4)}$$

$$Y_2 = 6.35 + 2.04X_2 - 1.158\ X_2\ X_2 \quad \text{- - - - (5)}$$

$$Y_3 = 74.47 - 3.66X_1 + 16.56X_2 - 4.10\ X_1\ X_2 - 15.64\ X_2\ X_2 \quad \text{- - - - (6)}$$

Carbopol 971 gels with β-CD

$$Y_1 = 0.5 - 0.028X_1 + 0.135X_2 - 0.055\ X_2\ X_2 \quad \text{- - - - (7)}$$

$$Y_2 = 5.89 - 0.185X_1 + 1.99X_2 - 0.0732\ X_2\ X_2 \quad \text{- - - - (8)}$$

Carbopol 971 gels with HPβ-CD.

$$Y_1 = 0.424 + 0.12X_2 \quad \text{- - - - (9)}$$

$$Y_2 = 5.02 - 0.48\ X_1 + 1.64X_2 - 0.85\ X_2\ X_2 \quad \text{- - - - (10)}$$

Polycarbophil gels with β-CD.

$$Y_1 = 0.37 + 0.12X_2 \quad \text{- - - - (11)}$$

$$Y_2 = 4.50 - 0.51X_1 + 1.36X_2 - 0.075\ X_2\ X_2 \quad \text{- - - - (12)}$$

Polycarbophil gels with HPβ-CD.

$$Y_1 = 0.39 + 0.14X_2 \quad \text{- - - - (13)}$$

$$Y_2 = 4.68 - 0.52\ X_1 + 1.53X_2 - 0.80\ X_2\ X_2 \quad \text{- - - - (14)}$$

The polynomial equations generated for the dependent and independent variables are shown above in equations 1-14. They represent the quantitative effect of process variables X_1 and X_2 and their interactions on the response Y_1, Y_2 and Y_3 that is D, K_p and F. The values of the coefficients were related to the effect of these variables on the particular response. Coefficients with more than one factor term and those with higher order terms represented interaction terms. A positive sign represented a synergistic effect, while a negative sign signified an antagonistic effect.

From the equations 1, 4, 7, 9, 11, 13 it could be seen that the presence of CD(X_2) had a significant effect and was the main effect on Y_1, diffusion coefficient as the coefficient for X_2 was found to be significant. The concentration of polymer (X_1) had less impact while the negative sign indicated that as the concentration of polymer increased the diffusion of the drug from the system decreases. The interaction terms were less significant.

In case of the next response, Y_2, the permeability coefficient, (equations 2, 5, 8, 10, 12, 14) it was seen that the sign and magnitude of the coefficient for X_2 indicated that presence of CD had the greatest influence on the response. This revealed that the method of addition of CD and its presence had a rather more positive and pronounced effect on the value of permeability coefficient. In case of X_1 the negative sign indicated that the concentration of polymer inversely affected

the permeation of the drug and its effect was less pronounced. The interaction terms were less significant.

The equations 3 and 6 indicated the response Y_3, for the bioavailability, where the CD played a positive role in influencing the response. The interaction term though significant was lower as compared to the coefficient of X_2.

The process was optimized for the response Y_1 to Y_3 and the optimized formulation was arrived at by maximizing the diffusion coefficient, permeability coefficient and bioavailability to obtain the desired levels of independent variables. The results from the optimization clarified the optimum settings as 1% for the concentration of the polymer and the addition of cyclodextrins as inclusion complexes.

5. Toxicological studies

5.1 Slug Mucosal Irritation Test

Effect on the mucosa of slugs treated for five successive days with different gels on the total amount of mucus production, mean protein, mean LDH and mean ALP released is shown in Table 82. To replace the laboratory animals an alternative mucosal irritation test was developed using slugs (*Arion lusitanicus*) which have high mucosal surface as a test organism (Fig.77). The slug mucosal irritation test enabled estimation of the irritation potential of a repeated treatment with bio-adhesive formulations on nasal mucosal tissue. This was also used to estimate local tolerance on buccal and ocular tissues. Adriaens and Remon[122] have reported excellent agreement between Slug Mucosal Irritation test and the *in vivo* data on local tolerance.

The body wall of slugs consists of a mucosal surface that contains cells with cilia, microvilli and mucus secreting cells covering a sub-epithelial connective tissue. Slugs that were placed on an irritant substance would produce mucus. Additionally, tissue damage was induced which resulted in the release of proteins and enzymes. Test preparations which caused tissue damage would result in the release of biomarkers into the PBS and the proteins and enzymes could be measured in these PBS samples.

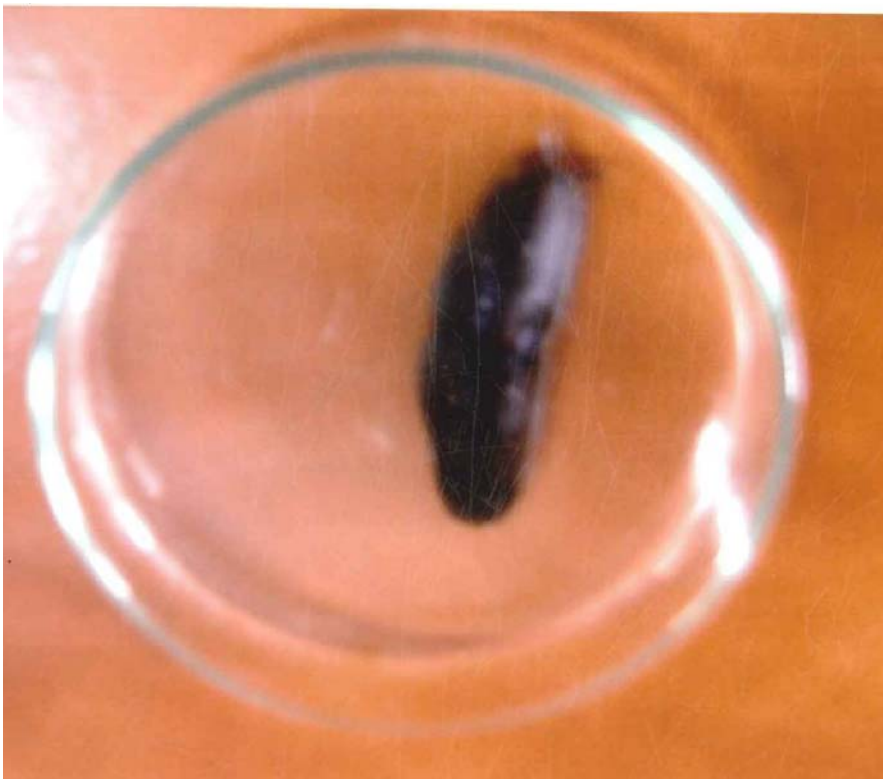

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Fig. 77. Slug used in Slug Mucosal Irritation (SMI) assay

The purpose of this study was to evaluate the local tolerance of the nasal gels. The irritation potential of the gels on the slug mucosa was analyzed by the mucus production caused by repeated exposure for 5 successive days. Additionally, membrane damage was estimated by the protein and enzyme release. By means of a classification prediction model they were classified into four irritation classes (non, mild, moderate and severe).

Table 82 shows the amount of mucus produced by the slugs and the mean protein and enzyme released from the slug body wall caused by a repeated treatment by gel formulations. Gel with 5% BKC was used as the positive control and slugs not treated as the negative control. All the slugs survived the 5 day treatment period except for a 40 % mortality rate for the positive control on day 5.

The percentages shown in Fig.78 correspond to the prediction models that distinguish between the irritation potency and tissue damage values.

Irritation Potency

The irritation potency was predicted based on the total mucus production (total MP) during the repeated contact periods. The amount of mucus produced by the slugs depended on the physical state of the test substance: however the physical state did not affect the release of biomarkers. The total mucus produced increased in all the gels as compared to the negative control. The negative control group had a total MP of 3.4% which was increased to 28.1% for the positive control group. In case of formulations C_1BF8, C_2BF8 and PBF8 the total MP was found to be 12.4%, 14.8% and 14.2% respectively. The total MP (%) was 13.9%, 14.9% and 11.2% for formulation C_1HF8, C_2HF8 and PHF8 respectively. As seen in the Fig.78 the values for semi solids was more indicating that the state of the sample has an effect on the amount of mucus produced. In all the formulations the mucus produced was less than 15% indicating a non irritating effect. In case of the positive control the mucus production was 28.1% which showed severe damage.

Tissue Damage

Tissue damage was predicted by the number of slugs that show ALP release, the mean LDH release of all the samples and the mean protein release.

ALP release

None of the slugs which received the formulation showed any release of ALP as this enzyme was found to be below detectable limits. In case of the positive control the mean ALP release was found to be 1.11±0.8 IU/l.g. Hence it showed that there was severe damage in case of positive control and not in case of the formulations tested.

Total protein release

The nasal gels resulted in protein release comparable to that of the negative control group ($P<0.05$). Positive control group showed an increase in release of proteins at 186.1µg/ml.g which was indicative of severe damage. In case of the formulation they were found to be between 10.77-19.73 µg/ml. This could be classified as non toxic as values of less than 25 µg/ml had no toxic effect and between 25-50 µg/ml was indicative of mild tissue damage.

LDH release

The LDH release in case of the samples was found to be below detectable limits indicating no tissue damage. In case of positive control it was 11.2 IU/I.g which was indicative of severe damage.

The nasal gels were hence classified as non-irritating since a 5 day treatment showed a low mucus production, low protein release comparable with negative control slugs, no enzyme (LDH and ALP) release and no mortality.

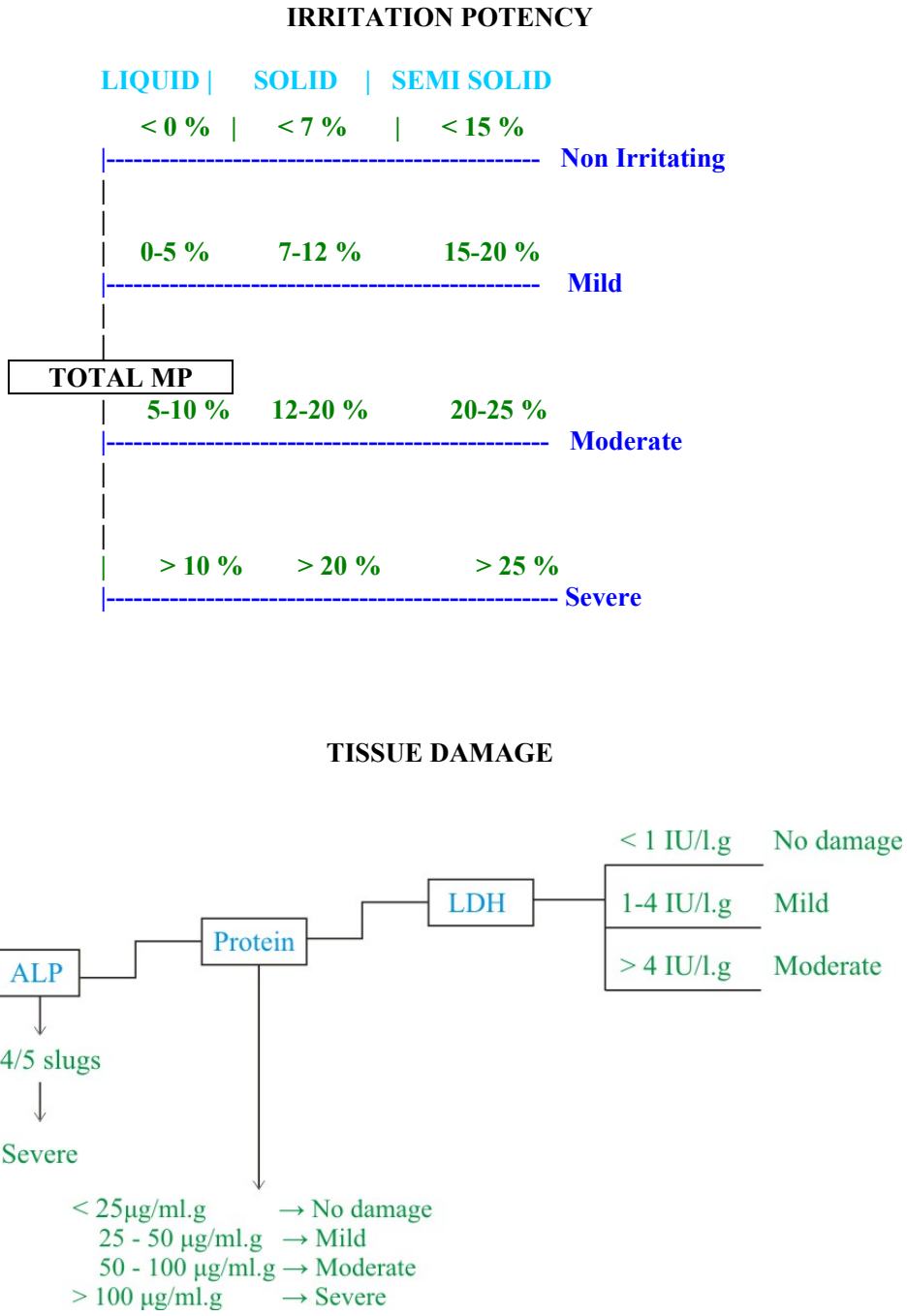


Fig.78 Prediction model that distinguishes between irritation potency and tissue damage

5.2 Rabbit nasal irritation test

The results are shown in Table 83. The wash out method used in the present study was a non-invasive method to evaluate the toxicological and possible adverse effects of the prepared formulations. The effectiveness of the non-invasive wash out method was used to evaluate the two selected formulations (C_1BF8 and C_1HF8) with a 5% BKC gel which had known damaging effects to different cell types and was used frequently a positive control. The two gels mentioned above were selected, as C_1HF8 and C_1BF8 are two classes of gels in which the drug, polymer and CD would be exposed to the nasal mucosa and were also chosen to compare any possible difference in effect due to change in the cyclodextrin used.

Influence of the treatment with gels on the mean protein and LDH released 1 h after repeated administration

The protein and LDH release increased markedly to 51.3 mg/ml and 3897.3 U/I in case of the positive control indicating that the 5% BKC gel could be used for evaluating the damage to the nasal mucosa. There was no mortality in any of the groups during the 12 day period. Prior to each administration the rabbits were examined and there was no nasal irritation or discharge from the groups that received progesterone gels and negative control group. In case of the positive control group there was nasal discharge which was found to increase from days 4 to 12 after administration. The mean protein release for C_1BF8 and C_1HF8 tested groups were 8.6 mg/ml and 9.2 mg/ml respectively. The rabbits which received C_1BF_8 and C_1HF_8 gels showed a mean LDH release of 589.1 IU/I and 601.4 IU/I respectively. The PBS samples of the positive control group, C_1BF8 and C_1HF8 showed a significant increase in mean protein and mean LDH release as compared to the negative control group in samples taken 1 h after administration ($P<0.05$).

Influence of the treatment with gels on the mean protein and LDH released 24 h after repeated administration

The PBS samples (at 24 h after administration of gels) of the negative control group and the C_1BF8 and C_1HF8 treated group showed no significant difference in

the mean protein release (P=0.053). Also the LDH release showed that there was no significant difference between the gel samples and negative control (P=0.09). This showed clearly that there was a reversibility of the effect on the nasal mucosa which was not seen in case of the positive control group administered with BKC gel. There was no significant difference in release of the marker compounds between the two progesterone gels C_1BF8 and C_1HF8 ($P<0.05$) and hence there was no difference in effect due to the presence of absorption enhancers β-cyclodextrin and hydroxy propyl β-cyclodextrin.

As expected the protein and LDH release of the treated group were significantly increased as compared to the negative control group when the measurements was done 1 h after the gel administration but importantly the protein and LDH released in the washings 24 h after an administration was comparable to those of the negative control group. This indicated that the effect of the nasal progesterone gels on the nasal mucosa was negligible due to its reversibility and hence was considered as a safe carrier.

5.3 Histological study

Fig.79 shows sections of the nasal tissue after the 12 day treatment period. The observed changes were classified as epithelial disruption and complete loss of some parts of the epithelium. The microscopy pictures for progesterone gel formulations C_1BF_8 and C_1HF_8 respectively showed that no severe damage was found on the integrity of the nasal mucosa and the mucosa retains a good morphology. An intact ciliated pseudostratified epithelium with slight increase in the number of granulocytes in the epithelium was seen. In case of the sample treated with BKC gel the epithelium containing many granulocytes was interrupted at some places and ulcerations were seen. Morphological changes in the epithelia exposed to the formulations were milder than those exposed to BKC gel. The nasal mucosa exposed to C_1BF_3 and C_1HF_3 were similar which showed that there was negligible effect due to exposure to β-cyclodextrin or hydroxy propyl β-cyclodextrin and the results obtained were in agreement with the results of other workers[65,155]

Table 82: Effect on the mucosa of slugs treated for five successive days with different gels on the total amount of mucus production, mean protein, mean LDH and mean ALP released

Group	Sample	Total MP (%)	Mean protein release (µg/ml)	Mean LDH release (IU/l.g)	Mean ALP release (IU/l.g)
1	C_1BF_8	12.4±1.2*	13.80±1.2	-	-
2	C_2BF_8	14.8±0.9*	14.97±0.9	-	-
3	PBF_8	14.2±1.2*	12.53±2.0	-	-
4	C_1HF_8	13.9±4.1*	11.73±3.1	-	-
5	C_2HF_8	14.9±0.9*	10.77±1.4	-	-
6	PHF_8	11.2±2.6*	11.30±3.0	-	-
7	Positive control	28.1±3.9*	186.1±3.8*	11.2±1.5	1.11±0.8
8	Negative control	3.4±0.8	10.4±3.1	-	-

Data are presented as the mean ±SD (n = 5)

* Significantly ($P<0.05$) different from negative control group.

MP is mucus production expressed as % w/w of the body weight

- Below detection limits.

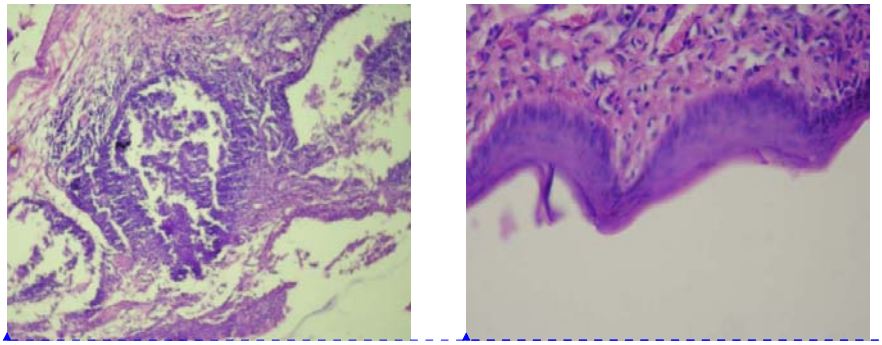
Table 83: Effect on the nasal mucosa of rabbits treated with the gels for 12 days on the release of mean proteins and mean LDH and the mean scoring[a] values from microscopy study.

Sl. No.	Sample	Mean protein release (mg/ml)		Mean LDH release (U/l)		Epithelial loss and atrophy
		After 1 h	After 24 h	After 1 h	After 24 h	Scoring[1]
1	C_1BF8	8.6 ±2.4*	2.6 ±1.3	589.1 ±2.6*	172.0± 3.1	1
2	C_1HF8	9.2 ±3.1*	2.5 ±2.0	601.4 ±3.6*	172.7 ±4.2	1
3	Positive control	51.3 ±5.4*	21.3 ±4.6*	3897.3 ±16.3*	581.5 ±6.3*	4
4	Negative control	2.4 ±2.6	2.1 ±0.9	176.3 ±3.8	170.5 ±4.3	0

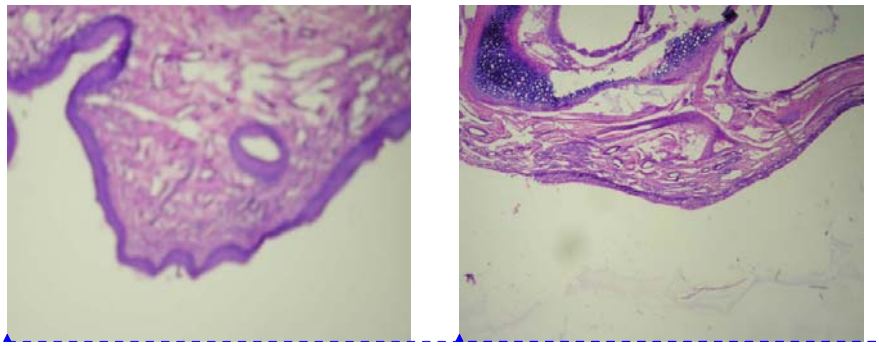
Data are presented as the mean± SD (n = 3)

* Significantly ($P<0.05$) different from negative control group.

[1] Where 0 = no damage and equal to control, 1 = less than 25% epithelial cells lost, 2 = less than 50% epithelial cells lost, 3 = less than 75% epithelial cells lost, 4 = only basal cells left, 5 = all basal and epithelial cells lost.



Positive control Normal rabbit nasal mucosa



Sample C_1HF3 Sample C_1BF3

Fig.79 Histological studies of nasal mucosa of rabbits treated during 12 days with 5% BKC gel (positive control), nasal progesterone gel C_1BF3 and C_1HF3

6. MECHANICAL STUDIES: RHEOLOGICAL AND TEXTURAL PROPERTIES

Composition of a gel can strongly influence its rheological properties and even only one different constituent can lead to significantly different rheological behavior[176,177]. Mucus is a weak viscoelastic gel[178,179] that adheres to and covers all the internal tracts of the body. The interactions at the functional group level between mucus and bioadhesive polymer often result in the formation of mixtures capable of exhibiting rheological synergy, that is, to demonstrate gel like properties when mixed, greatly in excess of when the mucin and polymer dispersions were examined separately. Hence rheological synergism[180] between mucin-polymer or mucus-polymer mixtures can be used as an *in vitro* parameter to determine the mucoadhesive properties of a material.

Table 84 and 85 shows the observed viscosity values of nasal gels and mucin solution (η_{obs}), the expected viscosity (η_{exp}) , viscosity enhancement ($\eta_{enhance}$) and relative viscosity enhancement of the combination system (η_{rel}) as calculated from equation given below

$$\eta_{exp} = \eta_p + \eta_m$$

$$\eta_{enhance} = \eta_{obs} - \eta_{exp}$$

$$\eta_{rel} = \eta_{obs} / \eta_{exp}$$

The η_p and η_m were the viscosities of the formulation and mucin alone, respectively. It could be seen that all the observed viscosity values were higher than the expected viscosity and the viscosity enhancement was calculated using the equation given above. The viscosity enhancement was equivalent to the viscosity component of bioadhesion (η_b) as given in the equation

$$\eta_t = \eta_m + \eta_p + \eta_b$$

where, η_t is the viscosity of the system, η_m and η_p are the individual viscosities of mucin and polymer, respectively. Consequently the force of mucoadhesion (F) represents the additional intermolecular frictional force per unit area and is

determined by the equation $F = \eta_b \sigma$ where σ is the shear rate (s^{-1}). The two[129] parameters, force of mucoadhesion (F) and viscosity component of bioadhesion (η_b) gave a direct estimate of the gel-mucin interaction, occurring in mucoadhesion.

6.1 Viscosity component of bioadhesion (η_b)

Effect of mucin on viscosity enhancement (synergy) and the calculated force of bioadhesion values are shown in Table 84 and 85. It was apparent from the table that viscosity of the system was greater than the sum of individual viscosities. This suggested that all the gels were able to interact strongly with mucin. The poly (acrylic) acid gels interact[181] with mucin by forming physical entanglements followed by hydrogen bonds with sugar residues on the oligosaccharide chains, which resulted in the formation of a strengthened mucus gel network. The rank order of $\eta_{enhance}$ of the gels-mucin combination for both β-cyclodextrin and hydroxy propyl β-cyclodextrin gels were Carbopol 974 > Carbopol 971 > Polycarbophil. In case of β-CD gels the highest $\eta_{enhance}$ value was shown by C_1F7 at 871.6 m Pa s. C_1F7 has carbopol 974 at 2% with no cyclodextrins. All the gels showed a similar pattern, that the presence of cyclodextrins decreased the $\eta_{enhance}$ vales. In case of C_1F7 the viscosity enhancement was 871.6 m Pa s whereas it was decreased to 584.3 m Pa s and 620 m Pa s in case C_1BF8 and C_1BF9 respectively. The $\eta_{enhance}$ vales for C_1F1 and C_1F4 were 752.2 and 790.2 m Pa s respectively as compared to 871.6 m Pa s for C_1F7. This increase in the value was due to the increase in initial viscosity of the systems. The decrease in viscosity was more in case of the HPβ-CD gels. In case of the HPβ-CD gels the C_1HF8 and C_1HF9 showed viscosity enhancement of 553.9 and 612.1 m Pa s respectively as compared to 871.6 m Pa s for C_1F7.

The next factor to consider was the magnitude of the rheological synergism by consideration of the relative viscosity enhancement η_{rel}. This allowed the viscosity enhancement effect to be expressed as a proportion of the unmixed materials viscosities. A $\eta_{rel} = 1$ meant[169] that there was no interaction between polymer and mucin. A higher value of η_{rel} showed rheological synergism between polymer and mucin and was indicative of potentially mucoadhesive association between them. The calculated value showed that the Carbopol 974 gels had higher

η_{rel} compared to the other two polymers. It was also seen that the initial viscosity affected the η_{rel}. As the concentration of the polymers was increased the initial viscosity was more and there was an increase in η_{rel} . Again it was evident that presence of cyclodextrin had a detrimental effect on the rheological synergism which caused a decrease in the η_{rel} values. The presence of cyclodextrins either as physical mixture or inclusion complex did not have a significant effect on the η_{rel} values ($P>0.05$).

Force of bioadhesion

A bioadhesive force is required between the drug device and the mucosal surface to successfully retain the device and retard the natural clearance processes. A single shear rate of 5 s^{-1} was selected to determine the viscosity. A stronger force of adhesion was seen in case of Carbopol 974 gels and this represents higher bond strength. The strongest force of adhesion was demonstrated by C_1F7 at 4358 m Pa. It was 3705.5 m Pa and 3500.5 m Pa for C_2F7 and PF7. Thus the formation of interfacial bonds between the gels prepared with Carbopol 974 and mucin was stronger that with Carbopol 971 and polycarbophil. In case of C_1BF9 the F value was 3100.0 m Pa while it was 3060.5 m Pa for C_1HF9. In case of carbopol 971 gels C_2BF6 and C_2HF6 gels showed a F value of 2721.0 m Pa and 2610 m Pa respectively. This pattern was repeated for all the gels. Hence it was inferred that there was no significant difference between the β-CD and HPβ-CD gels ($P>0.05$).

The pattern noticed in this study was that the gels without the CD showed the highest force of bioadhesion which was followed by the gels with CD as inclusion complex and then by the gels with CD as a physical mixture. This pattern was confirmed with the Texture Profile Analysis (TPA) as discussed below.

Table 84: Effect of mucin on viscosity enhancement (synergy) and calculation of force of bioadhesion

Sample	η_p (m Pa s)	η_m (m Pa s)	η_{exp} (m Pa s)	η_{obs}	$\eta_{enhance}$ (m Pa s)	η rel	F (m Pa)
C_1F1	285.2	56.7	341.9	1094.1	752.2	3.2	3761.1
C_1BF2	256.0	56.7	312.7	901.2	598.5	2.8	2992.5
C_1BF3	254.2	56.7	310.9	921.0	610.1	2.9	3050.5
C_1F4	302.5	56.7	359.2	1149.4	790.2	3.2	3951.0
C_1BF5	275.0	56.7	331.7	925.0	593.3	2.7	2966.5
C_1BF6	279.1	56.7	335.8	945.1	609.3	2.7	3046.5
C_1F7	322.1	56.7	378.8	1250.4	871.6	3.3	4358.0
C_1BF8	301.0	56.7	357.7	942	584.3	2.6	2921.5
C_1BF9	289.1	56.7	345.8	965.8	620.0	2.7	3100.0
C_2F1	251.2	56.7	307.9	925.0	617.1	3.0	3085.5
C_2BF2	221.0	56.7	277.7	745.5	467.8	2.7	2339.0
C_2BF3	220.2	56.7	276.9	721.1	444.2	2.6	2221.0
C_2F4	278.3	56.7	335.0	1071.9	736.9	3.2	3684.5
C_2BF5	249.1	56.7	305.8	839.3	533.5	2.7	2667.5
C_2BF6	251.1	56.7	307.8	852.0	544.2	2.8	2721.0
C_2F7	296.3	56.7	353.0	1094.1	741.1	3.1	3705.5
C_2BF8	255.3	56.7	312.0	873.6	561.6	2.8	2808.0
C_2BF9	251.0	56.7	307.7	892.3	584.6	2.9	2923.0
PF1	291.2	56.7	347.7	938.7	590.8	2.7	2954.0
PBF2	252.0	56.7	308.7	741.0	432.3	2.4	2161.5
PBF3	262.3	56.7	319.0	821.7	502.7	2.5	2513.5
PF4	312.0	56.7	368.7	957.0	588.3	2.6	2941.5
PBF5	281.1	56.7	337.8	776.7	438.9	2.3	2194.5
PBF6	271.2	56.7	327.9	858.5	530.6	2.5	2653.0
PF7	332.0	56.7	388.7	1088.8	700.1	2.8	3500.5
PBF8	282.1	56.7	338.8	871.0	532.2	2.5	2661.0
PBF9	290.5	56.7	347.2	907.1	559.9	2.6	2799.5

Table 85: Effect of mucin on viscosity enhancement (synergy) and calculation of force of bioadhesion

Sample	η_p (m Pa s)	η_m (m Pa s)	η_{exp} (m Pa s)	η_{obs} (m Pa s)	$\eta_{enhance}$ (m Pa s)	η rel	F (m Pa)
C_1F1	285.2	56.7	341.9	1094.1	752.2	3.2	3761.1
C_1HF2	240.0	56.7	296.7	881.0	584.3	2.9	2921.5
C_1HF3	242.2	56.7	298.9	900.1	601.2	3.0	3006.0
C_1F4	302.5	56.7	359.2	1149.4	790.2	3.2	3951.0
C_1HF5	262.0	56.7	318.7	892.3	573.6	2.7	2868.0
C_1HF6	270.1	56.7	326.7	938.2	611.5	2.8	3057.5
C_1F7	322.1	56.7	378.8	1250.4	871.6	3.3	4358.0
C_1HF8	264.3	56.7	321.0	874.9	553.9	2.7	2769.5
C_1HF9	274.0	56.7	330.7	942.8	612.1	2.8	3060.5
C_2F1	251.2	56.7	307.9	925.0	617.1	3.0	3085.5
C_2HF2	222.0	56.7	278.7	703.0	424.3	2.5	2121.5
C_2HF3	220.8	56.7	277.5	689.8	412.3	2.4	2061.5
C_2F4	278.3	56.7	335.0	1071.9	736.9	3.2	3684.5
C_2HF5	241.3	56.7	298.0	819.4	521.4	2.7	2607.0
C_2HF6	234.1	56.7	290.8	812.9	522.1	2.7	2610.5
C_2F7	296.3	56.7	353.0	1094.1	741.1	3.1	3705.5
C_2HF8	241.3	56.7	298.0	843.3	545.3	2.8	2726.5
C_2HF9	223.4	56.7	280.1	822.2	542.1	2.9	2710.5
PF1	291.2	56.7	347.7	938.7	590.8	2.7	2954.0
PHF2	241.0	56.7	297.7	716.0	418.3	2.4	2091.5
PHF3	262.6	56.7	319.3	841.9	522.6	2.6	2613.0
PF4	312.0	56.7	368.7	957.0	588.3	2.6	2941.5
PHF5	251.3	56.7	308.0	732.3	424.3	2.3	2121.5
PHF6	262.4	56.7	319.1	850.0	530.9	2.6	2654.5
PF7	332.0	56.7	388.7	1088.8	700.1	2.8	3500.5
PHF8	284.5	56.7	341.2	875.3	534.1	2.5	2670.5
PHF9	291.3	56.7	348.0	893.6	545.6	2.5	2728.0

6.2 Texture profile analysis (TPA)

Texture profile analysis (TPA) has been used as an interesting technique to characterize the mechanical properties[184-186] of pharmaceutical gels and semisolid systems. This simple and rapid technique can provide information related to the gel mechanical parameters, such as hardness and adhesiveness[187]. The mechanical properties of the gels are shown in Table 86 and Fig. 80-82.

Hardness

It was seen that the addition of HPβ-CD significantly altered the gel hardness as compared to plain gel ($P<0.0001$). The gel with HPβ-CD as physical mixture showed the lowest value of hardness as compared with the gel with no cyclodextrins. The hardness for C_1F1 was 91.3 g while it was 37.3 g for C_1HF2 and 81.8 g for C_1HF3. There was significant difference in the hardness of the gels with different methods of addition of CDs ($P<0.0001$). The gel which had inclusion complex had a higher hardness as compared with gels which had drug and CD as physical mixture. The addition of HPβ-CD considerably changed the hardness. The greatest effect on the decreasing hardness was observed with physical mixture of HPβ-CD with drug. The value of hardness follows the order: $C_1F1>C_1HF3>C_1HF2$.

Adhesiveness

The adhesiveness was found to be 15.3 gs, 11.4 gs and 15.1 gs for C_1F1, C_1HF2 and C_1HF3 respectively. The adhesiveness of gel formulation C_1F1 (gel without CD) was statistically different from C_1HF2 (gel with drug and CD as physical mixture) ($P<0.05$). The TPA analysis showed that there was no significant difference between the C_1F1 and C_1HF3 gel ($P = 0.39$). The adhesives of C_1F1 and C_1HF3 were similar, and therefore presence of inclusion complex does not affect the adhesiveness as opposed to C_1HF2. The gel C_1HF3 also had best compromise between hardness and adhesiveness for nasal application.

The hardness values and adhesiveness decreased significantly when various constituents were incorporated especially in the presence of modified cyclodextrins.

Hydrophobic interaction could occur between the polymer chains and the HPβ-CD resulting in a reduction of the polymer chains unfolding. Consequently it may modify the polymer affinity for the hydration medium, decreasing its firmness and swelling[188]. Qi *et al*[189] demonstrated that the addition of HPβ-CD in a pluronic gel increased the length of gelling but drastically reduced the gel strength and adhesiveness.

In conclusion, gel containing drug/ HPβ-CD inclusion complex showed a value of adhesiveness and hardness close to the gel without HPβ-CD suggesting that a gel with suitable properties was obtained by addition of inclusion complex.

Table 86: Mechanical properties of selected gels

Sl. No	Sample	Hardness (g) Cycle 1			Hardness (g) Cycle 2			*Mean ± S.D	Adhesiveness (gs)			*Mean ± S.D
1	C_1F1	89	90	90	92	90	97	91.3 ± 2.9	15.2	15.4	15.3	15.3 ± 0.4
2	C_1HF2	30	40	39	30	42	43	37.3 ± 5.9	13.2	8.9	12.1	11.4 ± 2.2
3	C_1HF3	79	81	83	80	83	85	81.8 ± 2.2	16.5	17.0	12.0	15.1 ± 2.8

*Values are expressed as mean ± SD, n = 3

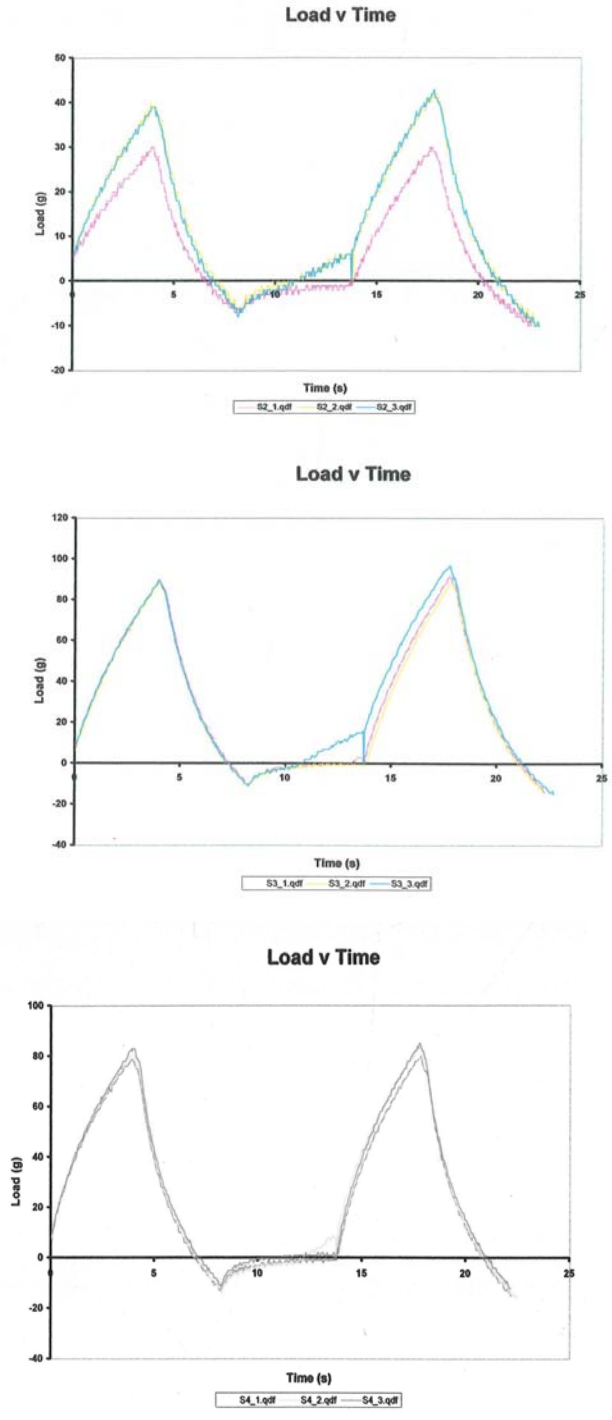


Fig. 80.Texture profile analysis of C_1HF2, C_1F1 and C_1HF3.

7. Mucociliary clearance

Mucociliary transport is an important lung defense mechanism protecting the bronchial mucous membrane from inhaled aero contaminants by trapping them in the mucus blanket, which is continuously propelled towards the pharynx by ciliary beating[190]. Efficient mucociliary clearance is dependent upon the complex interaction between ciliary function and the rheological properties of mucus[191]. The nasal mucociliary clearance system is particularly susceptible to damage. This can have serious consequences, because it plays an important role in the protection of the respiratory system. Therefore it is important to study the effects of formulations on nasal mucociliary clearance.

7.1 Frog Palate Method

The palate of the frog possesses a pseudostratified epithelium with mucus secreting cells and numerous ciliated cells covered with a continuous mucus blanket. The palate can be used as a simple model for studying mucus transport[192]. The ciliated epithelium of the frog palate is a frequently used model to evaluate the role of rheological properties of mucus on ciliary transport velocity[193]. The range of mucociliary transport velocity of the freshly excised frog (8-14 mm/min) is similar to that of human tracheal mucus transport velocity. The exogenous mucus provided is used to restore and maintain the transport of the tracer particle[194].

Implications of mucociliary clearance for nasal drug absorption:

The absorption of drugs from the nasal mucosa is influenced by the contact time between drug and epithelial tissue. Intranasally delivered drugs show a rapid rise to peak concentrations due to the high permeability of the nasal epithelium, while diffusional path length through the nasal mucosal epithelium is short, consisting of only two cell layers. Mucociliary clearance on the other hand limits the residence time of drugs administered into the nasal cavity, decreasing the time available for the drug to be absorbed. Therefore the strategy to increase the nasal bioavailability of drugs that are poorly absorbed from the nasal mucosa can be

aimed either at increasing the nasal membrane permeability or/and increasing the contact time for absorption by decreasing the mucociliary clearance rate[195-197].

The results are shown in Table 87. The MTR of all the three types of polymers were similar. Compared to the gels with cyclodextrins, the gels which did not contain the CDs were more effective at reducing the MTR, likely because of their higher viscosities. There was a direct relationship between the viscosity and the reduction in MTR. As the viscosity of the gels increased there was an increase in the percentage reduction in MTR. As the concentration of the carbomers used in the gels increased from 1 to 1.5 to 2% there was an increase in the MTR reduction. These studies were carried out to quantify the viscoelastic properties to delay the MTR. There was a need to maintain a balance between the % reduction in MTR and the irreversible damage to the mucociliary clearance mechanism. All the gels were capable of producing more than 55% reduction in MTR which thus provided a desired mucociliary transport rate for optimum release characteristics. The maximum MTR reduction was seen in case of C_1F7 at 71%. It was 62.9% and 66% for C1F1 and C_1F4. These were gels without any CD. The % reduction in MTR was decreased to between 54-65% for the carbopol gels with added CD. There was no significant difference ($P<0.05$) in the % reduction in MTR between gels prepared with the three type of polymers. This was true for gels prepared with both kinds of CDs.

Table 87: Mucociliary transport rates (MTR) of mucus control and nasal gels

Sample	MTR (cm/min)		% reduction
	Mucus Control	Gels	
C_1F1	0.62	0.23	62.9
C_1BF2	0.62	0.28	54.8
C_1BF3	0.62	0.27	56.5
C_1F4	0.66	0.25	66.0
C_1BF5	0.66	0.27	59.1
C_1BF6	0.66	0.28	57.6
C_1F7	0.69	0.20	71.0
C_1BF8	0.69	0.24	65.2
C_1BF9	0.69	0.25	63.7
C_2BF3	0.69	0.29	57.9
PBF3	0.69	0.31	55.1
C_1F1	0.56	0.22	60.7
C_1HF2	0.56	0.24	57.1
C_1HF3	0.56	0.24	57.1
C_1F4	0.54	0.18	66.6
C_1HF5	0.54	0.20	62.9
C_1HF6	0.54	0.20	62.9
C_1F7	0.67	0.22	67.2
C_1HF8	0.67	0.25	62.6
C_1HF9	0.67	0.26	61.2
C_2HF3	0.58	0.24	58.6
PHF3	0.58	0.17	56.9

8. Stability testing

The guidance and recommendations detailed in the ICH guidelines to propose a retest or shelf life was followed. Three batches of the product were tested. The gel preparations were packed in tight containers. The batches selected for the stability testing contained 100% of the label claim. The stability information presented includes results from physical, chemical and release profile tests. The attributes tested were appearance, assay, viscosity and *ex vivo* release profile. Each attribute was assessed separately.

The results are shown in table 88-90.

Appearance

There was no change in the appearance.

Assay

The assay of the three batches for the two formulations selected was determined individually at time intervals of 0, 1, 3 and 6 months. As per ICH guidelines the poolability of the results were tested to determine whether the data from the different batches could be considered for an overall estimate of a single retest period or shelf life. A preliminary statistical test was performed to determine whether the regression lines from different batches had a common slope and a common time–zero intercept The results show that for sample C_1BF3 the slope was 0.132, 0.134, 0.130 and for C_1HF3 it was 0.146,0.148,0.145 and the common time zero intercept was 100.2 and 100.1 respectively for all the batches.

The results showed the regression line for assay for the formulation with lower acceptance criteria of 95 percent with six months of accelerated stability data and the proposed shelf life calculated using linear regression interpolation was 38.41 and 33.8 months for C_1BF3 and C_1BF3.

pH

There was no significant change

Viscosity

There was a slight reduction in the viscosity of the samples which did not affect the consistency of the samples.

Ex vivo diffusion

The *ex vivo* release profile for the stability samples were compared with that of initial values. It was compared[182,183] according to FDA guidelines using the mathematical comparison by applying f2 factor. The factor f2 measures the closeness between two release profiles and is described as the similarity factor. If the profiles were identical, f2 = 100. An average difference of 10% at all measured time gave a value of 50%. According to FDA, guidance values for f2 between 50 and 100 ensured sameness or equivalence of two release profiles. The main advantage of calculating f2 (similarity) factor was to provide a simple way of data comparison. From the results of the f2 data seen in Table 91 it was proved that the release data was similar to that of the initial release profiles as the f2 values were between 78.7-91.6.

The results of the stability studies on nasal gels showed no degradation of the drug and also similar release profile between control samples and the samples exposed to stability test conditions.

Linear regression of line obtained by plotting time versus % drug content with unknown interpolation was carried out to determine the shelf life period. The data from all the factor combinations considered support the proposed shelf life of 30 months.

Table 88: Stability testing data for nasal gels C_1BF3 and C_1HF3 .

Sample	Appearance			*Drug content (%)		
	1 month	3 months	6 months	1 month	3 months	6 months
C_1BF3	No change	No change	No change	99.97±0.02	99.87±0.01	99.21±0.18
C_1HF3	No change	No change	No change	99.90±0.97	99.62±2.1	99.12±1.81

* Values are expressed as mean ± SD, n = 3

Table 89: Stability testing data for nasal gels C_1BF3 and C_1HF3 .

Sample	pH			Viscosity cps*			
	1 month	3 months	6 months	Initial	1 month	3 months	6 months
C_1BF3	7.0±0.3	7.0±0.3	6.9±0.4	4212±3.6	4201±8.2	4175±2.3	4123±6.1
C_1HF3	7.0±0.3	7.0±0.2	6.8±0.5	4423±7.9	4388±5.74.1	4314±3.7	4236±4.3

* Viscosity measured in Brookfield DV-E Model Viscometer, Spindle No.64 at 100 rpm at room temperature.

All values are expressed as mean ± SD, n = 3

Table 90: Stability testing data for nasal gels C_1BF3 and C_1HF3 .

Sample	*Ex vivo* diffusion Similarity factor (f2)		
	1 month	3 months	6 months
C_1BF3	83.34	82.08	78.69
C_1HF3	91.57	82.91	80.93

Progesterone is an active natural human sex hormone. Because of its very limited oral effectiveness, resulting from a high first pass effect, these natural sex hormones have been replaced by synthetic and semisynthetic derivatives for virtually all medical applications. It is however known that these synthetic derivatives have negative side effects, especially on protein synthesis. For the above reasons there is a clear and long felt need for better non-oral compositions and dosage forms of the natural sex hormone. In order to offer high flexibility in their therapeutic use such dosage forms should ensure low physiological levels of the natural hormone while at the same time leaving the option to adapt the dose of the hormone to the individual therapeutic needs. Parenteral administration circumvents the undesirable first pass effect. There are, however, obvious inconveniences like need for sterile delivery devices, pain and irritation, need for medical assistance in administering and potential risks of infections. Therefore alternative means of drug delivery, equally effective to circumvent the effect of first pass metabolism has been sought. Transdermal drug delivery could be an alternative to parenteral therapy but has problems of overcoming the barrier properties of stratum corneum causing incomplete absorption. It also leaves very limited possibilities to adjust the dose and to meet the therapeutic goals and individual needs.

One promising alternative to the above mentioned routes is drug administration via the transmucosal route. However there are problems associated with transmucosal delivery like the bioavailability of the drug being unpredictable depending on the nature of the drug and the drug delivery system. Nasal delivery is a promising alternative for systemic administration of drugs that are poorly absorbed via the oral route. The nasal epithelium has a relatively high permeability and only two cell layers separate the nasal lumen from the dense blood vessel network in the lamina propria. Intranasal delivery is a needle-free, patient-friendly administration route. Because needles are not involved, this method of drug delivery is virtually painless. For patients who fear injections, intranasal administration offers a more acceptable alternative. Additionally, the simplicity of nasal delivery would allow for self-administration in a home setting. In general, for patients, the intranasal dosage form provides comfortable, non-threatening, less invasive therapy. Another major

benefit of intranasal administration, in contrast to injectables, is that it does not contribute to biohazardous waste. When the drug has been delivered intranasally, the administration device may be disposed off in the normal garbage. There is no need for special waste containers. Again, this delivery method does not require needles.

From a pharmacokinetic standpoint, absorption is rapid, which should provide a faster onset of action compared to oral and intramuscular administration. Hepatic first-pass metabolism is also avoided, allowing increased reliable bioavailability. In this regard, good drug candidates for intranasal delivery are those that undergo extensive first-pass metabolism, display erratic absorption or require quick therapeutic onset. Lastly, patent life of a particular product may be extended via development of an alternative dosage form, providing companies the opportunity to maintain their market share. So from a drug development perspective, intranasal delivery should stimulate favorable profit outcomes.

Hence this work was undertaken to successfully design a transmucosal i.e. nasal drug delivery system in the form of a gel which would have excellent patient acceptability. Nasal drug delivery includes a large variety of pharmaceutical forms such as solutions, suspensions, powders, microspheres and semi-solids. Gels as the desired dosage form for this research work was selected as gels are known to be more suitable than powder and liquid formulations and are suitable carriers for nasal application of the drug. Gels are semi-solid systems comprising small amounts of solid, dispersed in relatively large amounts of liquid, yet possessing more solid-like character. These systems form a three-dimensional, polymeric matrix in which a high degree of physical (sometimes chemical) reticulation has been comprised. They are formed of long, disordered chains that are connected to specific points, but the connection is reversible. Gels are suitable carriers for nasal delivery due to their property to reduce MCC and hence provide for longer residence time of the formulation at the site of absorption. The advantages of nasal gels also include the reduction of post-nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation and target delivery to mucosa for better absorption. As far as nasal gels of progesterone were concerned, optimization of the formulations required more

attention, particularly characteristics like choice of the polymer, concentration of the polymer, type of absorption enhancers, viscosity and bioadhesion.

A prime objective sought by the development of new delivery devices or release systems for active ingredients was the increased efficacy of the active agent, with enhanced bioavailability at an optimum rate and therapeutic dose. The large variety of applications as well as the steadily increasing number of research activities carried out in polyacrylic acid copolymers due to their unique properties suggests the potential of carbopols as novel and versatile polymers with significant contributions to the future of drug delivery systems. Carbopols and polycarbophil[70,73,93] have been used for nasal delivery of drugs including female steroid hormones like estradiol and progesterone. Carbopols has been used as powder and solution form. As gels are a more suitable dosage form for nasal delivery, polyacrylic acid copolymers were chosen for the formation of the nasal gels. Cyclodextrins[198,199] were chosen as the absorption enhancers as they are solubilizers and absorption promoters in nasal drug delivery. β-CD and its derivative HPβ-CD were chosen for the study. The effect of the addition of both the CDs as a physical mixture or inclusion complex in the gels was studied.

An erythrocyte model has been used in investigating the membrane activity of various agents which were used in the various formulations. Erythrocytes were commonly used as a model system for investigating the membrane interactions. They were readily available in large amounts and their lysis was readily measured by colorimetric determination of haemoglobin release. The relative membrane activity of different substances identified this way provided information about their biological effects and possible mechanism of action. Cell lysis could occur by the removal of membrane lipids into mixed micelles with the lysate molecule or by local high densities of the lysate incorporated into the membrane which lead to disruption of the normal molecular organization and by formation of hydrophilic channels ion permeability changed and ultimately osmotic lysis occurred and caused leakage of hemoglobin. For example it was found that in absorption enhancers[128] like Laureth-9, the mechanism of action was by membrane disruption which was supported by the extensive epithelium damage seen *in vivo*. The identification of these materials

with reduced membrane perturbing potential was promising from the formulation and toxicological standpoint. Hence from the results it was concluded that carbomers, CDs and propylene glycol showed low membrane perturbing activity which could be safely used for nasal preparations as it was toxicological safe.

Inclusion complex with β-CD and its derivative HPβ-CD were prepared by freeze drying method. Generally, drugs were incorporated into cyclodextrins[155] as inclusion complexes in drug–cyclodextrin ratios of 1:1 or 1:2. Literature[65] shows that progesterone exhibits a B_S type solubility diagram for β-CD. Type B phase-solubility profiles are indicative of the formation of complexes with limited water solubility and are traditionally observed with naturally occurring CDs, especially β-CD. In case of the B_S type, as the CD concentration increases, soluble complex forms which increase the total solubility of the substrate. At a particular point the maximum solubility of the drug is achieved. The increase of the apparent progesterone solubility reaches a plateau, which represents the limit of complex solubility before starting to decrease as the concentration of cyclodextrin increase due to inclusion complex precipitation. The interaction studies between progesterone and hydroxypropyl beta cyclodextrin (HPβ-CD) exhibited typical A_L curve corresponding to progesterone/cyclodextrin 1:1 molar ratio. In A systems[156] the apparent solubilities of the substrate increases as a function of CD concentration. A_L profile indicates a linear increase in solubility as a function of solubilizer concentration. The A_L type diagram is typical for the formation of a solubility complex between progesterone and hydroxypropyl beta cyclodextrin corresponding to an apparent progesterone solubility level below 30 mg/ml. The inclusion compounds formed are entirely soluble in water in equimolar ratios. The reported solubility enhancement of progesterone was 400% for β-CD and 360000% for HPCD[65]. Progesterone has a molecular weight of 314.45 g/mol, beta cyclodextrin (β-CD) has a molecular weight of 1135 g/mol and HPβ-CD 1488 g/mol. The solubilities of β-CD and HPβ-CD are 18.5 mg/ml and 400 mg/ml respectively.

The inclusion compounds formed were entirely soluble in water in equimolar ratios. The solubility enhancement was 4.2 times for β-CD complex and 81.2 times for HPβ-CD complex. Dissolution of progesterone/HPβ-CD complex was

instantaneous. Almost the entire progesterone included in the complex was released in less than 20 min. The improvement in the dissolution with the progesterone/β-CD complex was less pronounced. The time taken for 90% drug to be released for HPβ-CD and β-CD complexes was 0.8 min and 70.9 min respectively. In case of the physical mixtures 59.2% and 22.8% of the drug was released at the end of 60 min for HPβ-CD and β-CD respectively. The formation of the complex was confirmed by FTIR, DSC,PXRD and SEM analysis.

The increased solubility of progesterone by formation of inclusion complexes with both β-CD and HPβ-CD is an important factor in the mechanism of absorption enhancement. However there is evidence that CD particularly β-CD and HPβ-CD can extract lipids from the nasal mucosa to facilitate nasal absorption [54]. The increased permeability of progesterone from nasal gels across sheep nasal mucosa was indicated by the upward shift of time-permeation profile, which was significant over the whole period of 180 min. This was also due to the importance of chelating calcium for enhancing the permeability of drugs. Anionic polymers like carbopols were reported[71] to demonstrate permeation enhancing properties. These polymers express a high calcium binding ability. Presences of carbopols caused an increase in calcium binding sites and also increase in interaccessibility of calcium binding sites owing to relaxation of the polymer network. Depletion of the calcium ions from the extracellular cell medium increased the permeation of drugs. The effective permeability of progesterone from gels was found to be was 7.56×10^{-5} cm/s for C_1BF3, 6.60×10^{-5} cm/s for C_2BF3 and 5.66×10^{-5} cm/s for PBF3. In case of HPβ-CD gels the permeability coefficient for C_1HF3, C2HF3 and PHF3 were 7.83×10^{-5} cm/s, 6.83×10^{-5} cm/s and 5.93×10^{-5} cm/s respectively. The release was in the order carbopol 974 > carbopol 971 > polycarbophil. The presence of CD significantly enhanced the penetration of active substance ($P<0.05$). The penetration enhancing activity of CD was due to its membrane effects on nasal mucosa. The nasal absorption of progesterone appeared to be markedly enhanced by the addition of β-CD and HPβ-CD. The increase in bioavailability from[92] less than 10% for oral route to 84 % for the gels with HPβ-CD as a complex showed enhanced improvement. Cyclodextrins[139] act as absorption enhancers by various effects

including disaggregation of protein aggregates or interaction with lipids and divalent cations on membrane surface or a direct effect on the paracellular pathway by a transient effect on tight junctions.

For many drugs a modified *in vivo* drug release is desired to improve the absorption profile and improve efficacy. Cyclodextrins offer an excellent approach to this effect. These cyclic oligosaccharides have the ability to form non-covalent complexes with a number of drugs and in so doing alter their physiochemical properties. It followed that the incorporation of these agents into polymeric drug delivery systems, as physical mixtures or inclusion complexes permitted a greater degree of control of drug release. They influence the mechanism by which drug was released. Physically mixed and complexed cyclodextrins can modify drug solubility or diffusivity, improve hydration of the polymer matrix or promote its erosion. Cyclodextrins have the potential to enhance the release of drug from polymeric systems by increasing the concentration of diffusible species within the matrix. It has been previously reported by Guo and Cooklock[200] that CDs were used to increase the solubility of the poorly water soluble drugs like opioid analgesic, buprenorphine and modify its release from buccal patches. It was found that there was more than two fold increase in release from a matrix with complexed drug than drug alone. It was reported by Samy and Safwat[201] that β-CD increased the release of piroxicam and diclofenac from both methyl cellulose and hydroxy propyl methyl cellulose gels. β-CD also enhanced the release of flurbiprofen from an aerosil gel compared to free drug.

In the case of progesterone nasal gels it was noted that the presence of CDs had an absorption enhancing effect. It was seen that the incorporation of CDs enhanced the release of drugs by the following mechanism

1. improving the aqueous solubility of drugs
2. acting as channeling agents and promoting erosion of the matrix
3. increasing the concentration of diffusible species

In case of the nasal gels, progesterone existed within the hydrated matrix after equilibrium had been established between drug and CDs, progesterone release was a result of the combined diffusion of the free drug and the drug-CD complex. This was seen in case of progesterone which was present in the gels in concentration above saturation. This was also the reason there was no significant difference in release kinetics of gels with drug as physical mixture or complex with CD. Total drug release was increased as the diffusion rates of free drug and drug-CD complex was additive. The addition of CDs to gels also enhanced drug release by acting as channeling or wicking agents and promoting erosion of the matrix. The enhanced release was a result of the incorporated CD dissolving on contact with water, increasing the porosity of the gels and by allowing the removal of the drug via its inclusion within the CD cavity. This was also the reason that HPβ-CD gels showed enhanced absorption effect as compared to β-CD gels. This type of result has also been reported by Villar-Lopez *et al.*[202]and Giunchedi *et al.*[203] Both groups of researchers concluded that the improved drug release was the result of the ability of the incorporated CD to enhance the aqueous solubility of drug whilst concomitantly acting as a water leachable component and promoting matrix erosion.

However, drug solubility enhancement was only one of the mechanisms by which increase in release was achieved. The other important mechanism was alteration of the nasal mucosal permeability. Also in case of gels[204-206] there was a possibility of greater contact time period with the nasal mucosal surface to allow for greater dissociation. This was due to the significant reduction in mucociliary clearance (MCC) by the nasal gels.

Mucosal toxicity potential of these formulations should be assessed during the regulatory safety evaluation. Animal studies were usually performed to assess this using various mammal species. To minimize the use of mammals during the initial studies involving a large number of samples of nasal gels, Slug Mucosal Irritation (SMI) assay was used as an alternative test to predict the mucosal tolerance of the prepared nasal gels thereby replacing use of laboratory mammals. The SMI assay could predict the local tolerance of semi-solid gels. Irritation potency was predicted based on the total amount of mucus produced during repeated contact

periods. The amount of mucus produced also depended on the physical state of the test substance. Hence the cut-off value for semi-solid gels was higher. However the physical state did not affect release of biomarkers. Tissue damage was predicted by the number of slugs that showed ALP release, the amount of mean LDH released and the mean protein released as compared to negative control. Based on the results of the SMI test on nasal gels of progesterone and the prediction model to distinguish for irritation potency and tissue damage all the nasal gels were classified as non-irritating and non toxic. To confirm the reliability of the SMI test, a validation was carried out using the rabbit model. The effectiveness of the non-invasive wash out method described above was evaluated using selected nasal gels with different absorption enhancers, beta cyclodextrin and hydroxypropyl beta cyclodextrin. The formulation F8 was chosen as in that gel, CD is present as physical mixture, thus exposing both drug and CD along with polymer to the nasal mucosa. 5% BKC gel was chosen as the positive control for its well known damaging effect to different cell types. The protein, LDH release increased significantly on administration of BKC gels indicating that the method could be used to evaluate damage to the nasal mucosa. The LDH and protein concentration returned to same level as negative control indicating that the formulation induced a reversible effect on the mucosa. Effectively the protein and LDH was higher when nasal cavity was rinsed one hour after administration of the formulation, but comparable levels to those of the negative control group, after 24 h of administration. From the results of the study with slugs it was concluded that nasal gels did not have any damaging effects on slugs. These results were also in agreement with the data on the toxicological evaluation in rabbits which showed only reversible changes. This was also confirmed microscopically with histological study. The test samples showed only mild changes like inflammation but no necrosis, squamous metaplasia or ciliary degeneration even after 12 day study.

Composition of a gel can strongly influence its rheological properties and even only one different constituent can lead to significantly different rheological behavior[176]. The ideal viscosity values of gels are hard to define. The gel optimization should not focus simply upon rheology of undiluted materials, but also

include a selection of macro-molecules that produce interactions[177]. Thus in this study, nasal gels were studied in interaction with mucus, with which the gel comes in contact on application. Mucus is a weak viscoelastic gel[178] that adheres to and covers all the internal tracts of the body. The main component of mucus layer includes water (up to 95%) mucin (0.5-5%), inorganic salts (1%), carbohydrates and lipids. The major structure-forming component of mucus, namely the mucin glycoprotein[179] is responsible for its rheological properties. The mucin glycoproteins are capable of associating with each other by means of non-covalent interactions to form the gel matrix. Strong mucoadhesion would most likely produce changes in the rheological properties of the interfacial region. The interactions at the functional group level often result in the formation of mixtures capable of exhibiting rheological synergy, that is, to demonstrate gel like properties when mixed, greatly in excess of when the mucin and polymer dispersions were examined separately. Hence rheological synergism[180] between mucin-polymer or mucus-polymer mixtures was used as an *in vitro* parameter to determine the mucoadhesive properties of a material.

The viscosity enhancement values were calculated which was equivalent to the viscosity component of bioadhesion. It was apparent from the results that the viscosity enhancement of systems containing carbopol 974 was higher than those that contain carbopol 971 and polycarbophil. This suggested that carbopol 974 was able to interact more strongly with mucin as compared with other polymer gels. Presence of cyclodextrin has a detrimental effect on the rheological synergism causing a decrease in the η_{rel} values. The presence of cyclodextrins either as physical mixture or inclusion complex did not have significant difference in its capacity to lower η_{rel} values. The mucoadhesive properties[108] of these types of polymers were capable of interacting with mucus in their fully hydrated state. This was probably due to the interaction between gels[207] and mucin by formation of physical entanglements followed by hydrogen bonds with sugar residues on the oligosaccharide chains which resulted in the formation of a strengthened mucus gel networks. The viscosity was also found to be dependent on the initial viscosity of the systems.

A second way to consider the magnitude of the rheological synergism was by a consideration of the relative viscosity enhancement. This would allow the viscosity enhancement effect to be expressed as proportion of the unmixed materials viscosities. A $\eta_{rel} = 1$ meant there was no interaction between gel and mucin. A higher value of η_{rel} showed synergism between the polymer and mucin. The η_{rel} values of carbopol 974 gels were higher. Thus the formation of interfacial bonds between the gels prepared with Carbopol 974 and mucin was stronger that with Carbopol 971 and polycarbophil. Also there was no significant difference between the β-CD and HPβ-CD gels ($P>0.05$).

Texture profile analysis (TPA) is used as an interesting technique to characterize the mechanical properties of pharmaceutical gels and semisolid systems[208, 209]. This simple and rapid technique can provide information related to the gel mechanical parameters, such as hardness and adhesiveness[187]. Hardness is the force required to attain a given deformation and can be obtained from the maximum force during the first compression cycle. Adhesiveness is the work required to overcome the attractive forces between the surface of the product and the surface of the probe with which the sample comes into contact and is represented by the negative area of the force-time curve. Ideally gels designed for mucosal drug delivery should have low hardness yet high adhesiveness. Low gel hardness[187] and compressibility will ensure that minimum work is required for gel removal and administration. Adhesiveness is an important parameter on the design of a nasal gel, since a desirable gel contact and retention at the mucosal surface would ensure better clinical efficacy. Hence high gel adhesiveness would ensure prolonged adhesion of the gel onto the nasal mucosa and a complete structural recovery of the gel following application. The hardness values and adhesiveness decreased significantly when various constituents were incorporated especially in the presence of modified cyclodextrins. An interesting finding was that gels containing drug/ HPβ-CD inclusion complex showed a value of adhesiveness and hardness close to the gel without HPβ-CD suggesting that a gel with suitable properties can be obtained by addition of inclusion complex.

From the mechanical studies of gels based on their rheological properties and texture profile analysis it is seen that the carbopol 974 gels showed the greatest η_{rel} values which was affected by the addition of CD with a lowering of the $\eta_{rel.}$ The type of CD added does not influence the η_{rel} values. An important finding which has implications in nasal delivery is that hardness and adhesiveness of the gels were less affected when the drug was added as a complex with CD than as a physical mixture. This was confirmed both in case of the rheological studies and TPA studies. Hence the good release and absorption profile of C_1HF3 and C_1BF3 gels could also be due to their increased adhesiveness to the nasal mucosa.

Nasal mucociliary clearance[167] also largely determined the absorption profile of nasal drug delivery since the residence time of drugs administered to the nasal cavity is limited by mucociliary clearance. The nasal mucociliary clearance system transports the mucus layer that covers the nasal epithelium towards the nasopharynx by ciliary beating. Its function is to protect the respiratory system from damage by inhaled substances Impairment of nasal mucociliary clearance can result in diseases of the upper airway. Therefore it was important to study the effects of progesterone nasal gel on nasal mucociliary clearance and establish that nasal drug formulations do not disturb the ciliary movement. On the other hand nasal mucociliary clearance also largely determines the absorption profile of the nasal drug delivery, since residence time of drugs administered to the nasal cavity is largely limited by mucociliary clearance. Since nasal mucociliary clearance is a complex system of interactions between mucus, cilia and the periciliary fluid, studies were conducted to investigate the effects of gels on the mucociliary system. Studying the nasal mucociliary clearance is therefore important in order to optimize nasal drug delivery with respect to both safety and the amount and rate of absorption. When nasal mucociliary clearance is irreversibly impaired by the components of nasally administered drug formulations, this can prohibit their therapeutic usage. A large number of methods are used to assess mucociliary clearance. These methods study the effects of drugs and excipients on the mucociliary system *in vitro* or *in vivo*, in animals or humans. The *in vitro* model involves the excised frog palate model. The ciliated mucosa of the frog palate closely resembles that of mammalian respiratory

epithelium with respect to morphology, function and histochemistry. However in the absence of mucus or extracellular Ca^{2+}, the cilia of the frog palate do not beat, in contrast to cilia of the mammalian respiratory epithelium. There are two kinds of frog palate experiments; mucus depleted and mucus non-depleted. The mucus non depleted method was used for this study[192-195]. This mucus layer is essential for transport, and it also offers an intact barrier that protects the underlying cilia. Because of these characteristics, the non-depleted frog palate model was used to study the effects of nasal gels on intact mucociliary clearance systems.

In the *in vitro* method compared to the gels with cyclodextrins, the gels which did not contain the CDs were more effective at reducing the MTR, likely because of their higher viscosities. There was a direct relationship between the viscosity and the reduction in MTR. These studies were carried out to quantify the viscoelastic properties to delay the MTR. All the gels are capable of producing more than 55% reduction in MTR which thus provides a desired mucociliary transport rate for optimum release characteristics. Hence studying the nasal mucociliary clearance system was important in optimizing nasal gels with respect to both safety and amount and rate of absorption. There was a need to maintain a balance between the % reduction in MTR and the irreversible damage to the mucociliary clearance mechanism.

The results of the stability studies on nasal gels showed no degradation of the drug and also similar release profile between control samples and the samples exposed to stability test conditions. Linear regression of line obtained by plotting time versus percentage drug content with unknown interpolation was carried out to determine the shelf life period. The data from all the factor combinations considered, support the proposed shelf life of 30 months.

In this study, CDs significantly enhanced the nasal absorption of progesterone in rabbits. From these results it is suggested that the absorption enhancing effect is mediated through the solubility enhancing and permeation enhancing properties of the CDs. Similar results have been reported by Henry[131] *et al* in which dimethyl-β-cyclodextrin is used in nasal product containing 17-β-estradiol. The solubilized 17-β-estradiol gave significantly higher nasal bioavailability in rabbits as compared to non-suspension formulations. A similar work by Merkus[145] *et al* demonstrated that formulation in which dimethyl-β-cyclodextrin was used to solubilize progesterone for nasal delivery has been tested in human volunteers and has been shown to result in blood levels of progesterone comparable to those after intravenous administration. In addition to the solubilizing and stabilizing effects of the cyclodextrins, these materials may also have an absorption enhancing effect on the nasal membrane. Gu[72] *et al* investigated the solubilization of prostaglandin with hydroxypropyl β-cyclodextrin. The complex first increased the solubility and stability of the drug which lead to increase in bioavailbility to 25% on nasal administration. In this study the effect of the CDs on the integrity of nasal mucosa was evaluated and found to be safe. This was similarly demonstrated in the study by Kazunori[121] *et al* who showed that there was no tissue damage with 1.5% β-cyclodextrin and 10% hydroxypropyl β-cyclodextrin. This work stives to show the effect of the method of addition of CDs on the absorption enhancement properties on the nasal mucosa. The progesterone absorption-enhancing effect of CDs showed a trend towards its method of addition either as a complex or as a physical mixture.

Gels have been used for quite a long time as drug carriers and recent advances in gel and polymer technology have attracted researchers' interest in these polymeric systems. The nasal route was no exception. The nasal route for administration of gels for systemic delivery of progesterone is proved to be an effective mode of drug delivery. The increased bioavailability of progesterone following nasal instillation of the gel formulation using bioadhesive PAA polymers along with beta cyclodextrin and hydroxypropyl beta cyclodextrin, together with observed minor toxicity demonstrate the potential of these nasal gels to systemically deliver the hormone. PAA polymers are a promising drug delivery system for the steroidal hormone progesterone, which enhances nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore PAA polymers along with absorption enhancers beta cyclodextrin and hydroxypropyl beta cyclodextrin exhibit permeation enhancing effect.

Gels for nasal delivery require careful study because of the special delivery required. According to this work the formulative approaches for improving nasal delivery of progesterone consist in the use of suitable absorption enhancers. As cyclodextrins were chosen for absorption enhancement, the method of addition is important to the study. Statistical optimization reveals that addition of cyclodextrins as an inclusion complex with the drug in the gels significantly affects the absorption profile. *In vivo* studies in rabbits for nasal absorption of progesterone show a rapid increase in plasma levels and promising bioavailability values. Hence the formulations represent a promising and novel tool for the nasal delivery of progesterone and can be used instead of injectables for the administration of the hormone.

As drugs and biotechnology markets continue to grow, in the future we should expect a range of novel nasal products to escalate in the market. It is envisaged that these products will be used in crisis management as well as in treatment of long term conditions. Most of the drugs which will be available as nasal products in the future, are those, which at present are given as injections or have a low bioavailability and display unwanted pharmacological effects due to inappropriate dosing regimen.

In this study detailed characterizations of the formulations along with toxicological and bioavailability studies are carried out in animals which has been promising. However there is a tendency for the absorption promoting effect to be different in larger animals. Accordingly, investigations in humans are necessary in order to prove the pharmaceutical performance and allow excellence of clinical outcome.

1. Alison BL, Gary PM. Nasal Drug Delivery. In: Anya M Hillery, Andrew W Lloyd, James Swarbrick, editors. Drug delivery and targeting. CRC Press; 2001.

2. Jack Arora D. Development of nasal delivery systems: a review. Drug Del Tech 2002;2(7).

3. Daniel PW, Jodi LM. Intranasal drug delivery. In: Michael J, Jonathan Hadgraft, Michael S Roberts, editors. Modified release drug delivery technology. New York: Marcel Dekker;2003.

4. Ugwoke MI, Agu RU, Verbeke N, Kinget R. Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives. Adv Drug Delivery Rev 2005;57:1640-65.

5. Bernstein JM, Reddy MS, Scannapieco FA, Faden HS, Ballow M. The microbial ecology and immunology of the adenoid: implications for otitis media. Ann NY Acad Sci 1997;830:19-31.

6. Proctor DF. The upper airways: In nasal physiology and defense of the lungs. Am Rev Respir Disord 1977;115:97-129.

7. Emmeline M, Nicolaas GM, Schipper J, Coos V, Merkus FWHM. Nasal mucociliary clearance as a factor in nasal drug delivery. Adv Drug Delivery Rev 1998;29:13-38.

8. Van de Donk HJM, Muller Plantema IP, Zuidema J, Merkus FWHM. The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. Rhinology 1980;18:119-30.

9. Huang C, Kimura R, Nassar A, Hussain A. Mechanism of nasal drug absrption of drug II: absorption of L-tyrosine and the effect of structural modification on its absorption. J Pharm Sci 1985;74:1298-301.

10. Jadhav KR, Gambhire MN, Shaikh IM, Kadam VJ, Pisal SS. Nasal drug delivery systems-factors affecting and applications. Current Drug Therapy 2007;2:27-38.

11. Behl CR, Pimplaskar HK, Sileno AP, Xia WJ, Gries WJ, DeMeireles JC *et al.* Optimization of systemic nasal drug delivery with pharmaceutical excipients. Adv Drug Delivery Rev 1998;29:117-33.

12. Mathiowitz E, Chickering D, Jacob SJ, Santos C. Encyclopedia of controlled drug delvery. In:Mathiowitz E, editor. New York: John Wiley; 1999. p.9-42.

13. Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 2001;53:3–22.

14. Illum L. Bioadhesive formulations for nasal peptide delivery. In: Mathiowitz E, Chickering DE, Lehr C, editors. Bioadhesive drug delivery systems. New York: Marcel Dekker;1999. p.507–41.

15. Janes KA, Calvo P, Alonso MJ. Polysaccharide colloidal particles as delivery systems for macromolecules. Adv Drug Delivery Rev 2001;47:83–97.

16. McNeela EA, O'Connor D, Jabbal-Gill I, Illum L, Davis SS, Pizza M *et al*. A mucosal vaccine against diphtheria. Vaccine 2001;19:1188–98.

17. Edman P, Bjork E, Ryden L. Microspheres as a nasal delivery system for peptide drugs. J Controlled Release 1992;21:165–72.

18. Ugwoke MI, Kaufmann G, Verbeke N, Kinget R. Intranasal bioavailability of apomorphine from carboxymethylcellulose-based drug delivery systems. Int J Pharm 2000;202:125–31.

19. Nakamura K, Maitani Y, Lowman AM, Takayama T, Peppas NA, Nagai T. Uptake and release of budesonide from mucoadhesive pH-sensitive copolymers and their application to nasal delivery. J Controlled Release 1999;61:329–335.

20. Brime B, Ballesteros MP, Frutos P. Preparation and *in vitro* characterization of gelatin microspheres containing levodopa for nasal administration. J Microencapsul 2000;17:777–84.

21. Aikawa K, Matsumoto K, Uda H, Tanaka S, Shimamura H, Aramaki Y *et al*. Prolonged release of drug from o/w emulsion and residence in rat nasal cavity. Pharm Dev Technol 1998;3:461–69.

22. Law SL, Huang KJ, Chou HY. Preparation of desmopressin-containing liposomes for intranasal delivery. J Controlled Release 2001;70:375–82.

23. Betbeder D, Spe´randio S, Latapie J, J de Nadaı´, Etienne A, Zajac J *et al*. Biovector nanoparticles improve antinociceptive efficacy of nasal morphine. Pharm Res 2000;17:743–48.

24. Joel G Hardman, Lee E Limbird, Alfred Goodman Gilman. Goodman & Gilman's The pharmacological basis of therapeutics. 10th ed. New York: McGrawHill; 2001.

25. Sharma HL, Sharma KK. Principles of Pharmacology. New Delhi: Paras Medical Publishers; 2007.

26. James EF Reynolds. Martindale: The complete drug reference. 35th ed**.** London: Pharmacetutical Press; 2007.

27. Joseph P Remington. Remington's pharmaceutical sciences. 17th ed. Easton: Mack Publishing; 1985.

28. At Florence, Pu Jani. Novel oral-drug formulations-their potential in modulating adverse-effects. Drug Saf 1994;410:233-66.

29. Carnali JO, Naser MS. The use of dilute solution viscosity to characterize the network properties of carbopol microgels. Colloid Polym Sci 1992;270:183-93.

30. Garcia-Gonzalez N, Kellaway IW, Blanco Fuente H, Anguiano Igea S, Delgado Charro B, Otero Espinar F J et al. Influence of glycerol concentration and carbopol molecular weight on swelling and drug release characteristics of metoclopramide hydrogels. Int J Pharm 1994;104,107-13.

31. B F Goodrich Company Technical Literature: Carbopol Resin Handbook, 1991.

32. Raymond C Rowe, Paul J Sheskev, Paul J Weller, Ray Rowe, Paul Sheskev. Handbook of Pharmaceutical Excipients. 4th ed. Washington DC: American Pharmaceutical Association / Pharmaceutical Society of Great Britain;1986.

33. Lochhead RY, Davidson JA, Thomas GM. Cabopol and its pharmaceutical significance-a review. Polymers in aqueous media 1989;13:147.

34. Meyer RJ, Cohen L.The rheology of natural and synthetic hydrophilic polymer solutions as related to suspending ability. J Soc Cosmetic Chemists, presented November 1958, New York.

35. Flory, Paul J. Principles of Polymer Chemistry. New York (NY): Cornell University Press; 1953.

36. Reloar LRG. The physics of rubber elasticity. New York (NY): Oxford University Press;1958.

37. Akiyama Y, Nagahara N, Hirai S, Toguchi H. *In Vitro* and *In Vivo* evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and a poly(acrylic acid) derivative. Pharm Res 1995;12(3):397-405.

38. Micheal IU, Exaud S, Guy VDM, Norbert V, Renaat K. Nasal mucoadhesive delivery systems of the anti-parkinsonian drug, apomorphine : influence of drug – loading on *in vitro* and *in vivo* release in rabbits. Int J Pharm 1999;181:125-38.

39. Claudia V, Constantia EK, Irene H, Andreas B. Development and *in vitro* evaluation of a mucoadhesive vaginal delivery system for progesterone. J Controlled Release 2001;77:323–32.

40. ElHady SSA, Mortada ND, Awad GAS, Zaki NM, Taha RA. Development of *in situ* gelling and mucoadhesive mebeverine hydrochloride solution for rectal administration. Saudi Pharm J 2003;11(4):59-169.

41. Anlar S, Capan Y, Hincal AA. Physico-chemical and bioadhesive properties of polyacrylic acid polymers. Pharmazie 1993;48(4):285-87.

42. Chang HS, Park H, Kelly P, Robinson JR. Bioadhesive polymers as platforms for oral controlled release drug delivery-II: Synthesis and evaluation of some swelling, water- insoluble bioadhesive polymers. J Pharmacol Sci 1985;74:229.

43. Smart JD. Some formulation factors influencing the rate of drug release from bioadhesive matrices. Drug Dev Ind Pharm 1992;18(2):223-32.

44. Smart JD. An *in vitro* assessment of some mucosa-adhesive dosage forms. Int J Pharm 1991;73(1):69-74.

45. Brewster ME, Simpkins JW, Hora MS. The potential use of cyclodextrins in parenteral formulations. J Parenter Sci Technol 1989;43:231–40.

46. Duchêne D, Wouessidjewe D. Physicochemical characteristics and pharmaceutical uses of cyclodextrin derivatives, part I. Pharm Technol 1990;14(6):26-34.

47. Duchêne D, Wouessidjewe D. Physicochemical characteristics and pharmaceutical uses of cyclodextrin derivatives, part II. Pharm Technol 1990;14(8):14-26.

48. Brewster ME, Hora MS, Simpkins JW, Bodor N. Use of 2-hydroxypropyl-β-cyclodextrin as a solubilizing and stabilizing excipients for protein drugs. Pharm Res 1991;8(6):792–95.

49. Choudhury S, Nelson KF. Improvement of oral bioavailability of carbamazepine by inclusion in 2-hydroxypropyl-β-cyclodextrin. Int J Pharm 1992;85:175–80.

50. Szejtli J. Cyclodextrin technology. Boston: Kluwer Academic Publisher; 1988.

51. Marcus EB, Thorsteinn L. Cyclodextrrin as pharmaceutical solubilizers. Adv Drug Delivery Rev 2007;59:645-66.

52. Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem Toxicol 2005;43:1451–459.

53. Brewster ME, Mackie C, Lampo A, Noppe M, Loftsson T. The use of solubilizing excipients and approaches to generate toxicology vehicles for contemporary drug pipelines.In: Augustijns P,Brewster ME, editors. Solvent systems and their selection in pharmaceutics and biopharmaceutics. New York: American Association of Pharmaceutical Scientists and Springer;2007.p.221-56.

54. Uekama K, Otagiri M. Cyclodextrins in drug carrier sytems. Drug Carrier Syst 1987;3:1-40.

55. Duchene D, Wouessidjewe D. Pharmaceutical uses of cyclodextrins and derivatives. Drug Dev Ind Pharm1990;16:2487-499.

56. Duchene D, Wouessidjewe D. Physiochemical characterization and pharmaceutical uses of cyclodextrin derivatives. Pharm Technol Int 1990;21-29.

57. Eunsook Cho, Hyesun G, Inkoo C. Formulation and evaluation of ondansetron nasal drug delivery systems. Int J Pharm 2008;349:101-07.

58. Rathananand M, Kumar DS, Shirwaikar A, Kumar R, Sampath Kumar D, Prasad DS. Preparation of mucoadhesive microspheres for nasal delivery by spray drying. Indian J Pharm Sci 2007;69:651-57.

59. Dandagi PM, Mastibolimath VS, Gadad AP, Iliger SR. Mucoadhesive microspheres of propranolol hydrochloride for nasal delivery. Indian J Pharm Sci 2007;69:402-07.

60. Yadav AV, Mote HH. Development of biodegradable starch microspheres for intranasal delivery. Indian J Pharm Sci 2007;70:170-74.

61. Jain SK, Jain NK, Gupta Y, Jain A, Jain D, Chaurasia M. Mucoadhesive chitosan microspheres for non-invasive and improved nasal delivery of insulin. Indian J Pharm Sci 2007;69:498-504.

62. Shelke VB, Thopte GM, Pisal SS, Kadam SS. Nasal delivery of propranolol hydrochloride from sorbitan monosterate organogels : preformulation study. Indian J Pharm Sci 2005;67:200-05.

63. Zoppetti G, Puppini N, Pizzutti A, Fini T, Giovani T, Comini S. Water soluble progesterone-cyclodextrin complex for injectable formulations. J Incl Phenom Macrocycl Chem 2007;57:283-88.

64. Robert JG, Herman FS, Anthony JH. Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 2007; 8(4).

65. Malika LS, Cecile B, Frederic B, Same J, Mohamed S. Solubility and Disolution rate of progesterone–cyclodextrin polymer systems. Drug Dev Ind Pharm 2006;32:1043-56.

66. Krauland AH, Guggu D, Bernkop SA. Thiolated chitosan microparticles: A vehicle for nasal peptide drug delivery. Int J Pharm 2006;307(2):270-77.

67. Varshosaz J, Sadrai H, Heidari A. nasal delivery of insulin using bioadhesive chitosan gels. Drug Delivery 2006;13(1):31-38.

68. Patil SB, Murthy RSR. Preparation and *in vitro* evaluation of mucoadhesive chitosan microspheres of amlodipine besylate for nasal administration. Ind J Pharm Sci 2006;68(1):64-67.

69. Gavini E, Hegge AB , Rassu G, Sanna V, Testa C, Pirisino G, Karlsen J, Giunchedi P. Nasal administration of carbamazepine using chitosan microspheres: *In vitro/in vivo* studies. Int J Pharm 2006;307:9-15.

70. Cetin T, Cansel KO, Ayhan S, Yalcin O, Umut T, Hikmet A. Nasal absorption of metoclopramide from different carbopol 981 based formulations: *in vitro, ex vivo* and *in vivo* evaluation. Eur J Pharm Biopharm 2006;64:246-54.

71. Rita JM, Pradip KG, Manish LU, Murthy RSR. Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. AAPS PharmSciTech 2006;7(3):67.

72. Fu-Gen Gu, Fu-de Cui, Yong-liang Gao. Preparation of prostaglandin E1-hydroxy propyl-β-cyclodextrin complex and its nasal delivery in rats. Int J Pharm 2005;290:101-08

73. D'Souza R, Mutalik S, Venkatesh M, Vidyasagar S, Udupa N. Insulin gel as an alternate to parenteral insulin: formulation preclinical and clinical studies. AAPS PharmSciTech. 2005;06(02):184-89.

74. Baboota S, Dhaliwal M, Kohli K. Physiochemical characterization, *in vitro* dissolution behavior and pharmacodynamics studies of refecoxib-cyclodextrin inclusion compounds. AAPSPharmSciTech 2005;06(01):83-90.

75. Abdolhossein RN, Payam M, Hosnieh T. Intranasal bioavailability of insulin from carbopol –based gel spray in rabbits. Drug Delivery 2004;l11:295-300.

76. Sambhaji SP, Anant RP, Kakasaheb RM, Shivajirao SK. Pluronic gels for nasal delivery of Vitamin B_{12}. Preformulation study. Int J Pharm 2004;270:37-44.

77. Brent LV,Frank F, Brad B, Kelly HR. Partition-controlled progesterone release from water borne *in situ*-gelling materials. Int J Pharm 2004;274:191-200.

78. Shirui M, Jianming C, Zhenping W, Huan L, Dianzhou B. Intranasal administration of melatonin starch microspheres. Int J Pharm 2004;272:37-43.

79. Cerchiara T, Luppi B, Bigucci F, Zecchi V. Effect of chitosan on progesterone release from hydroxypropyl-β-cyclodextrin complexes. Int J Pharm 2003;258:209-15.

80. Dyer AM, Hinchcliffe M, Watts P, Castile J, Illum L. Nasal delivery of insulin using novel chitosan based formulation. Pharm Res 2002;19(7):998-1008.

81. Karen EEH, Kathy P, Sandra MB, Milton M, Dominique Z. Seqential hormonal supplementation with vaginal estradiol and progesterone gel corrects the effect of clomiphene on the endometrium in oligo-ovulatory women. Hum Reprod 2002;17(2):295-98.

82. Fusashi I, Mika M, Minoru H, Toshikazu Y,Ikumi T, Akira T. Insoluble powder formulation as an effective nasal drug delivery system. Pharm Res 2002;19:8.

83. Emilio S, Erika R. Measurement of nasal residence time by microdialysis. Current Separations 2002;19(4):127-30.

84. David CB, Nigel MD, Ian GT. Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. Int J Pharm 2001;197:1-11.

85. Fabiana Q, Giovanna V, Agnese M, Maria ILR, Domenico L, Giuseppe M. Modulation of the drug release from hydrogels by using β-cyclodextrins: the case of nicardipine/β-cyclodextrin system in crosslinked polyethyleneglycol. J Controlled Release 2001;71:329-37.

86. Garcia A, Santiago TS, Goya L, Torrado JJ. Spray dried powders as nasal absorption enhancers of cyanocobalmin. Biols Pharm Bull 2001;24(12):1411-16.

87. Michael V, Hagan DT. Microparticles for intranasal immunization. Adv Drug Delivery Rev 2001;51:127–41.

88. Illum L. Nasal vaccination: a non-invasive vaccine delivery method that holds great promise for the future. Adv Drug Delivery Rev 2001;51:1–3.

89. Illum L, Fisher AN, Jabbal-Gill I, Davis SS. Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides. Int J Pharm 2001;222:109-19.

90. Callens C, Remon JP. Evaluation of starch-maltodextrin-carbopol 974P mixtures for the nasal delivery of insulin in rabbits. J Contolled Release 2000;66:215-20.

91. Michael IU, Giana K, Norbert V, Renaat K. Intranasal bioavailability of apomorphine from carboxymethylcellulose-based drug delivery systems. Int J Pharm 2000;202:125-31.

92. Schoolcraft WB, Hesla JS, Gee MJ. Experience with progesterone gel for luteal support in a highly successful IVF programme. Hum Reprod 2000;15(6):1284-88.

93. Claudia W, Randall JM. *In vitro* evaluation of microparticles and polymer gels for use as nasal platforms for protein delivery. Pharm Res 1999;16(3):382-90.

94. Kouji N, Yoshie M, Anthony ML, Kozo T, Nicholas AP, Nagai T. Uptake and release of budesonide from mucoadhesive, pH-sensitive copolymers and their application to nasal delivery. J Controlled Release 1999;61:329-35.

95. Cavalli R, Peira E, Caputo O, Gasco MR. Solid lipid nanoparticles as carriers of hydrocortisone and progesterone complexes with β-cyclodextrin. Int J Pharm 999;182:59-69.

96. Takenaga M, Serizawa Y, Azechi Y, Ochiai A, Mizushima Y *et al*. Microparticle resins as a potential nasal drug delivery system for insulin. J Controlled Release 1998;52:81-87.

97. Alessia DA, Ruggero B, Giovanni C, Pier LC, Maria TP, Patrizia S *et al*. Delivery of nasal powders of cyclodextrin by insufflation. Pharm Res 1996;13(5):734-38.

98. Lena PM, Peter E. Influence of osmolarity on nasal absorption of insulin from the thermogelling polymer ethyl(hydroxyethyl) cellulose. Int J Pharm 1995;125:205-13.

99. Pereswetoff ML, Edman P. Dextran microspheres as a potential nasal drug delivery system for insulin-*in vitro* and *in vivo* properties. Int J Pharm 1995;124:37-44.

100. Daniela P, Alessia DA, Antonia M, Paolo C, Pier LC. Nasal powders of progesterone: manufacturing and bioavailability. Eur J Pharm Biopharm 1994;40(4):223-27.

101. Schipper NGM, Hermens WAJJ, Romeyn SG, Verhoef J, Merkus FWHM. Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats. Int J Pharm1990;64:61-66.

102. Cicinelli E, Cignarelli M, Resta L, Scorcia P, Petruzzi D, Santoro G. Efects of the repetitive administration of progesterone sprays in post meneopausal women. Fertil Steril 1993;60(6):1020-24.

103. Ting TY, Gonda I, Gipps EM. Microparticles of poly vinyl alcohol for nasal delivery: generation by spray drying and spray desolvation. Pharm Res 1992;9:10.

104. Edman P, Bjork E, Ryden L. Microspheres as a nasal delivery system for peptide drugs. J Controlled Release 1992;21:165-72.

105. Schipper NGM, Verhoef J, Romeijn SG, Merkus FWHM. Absorption enhancers in nasal insulin delivery and their influence on nasal ciliary functioning. J Controlled Release 1992;21:173-86.

106. Cicinelli E, Fanelli F, Ragno G, Vetuschi C, Cagnazzo I, Schonauer S. Progesterone administration by nasal spray. Fertil Steril 1991;56(1):139-41.

107. Illum L, Farraj NF, Davis SS, Johansen BR, O'Hagan DT. Investigation of the nasal absorption of biosynthetic human growth hormone in sheep-use of a bioadhesive microspheres delivery system. Int J Pharm 1990;63:207-11.

108. Walter AJJH, Marc JMD, Stefan GR, Coos JV, Frans WHMM. Nasal absorption enhancement of 17 estradiol by dimethyl β-cyclodextrin in rabbits and rats. Pharm Res 1990;7(5):500-03.

109. Basarshi-Nassar RN, Hussain AA, Crooks PA. Nasal absorption and metabolism of progesterone and 17 beta estradiol in the rat. Drug Metab Dispos 1989;17(3):248-54.

110. Diane CC, Jue CL, Yie WC. Drug absorption through mucosal membrane: effect of mucosal route and penetrant hydrophilicity. Pharm Res 1989;6:10.

111. Anand KTC, David GFX, Sankaranarayanan A, Puri V, Sundram KR. Pharmacokinetics of progesterone after its administration to ovariectomised rhesus monkeys by injection, infusion and nasal spraying. Proc Nat Acad Sci 1982;79:4185-89.

112. Jian W, Shigeko S, Yoshiharu D, Dianzhou B, Yasuhiko T, Kazuhiro M. Aminated gelatin as a nasal absorption enhancer for peptide drugs: evaluation of absorption enhancing effect and nasal mucosa perturbation in rats. J Pharm Pharmacol 2002;54:181-88.

113. Nartaya T, Ross AK, Pornsak S. Viscometric study of pectin–mucin interaction and its mucoadhesive bond strength. Carbohydr Polym 2008;71:170–79.

114. Pornsak S, Nathaya W. Rheological synergy in aqueous mixtures of pectin and mucin. Carbohydr Polym 2008;03:021.

115. Shah AJ, Maureen DD. Formulating gels for decreased mucociliary transport using rheologic properties: polyacrylic acids. AAPS PharmSciTech 2007;8(2): Article 33. Available from URL http://www.aapspharmscitech.org.

116. Shah AJ, Donovan MD. Rheological characterization of neural and anionic polysaccharides with reduced mucociliary transport rates. AAPS PharmSciTech 2007;8(2): Article 32. Available from URL http://www.aapspharmscitech.org.

117. Mallants R, Jorissen M, Augustijns P. Effect of preservatives on cilliary beat frequency in human nasal epithelial cell culture: single versus multiple exposure. Int J Pharm 2007;338:64-69.

118. Catherine T, Jeffrey PP, Kurt ID, Peter WD, Olay S. Rheological characterization of mixed gels of mucin and alginate. Carbohydr Polym 2005;59:189-95.

119. Giulia B, Sante M, Giovanni FP. Rheological mucoadhesive and release properties of carbopol gels in hydrophilic cosolvents. Int J Pharm 2004;282:115-30.

120. Bounmany KK, Mai Hoang N, Mathew WR. Effects of buffered saline solution on nasal mucociliary clearance and nasal airway patency. Annual Meeting of the American Academy of Otolaryngology-Head and Neck Surgery; 2004 Sep 19-22; New York..

121. Kazunori A, Mariko M, Hideo K, Shoji H, Katsuhiko S, Masahiro N *et al*. The effects of water soluble cyclodextrins on the histological integrity of the rat nasal mucosa. Int J Pharm 2002;246:25-35.

122. Adriaens E, Remon JP. Evaluation of an alternative mucosal irritation test using slugs. Toxicol Appl Pharmacol 2002;182:169-75.

123. Callens C, Adriaens E, Dierckens K, Remon JP. Toxicological evaluation of a bioadhesive nasal powder containing a starch and carbopol 974P on rabbit nasal mucosa and slug mucosa. J Controlled Release 2001;76:81-91.

124. Hagerstrom H, Paulsson M, Edsman K. Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using rheological method. Eur J Pharm Sci 2000;9:301-09.

125. Flemming M, Kirsten E, John DS. A rheological examination of the mucoadhesive / mucus interaction: the effect of mucoadhesive type and concentration. J Controlled Release 1998;50:167-78.

126. Slobodanka T, Duncan QMC. Thermorheological and thermogravimetric analysis of bioadhesive polymer/mucin mixtures. Thermochim Acta 1997;294:99-106.

127. Morath LP, Edman P. Immunological consequences of nasal drug delivery in dextran microspheres and ethyl (hydroxyethyl) cellulose in rats. Int J Pharm 1996;128:23-28.

128. Chandler SG, Thomas NW, Illum L. Nasal absorption in the rat: IV. Membrane activity of absorption enhancers. Int J Pharm 1995;117:139-46.

129. Emad EH, Gallo JM. A simple rheological method for *in vitro* assessment of mucin polymer bioadhesive bond strength. Pharm Res 1990;7:491-95.

130. Bhise SB, Yadav AV, Avachat AM, Malayandi R. Bioavailability of intransal drug delivery system. Asian J Pharm Sci 2008;2(4):201-15.

131. Henry RC, Illum L, Gordon B, Paul HJ, Steven CQ. Intranasal delivery: Physicochemical and therapeutic aspects. Int J Pharm 2007;337:1-24.

132. Varshosaz J. Insulin delivery systems for controlling diabetes. Recent patents on. Endocrine, metabolic and immunology. Drug Discovery 2007;1:25-40.

133. Sarasija S, Bhaskaran S. Nasal drug delivery: an overview. Indian J Pharm Sci 2005;67:19-25.

134. Michael IU, Remigius UA, Norbert V, Renaat K. Nasal mucoadhesive drug delivery:background, applications, trends and future perspectives. Adv Drug Delivery Rev 2005;57:1640-65.

135. Oya AH, Somavarapua S, Atuahb KN, Bramwella VW. Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery. Adv Drug Delivery Rev 2005;57:411-30.

136. Challa R, Ahuja A, Javed Ali, Khar RK. Cyclodextrins in Drug Delivery: An updated review AAPS PharmSciTech 2005;06(02):329-57.

137. Andreas BS. Thiomers: A new generation of mucoadhesive polymers. Adv Drug Delivery Rev 2005;57:1569-82.

138. Stephen PN, Gary RP, Richard ND. Drug delivery to the nasal cavity: *in vitro* and *in vivo* assessment. Ther Drug Carrier Syst 2004;21(1):21-66.

139. Davis SS, Illum L. Absorption enhancers for nasal drug delivery. Clin Pharmacokinet 2003;42(13):1107-28.

140. Illum L. Nasal drug delivery—possibilities, problems and solutions. J Controlled Release 2003;87:187-198.

141. Illum L. Nasal drug delivery: new developments and strategies. Drug Discov Today 2002;7(23):1184-89.

142. Michael IU, Norbert V, Renaat K. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 2001;53:3-22.

143. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Delivery Rev 2001;51:81-96.

144. Davis SS. Nasal vaccines. Adv Drug Delivery Rev 2001;51:21-42.

145. Merkus FWHM, Verhoef JC, Martin E, Romeijn SG, Van der Kuy PHM, Hermens WAJJ *et al*. Cyclodextrins in nasal drug delivery. Adv Drug Delivery Rev 1999;36:41-57.

146. Morath LP. Micorspheres as nasal drug delivery systems. Adv Drug Delivery Rev 1998;29:185-94.

147. Gonda I. Mathematical modeling of deposition and disposition of drugs administered via the nose. Adv Drug Delivery Rev 1998;29:179-84.

148. Behl CR, Pimplaskar HK, Sileno AP, Xia WJ, Gries WJ, DeMeireles JC *et al*. Optimization of systemic nasal drug delivery with pharmaceutical excipients. Adv Drug Delivery Rev 1998;29:117-33.

149. Stanley SD, Illum L. Drug delivery systems for challenging molecules.Int J Pharm 1998;176:1-8.

150. Kublika H, Vidgrenb MT. Nasal delivery systems and their effect on deposition and absorption. Adv Drug Delivery Rev 1998;29:157-177.

151. Jerome H, Artursson P. Mechanisms of absorption enhancement and tight junction regulation. J Controlled Release 1994;29:253-67.

152. Drug-Excipient interaction. Available from URL: www.usp.org/pdf/EN/meetings/asMeetingIndia/drugExcipient/Interaction.pdf.

153. The International Pharmacopoeia, Fourth Edition WHO Geneva.

154. Lloyd R Synder, Joseph J Kirkland, Joseph L Glajch. Practical HPLC method development. 2nd ed. New York: John Wiley & Sons; 1997.

155. Uekama K, Fujinaga F, Otagiri H, Yamasaki M. Inclusion complexations of steroidal hormones with cyclodextrins in water and in solid phase. Int J Pharm 1982;10(1):1-15.

156. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Del Rev 2007;59:645-66.

157. Patric B O'Donell, James W McGinity. Preparation of microspheres by solvent evaporation technique. Adv Drug Delivery Rev 1997;28:29-42.

158. Bilensoy E, Rouf MA, Sen M, Hincal AA. Mucoadhesive, thermosensitive, prolonged release vaginal gel for clotrimazole:cyclodextrin complex. AAPSPharmSci Tech 2006;7(2): Article 38. Available from URL http://www.aapspharmscitech.org.

159. Avdeef A. Solubility of sparingly soluble ionizable drugs. Adv Drug Delivery Rev 2007;59:568-90.

160. Mockel JE, Lippold BC. Zero order release from hydrocolloid matrices. Pharm Res 1993;10:1066-070.

161. Keck T, Leiacker R, Riechelmann H, Rettinger G. Temperature profile in the nasal cavity. Laryngoscope 2000;110:651-54.

162. Lang S, Peter L, Rainer O, Birgit T, and Hans M. Transport and metabolic pathway of thymocartin (TP4) in excised bovine nasal mucosa. J Pharm Pharmacol 1996;48:1190-196.

163. Dhondt MMM, Adriaens E, Roey JV, Remon JP.The evaluation of the local tolerance of vaginal formulations containing dapivirine using slug mucosa irritation test and the rabbit vaginal irritation test. Eur J Pharm Biopharm 2005;60:419-25.

164. NC3Rs-The slug mucosal irritation assay: an alternative assay for local tolerance testing. Available from URL: http://www.nc3rs.org.uk/news.asp?id=308.

165. Sriamornsak P, Wattanakorn N. Rheological synergy in aqueous mixtures of pectin and mucin. Carbohydrate Polymers 2008;3:474-81.

166. Thiarawong N, Kennedy RA, Sriamornsak. Viscometric study of pectin-mucin interactions and its mucoadhesive bond strength. Carbohydr Polym 2008;71:170-79.

167. Marttin E, Schipper NGM, Verhoef JC, Merkus FWHM. Nasal mucociliary clearance as a factor in nasal drug delivery. Adv Drug Delivery Rev 1998;29:13-38.

168. Mortazavi SA. An *in vitro* assessment of mucus mucoadhesive interactions. Int J Pharm 1995;124:173-82.

169. Tay HL, Armoogum N, Tan LKS. Nasal mucociliary clearance and salmetrol. Clin Otolaryngol 1997;22:68-70.

170. Lin SY, Amidon GL, Weiner ND, Goldberg AH. Viscoelasticity of anionic polymers and their mucociliary transport on the frog palate. Pharm Res 1993;10(3):411-15.

171. ICH Guidance for Industry: Q1E Evaluation of Stability data. Available from URL: http://www.fda.gov/Cber/gdlns/ichstabdta.htm.

172. Gary RP, Feray JC, Nazaret C, Fanous K, Villegas MJ, Letavernier JF. A new approach for the *in vitro* evaluation of cyclodextrins effects on cellular membranes of human cells. In: Osa T, editor. Proceedings 7th international cyclodextrins symposium. Tokyo: Komiyama Printing Co;1994. p.373-76.

173. Sarasija S, Shivakumar HN, Kiran K. Effect of β-cyclodextrin complexation on the solubility and dissolution rate of carbamazepine from tablets. Indian J Pharm Sci 2006;68(3):301-07.

174. Zannou EA, Streng WH, Stella VJ. Osmotic properties of sulfobutylether and hydroxypropyl cyclodextrins. Pharm Res 2001;18(8):1226-31.

175. Gonzalez-Gaitano G, Rodriguez P, Isasi JR, Fuentes M, Tardajos G, Sanchez M. The aggregation of cyclodextrins as studied by correlation spectroscopy. J Incl Phen and Macrocyclic Chem 2002;44:101-05.

176. Madsen F, Eberth K, John DS. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. J Controlled Rel 1998;50:167-78.

177. Owen DH, Peters JJ, Katz DF. Comparison of the rheological properties of Advantage-S and Replens Contraception 2001;64:393-96.

178. J das Neves, Bahia MF. Gels as vaginal drug delivery systems. Int J Pharm 2006;318:1-14.

179. Marriott C, Gregory NP. Mucus physiology and pathology. In: Lenaerts, Gumy R, editors.Bioadhesive Drug Delivery Systems. CRC Press; 1999. p.1-24.

180. Kerr LJ, Kellaway IW, Rowlands C, Parr GD. The influence of poly(acrylic) acids on the rheology of glycoprotein gels. Proc Int Symp Control Release Bioact Mater 1990;17:122-23.

181. Mortazavi SA. An *in vitro* assessment of mucus/mucoadhesive interactions. Int J Pharm 1995;124:173-82.

182. Koester LS, Ortega GG, Mayorga P, Bassani VL. Mathematical evaluation of in vitro release profiles of hydroxypropylemethyl cellulose matrix tablets containing cabamazepine associated to beta cyclodextrin. Eur J Pharm Biopharm 2004;58:177-79.

183. O'Hara T, Dunne A, Butler J, Devane J. A review of methods used to compare dissolution profile data. Pharm Sci Technol 1998;1:214-23.

184. Jones DS, Woolfson AD, Brown AF. Textural analysis and flow rheometry of novel, bioadhesive antimicrobialoral gels. Pharm Res 1997;114(4):450-57.

185. Jones DS, Woolfson AD, Djokic J, IrwinCR. Bioadhesive, semi-solid systems containing flurbiprofen for the treatment of gingivitis. Eur J Pharm Sci. 1996;4(1):145.

186. Jones DS, Woolfson AD, Brown AF, O'Neil MJ. Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: *in vitro* release kinetics, syringeability, mechanical and mucoadhesive properties, J Controlled Release 1997;49(1):71-79.

187. Yvonne TF Tan, Kok Khiang Peh, Othman Al-Hanbali. Effect of carbopol and polyvinylpyrrolidone on the mechanical, rheological and release properties of bioadhesive polyethylene glycol gels. AAPS PharmSciTech 2000;1(3):Article 24.

188. Boulmedarat L, Grossiord JL, Fattal E, Bochot A.Influence of methyl beta cyclodextrins and liposomes on rheological properties of Carbopol 974P NF gels. Int J Pharm 2003;254:59-64.

189. Qui HY, Li L, Huang CY, Li WM, Wu CJ. Optimization and physiochemical characterization of thermosensitive polaxamer gel containing puerarin for ophthalmic use. Chem Pharmaceut Bull 2006;54:1500-07.

190. Sleigh MA, Blake JR, Liron N. The propulsion of mucus by cilia. Am Rev Respir Dis 1988;137:726-741.

191. Puchelle E, Tournier JM, Petit A, Zahm JM, Lauque D, Vidailhet M, Sadoul P. The frog palate for studying mucus transport velocity and mucociliaty frequency. Eur J Respir Dis 1983;64:293-03.

192. Batts AH, Marrott C, Martin GP, Bond SW. The effect of some preservatives used in nasal preparations on mucociliary clearance. J Pharm Pharmcol 1989;41:156-59.

193. Gizurarson S, Marriott C, Martin GP, Bechgaard E. The influence of insulin and some excipients used in nasal insulin preparations on mucociliary clearance. Int J Pharm 1990;65:243-47.

194. Bechgaard E, Gizurarson S, Hjortkjaer RK, Sorensen AR. Intranasal adminstrationof insulin to rabbits using glycofurol as an absorption promoter. Int J Pharm 1996;128:87-289.

195. Aspden TJ, Adier J, Davis SS, Skaugrud O, Illum L. Chitosan as a nasal delivery system: evaluation of the effect of chitosan on mucociliary clearance rate in the frog palate model. Int J Pharm 1995;122:69-78.

196. Batts AH, Marriott C, Martin GP, Bond SW, Bond, Greaves JL, Wilson CG. The use of a radioactive saccharine solution to monitor the effect of the preservatives thiomersal, benzalkonium chloride and EDTA on human nasal clearance. J Pharm Pharmcol 1991;43:180-85.

197. Boek WM, Keles N, Graamans K. Physiological and hypertonic saline solutions impair ciliary activity *in vitro*. Laryngoscope 1999;109:396-99.

198. Yetkin G, Celebi N, Agabeyoglu I, Gokcora N. The effect of dimethyl β-cyclodextrin and sodium taurocholate on the nasal bioavailability of salmon calcitonin in rabbits. STP Pharm Sci 1999;9:249-52.

199. Irie T, Wakamatsu K, Arima H, Aritomi H, Uekama K. Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. Int J Pharm 1992;84:129-39.

200. Guo JH, Cooklock KM. Bioadhesive polymer buccal patches for buprenorphine controlled delivery; solubility consideration. Drug Dev Ind Pharm 1995;21:2013-19.

201. Samy EM, Safwat SM. *In vitro* release of anti-inflammatory drugs with beta cyclodextrin from hydrophilic gel bases. STP Pharma Sci 1994;4:458-65.

202. Villar Lopez ME, Nieto Reyes L, Anguiano Igea S, Otero Espinar FJ, Blanco Mendez J. Formulation of triamcinolone acetonide pellets suitable for coating and colon targeting. Int J Pharm 1999;179:229-35.

203. Giunchedi P, Maggi L, La Manna A, Conte U. Modification of the dissolution behavior of a water insoluble drug naftazone for zero order release matrix preparation. J Pharm Pharmacol 1994;46:476-80.

204. Agerholm C, Bastholm L, Johansen PB, Nielsen MH, Elling F. Epithelial transport and bioavailability of intranasally administered human growth hormone formulated with absorption enhancers didecanoyl L-α-phosphatidylcholine and α-cyclodextrin in rabbits. J Pharm Sci 1994;83:1706-11.

205. Morimoto K, Tabata H, Morisaka K. Nasal absorption of nifedipine from gel preparation in rats. Chem Pharm Bull 1987;35:3041-44.

206. Morimoto K, Morisaka K, Kamada A, Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. J Pharm Pharamcol 1985;37:134-36.

207. Lorenzi G, Bohm G, Guimaraes E, Vaz M, King M, Saldiva P. Correlation between rheological properties and *in vitro* ciliary transport of rat nasal mucus. Biorheology 1992;29:433-40.

208. Jones DS, Lawlor MS, Woolfson AD. Examination of the flow rheological and textural properties of polymer gels composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone): rheological and mathematical interpretation of textural parameters. J Pharm Sci 2002;91(9):2090-101.

209. Jones DS, Woolfson AD, Brown AF. Textural analysis and flow rheometry of novel, bioadhesive antimicrobial oral gels. Pharm Res 1997;14(4):450-57.