CHAPTER - 1

INTRODUCTION

1.1 INTRODUCTION

Drugs are administered traditionally by oral and parenteral routes for systemic delivery. The oral route is widely used as it is the preferred choice of administration by both patients as well as nursing staff. However, oral route presents certain challenges based on the physicochemical properties of the active moiety.It is often hostile as there is a wide array of enzymes, wide range of pH conditions and every patient presents diverse physiological variation. The rate of drug absorption varies in the presence or absence of food.Drugs may be susceptible to acid hydrolysis or extensive liver metabolism and thus exhibit poor bioavailability when administered by this route. Drugs administered parenterally have direct access to the systemic circulation and produce maximum plasma levels, but this pathway is often associated with pain and discomfort and can only be administered by trained nursing staff. Patients often experience adverse reactions like psychological distress, occasional allergies, and hypertrophy or atrophy of subcutaneous fat at the injection site, especially in chronic administration.

Researchers globally are exploring alternative pathways like transdermal, rectal, buccal and nasal routes to bypass first-pass hepatic metabolism and optimize the therapeutic outcome. However, the transdermal route does not provide rapid blood levels and is limited to the controlled administration of potent lipophilic drugs. The rectal path suffers from variable absorption profiles as well asthe acceptability of the patient. Buccal and sublingual routes of drug administration have gained recent interests but often has compliance issues. Hence, the nasal route holds potential for administration of various drugs withthe first-pass metabolism avoided and improved bioavailability and therapeutic profile.

1.2 NASAL ROUTE FOR DRUG ADMINISTRATION

Intranasal drugs (IN) represents a non-invasive method of administration for the local and systemic administration of various therapeutic compounds ranging from small molecules to large peptides (1-10). Nose, as an organ, offersa large absorptive surface area for rapid diffusion ofthe drug, thus leading toa quickonset of therapeutic effect. The drug also has thepotential to reach the central nervous system via the olfactory pathway. Owing to bypass of first-pass metabolism, dose reduction as compared tothe oral route is feasible. This may minimize drug related ADRs often seen with oral route, thus leading to improvement in drug tolerability as well as adherence to treatment. Thepermeability of

high molecular weight active moieties by nasal route is a challenge, and this can be overcome by using permeation enhancers in formulations where the safety of these permeation enhancers are well established (11). The extensiveclinical pipeline of new molecules, as well as repositioned molecules,indicate that the nasal route is gaining wider acceptance.

1.3 LOCAL DELIVERY VS SYSTEMIC DELIVERY

Intranasal delivery has been widely used since eons for theadministrationof antihistamines and corticosteroids for allergicrhinitis(12).Few examples arelevocabastine H_1-histamine (13), anticholinergic agent ipatropium bromide (14), and diverse range of steroids likebudesonide(15),Mometasonefuroate(16), triamcinoloneand beclomethasone(17).Intranasal corticosteroids and antihistamines (18) exhibit minimal potential for systemic adverse effects due to dose reduction when compared to oral. Intranasal antihistamines do not cause sedation or significantly impair of psychomotor function, which is often seen with oral therapy.

1.4 FACTORS AFFECTING THE PERMEABILITY OF DRUGS THROUGH NASAL MUCOSA

The factors affecting permeability of drug through the nasal mucosa can broadly be classified into three categories (19) shown in the Table1.1

Table 1.1 :Variable factors affecting the permeability of drugs through the nasal mucosa

<table>
<tr><th rowspan="6">Biological Factors</th><th>Physiological factors</th><th>Environmental factors</th><th>Biological and structural features</th></tr>
<tr><td>Blood supply and neuronal regulation</td><td rowspan="2">Temperature</td><td rowspan="6">Biochemical changes</td></tr>
<tr><td>Nasal secretions</td><td>Humidity</td></tr>
<tr><td>Nasal cycle</td><td rowspan="3"></td></tr>
<tr><td>pH of the nasal cavity</td></tr>
<tr><td>Mucociliary clearance and ciliary beat frequency</td></tr>
<tr><td>API related factors</td><td colspan="3">Molecular weight, Molecular size, Solubility, Lipophilicity, pKa and partition coefficient</td></tr>
<tr><td>Formulation related factors</td><td colspan="3">pH and mucosal irritancy, osmolarity, viscosity, drug distribution, area of nasal membrane exposed, area of solution applied, dosage form,device related, particle size of the droplet/powder</td></tr>
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1.5 ADVANTAGES AND LIMITATIONS OF INTRANASAL DRUG DELIVERY

The comparative advantages and disadvantages of intra -nasal drug delivery is enumerated as under

Table 1.2: Advantages and limitations of nasal drug delivery system

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

1.6 ANATOMY AND PHYSIOLOGY

The nasal cavity is separated bya nasal septum into two halves and extends to the nasopharynx, whereas the most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nasal fossa as depicted in Figure 1.1. The atrium is an intermediate region between the vestibule and the respiratory region. The respiratory region, nasal turbinate, occupies most of the nasal cavity and has lateral walls that divide it into 3 sections: the upper nasal middle and lowerturbinates. These folds give the nasal cavity a very high surface area when compared to its small volume. Epithelial cells in the nasal vestibule are stratified, squamous and keratinized with sebaceous glands. Due to its nature, the nasal vestibule is very resistant to dehydration and can withstand noxious environmental substances and limits the penetration of pernicious substances. The atrium

is a transitional epithelial region with anteriorly stratified squamous cells and pseudo-stratified columnar cells with posterior microvillus.Pseudostratified columnar epithelial cells as depicted in Fig.1.2interspersed with goblet cells, seromucous ducts, and openings of the sub epithelial seromucousal glands cover the respiratory region (the turbinates). In addition, many of these cells actively possess the beating of the cilia with microvilli. Each hair cell contains about 100 cilia, while the hair and non-hair cells have approximately 300 micro-villi each. Table 1.3 describes the structural characteristics of different nasal anatomic regions and their relevance in drug permeability and Table1.4 describe the barriers to nasal absorption (20).

Table 1.3: Structural features of different sections ofthe nasal cavity and their relative impact on permeability; Image Courtesy (20).

Region	Structural Features	Permeability
Nasal vestibule	Nasal hair, Epithelial cells keratinized	Least Permeable
Atrium	Transepithelial region	Less permeable, lesser surface area
	Stratified squamous cells with microvilli	
Respiratory region	Ciliated columnar cells with microvilli 300/cell receives maximum nasal secretions	Most permeable
Olfactory region	Ciliated olfactory nerve cells	Direct access to cerebrospinal fluid
Nasopharynx	upper part with ciliated cells and lower with squamous	Receives nasal cavity drainage

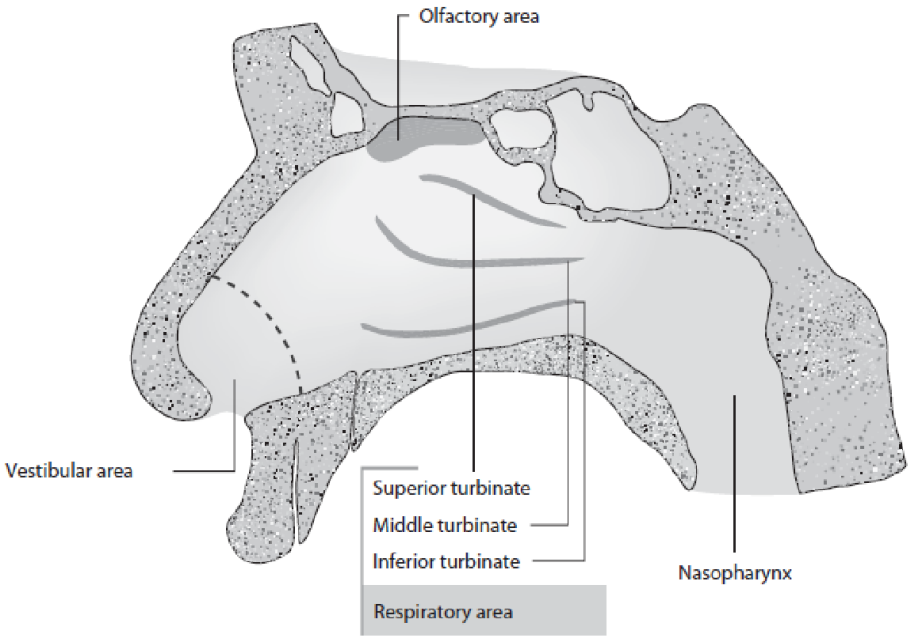

Figure 1.1: Schematic of a sagittal section of human nasal cavity showing the nasal vestibule respiratory region: inferior turbinate, middle turbinate and the superior turbinate, the olfactory region, and naso-pharynx.

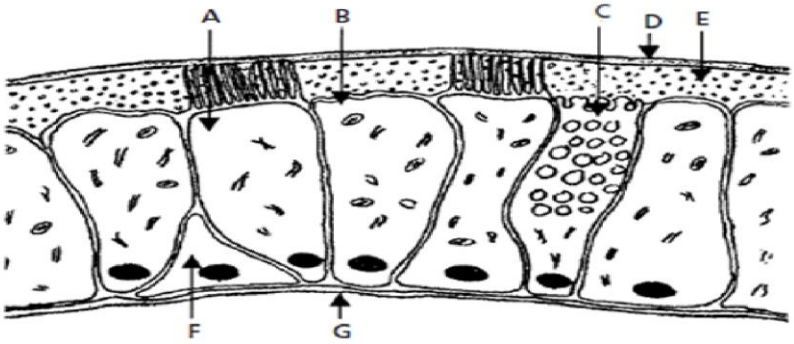

Figure 1.2: Cell types of the nasal epithelium with covering mucous layer showing ciliated cell (A), non-ciliated cell (B), goblet cells (C), mucous gel-layer (D), sol layer (E), basal cells (F) and basement membrane (G).

The main functions of the nose are to smell, humidify inhaled air and trigger immunological response to any antigens inhaled.

Table 1.4: Barriers to drug crossing the nasal membrane

Barriers in the nose	**Small Molecules (% loss)**	**High molecular weight molecules (% loss)**
Degradation	0-15	0-5
Clearance [a]	0-30	20-50
Deposition (anterior loss)	10-20	10-20
Health status and environment	10-20	10-40
Membrane permeability	0-30	20-50
Mucus layer	Less than or equal	Less than or equal

[a] Depends on excipient

[b] Depends on the characteristics of the drug e.g. partition coefficient, polarity etc.

1.7 NASAL ABSORPTION

The drug disappearance rate from the site of administration depends on the nasal cavity can be expressed by the following differential equation (see Figure 1.3).

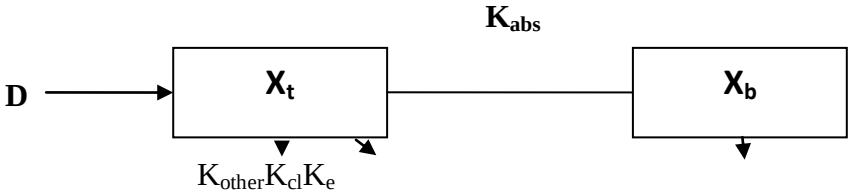

Figure 1.3: Disappearance from the absorption site in the nasal cavity

Where D=dose administered; K_{cl} =clearance rate constant; K_{other}=rate constant for the other process responsible for the decrease in the concentration; K_e=elimination rate constant; X_t=the amount of drug in the body; V_{max}=maximum velocity of enzymatic reaction; K_m=MichaelisMenten constant, X_b is amount of drug in brain

$$\frac{dX_t}{d_t} = -(K_{cl}+K_{abs}+K_{other})X_t - \frac{V_{max}X_t}{K_m+X_t}$$

The rate of change of drug levels in the body can be described by the following differential equation, assuming one compartmental model: $\frac{dX_b}{dt} = k_{abs}Xt - KeXb$

For the application of specific dose (D) ofthe drug in the nasal cavity, the plasma concentration can be estimated from the following equation.

Molecules that are cleared from the nasal cavity to the gastrointestinal tract (GI) by mucociliary clearance can be absorbed from the GI tract if not metabolized. This can be seen as a second peak in the plasma profile. Figure 1.4 shows a diagram expressing this additional absorption mechanism, where possible.

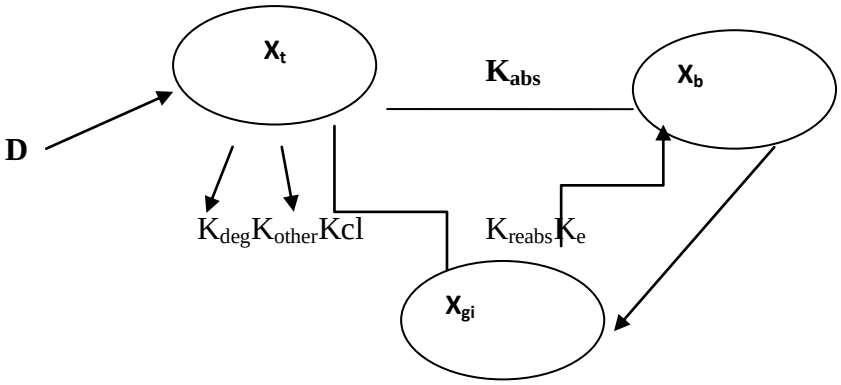

Figure 1.4: Diagram of disappearance from the absorption site in the nasal cavity assuming subsequent absorption from the GI tract

D = dose administered; X_t=the amount of drug at the absorption site at any given time; X_b the amount of drug in the body; X_{gi} = the amount of drug in the GI tract; K_{cl}= the clearance rate constant; K_{deg} = the rate of enzymatic degradation; K_{abs}= the systemic absorption rate constant; K_{other}=other factors that decrease the absorption rate; K_c = the elimination rate constant; and K_{reabs}= the gastrointestinal absorption rate constant.

1.8 MUCUS AND MUCOCILIARY CLEARANCE

The net amount of drug absorbed through the nasal route is dependent on mucociliaryclearance. The turnaround time of mucus flow determines the half-life of the drug within the nasal cavity. Various environmental factors such as humidity, temperature, toxins and airborne chemicals and many pharmaceutical excipients can alter the mucous flow rates. Although ciliary beating is the main force in mucociliary clearance, there is also the influence of mucus secretion, swallowing, inhaling and severity. The absorption process should be rapid, preferably within the first 15 minutes after administration (23).

1.9 NASAL ENZYMATIC DEGRADATION

The existence of different proteolytic enzymes in the nasal fluid was demonstrated. Numerous reports describe the importance of inhibiting the proteolytic degradation of insulin, for example through the nasal mucosa, caused by various enzymes or bacteria present in the mucus (23).

1.10 IMMUNOLOGY

Immune reaction is triggered when an antigen is present nasal cavity andantibodiesare generated and transported through the mucous membrane in considerable amounts to be found in high concentrations in the mucus layer. Any immune response to administered substances such as insulin or pharmaceutical excipients such as albumin is undesirable (23).Hence,it's an important parameter to screen excipients as well active moieties to be delivered through this route.

1.11 BLOOD FLOW

The nasal mucosa is highly perfused with blood flow rates greater than the upper respiratory tract,muscle,brain, and liver. These characteristics make the nose an interesting organ for the absorption of drugs. The extensive network of blood capillaries under the nasal mucosa and a large amount of blood flowing through this zone facilitate effective systemic absorption of drugs (23).

1.12 DEPOSITION

The drug delivery dosage form, as well as the device, determines the deposition of an applied drug. The droplet size distribution, plume geometry of the nasal spray, viscosity and bioadhesive properties are important parameters for nasal deposition. For any particle administered with a velocity q, which is pumped to a stream of air moving at a velocity u, directed at an angle θ, the resistance to particle motion is given by the Stokes'

Law and the stopping distance x can be calculated $$x = \frac{(u\sin\theta)(\rho_{\text{part}} - \rho_{\text{air}})d^2}{18\eta}$$

Where p_{part} and p_{Pair} are the density of the particle and air respectively, and d is the diameter of the particle.Recent studies investigating whether procedures for intranasal

administration or application to different regions of the nose affect the bioavailability of the drug have been inconclusive (23).

1.13 NASAL METABOLISM

The nasal cavities represent an important point of entry into the body of volatile xenobiotics, including carcinogens, present in the environment. Many authors have shown that respiratory and olfactory nasal membranes from a variety of mammalian species contain cytochrome P450 and are able to metabolize many chemicals (24-28). Recently, preliminary results have been published showing that human respiratory epithelium contains detectable levels of cytochrome P450 and associated mono-oxygenaseswhichacts on many chemical compounds particularly towards carcinogenic diethyl nitrosamine (26).

1.14 NOSE TO BRAIN TRANSPORT

Nose-to-brain transport hypothesized is through three pathways after nasal instillations: olfactory, trigeminal and trigeminal systemic pathways. The olfactory epithelium is located in the upper part of the nasal cavity and the drug can cross the olfactory region through neural or extra neuronal pathways and reach the cerebral parenchyma and the CSF. The trigeminal nerve pathways provide an additional route to transport the drug to brain tissues. Both olfactory and trigeminal pathways provide direct drug-to-brain delivery with extra-neuronal pathways that deliver the drug much faster than neural pathways.Nasal drugs could reach the brain / cerebrospinal fluid (CSF) through one or more transport mechanisms apart from thesystemic route (29).

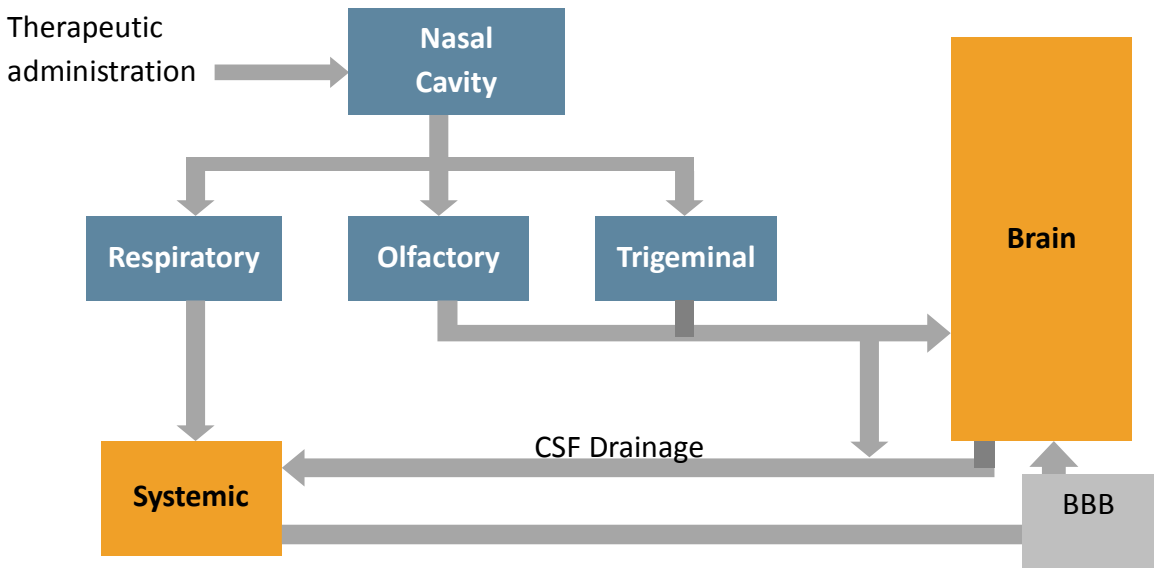

Figure 1.5:Different pathways for reaching the brain after intranasal administration

1.15 SOLID LIPID NANO-PARTICLES (SLN)

1.15.1 INTRODUCTION, ADVANTAGES

SLNs are colloidal particles consisting of a solid lipid composite matrix at room temperature and formulated as dispersion in an aqueous surfactant solution with a particle size distribution of 10-1000 nm(28). Both hydrophilic as well hydrophobic drugs can be encapsulated in SLN with GRAS listed excipients, reducing the risk of acute and chronic toxicity (29,30). Delivery of SLN throughtrans-nasal route poses challenges, including damage to the nasal mucosa through the frequent use of this route, rapid removal from the nasal cavity because ofmucociliary clearance mechanism, interference in absorption due to nasal congestion,and the possibility of partial degradation or irritation of the nasal mucosa (5,31).

In the brain, optimum targeting actions can be obtained because of the direct movement of the SLN particles from the sub-mucosal space of the nose into the CSF compartment of the brain (32). The highly permeable nasal epithelium allows rapid absorption of the SLN particles to the brain due to high blood flow, porous endothelial membrane, large surface area and avoidance of first pass metabolism.

Thus, SLNs can transport a wide variety of therapeutic agents (small molecules and macromolecules) to the CNS (33).

1.15.2 SLN TRANSPORT IN THE BODY

Nanoparticles located in the external environment of a cell can interact with the plasma membrane, which can lead to the uptake of these nanoparticles by the cells through a process called endocytosis. Understanding endocytic mechanisms are then crucial for the development of nanoparticles for clinical therapies. In addition, it has been shown that most of the nanoparticles exploit more than one pathway to obtain cell entry (34). The endocytosis of the nanoparticles also depends on the type of cells treated (35). Endocytosis is known as a general input mechanism for various extracellular materials and can be divided into two main categories: phagocytosis (uptake of large particles) and pinocytosis (uptake of fluids and solutes) (36,34). Phagocytosis is followed by specialized professional phagocytes, such as macrophages, monocytes or dendritic cells.

Pinocytosis, on the other hand, is present in all cell types and has multiple forms depending on the origin and function of the cell. Pinocytosis can be classified as clathrin-mediated endocytosis, caveolae-mediated endocytosis, clathrin-dependent endocytosis

and caveolaein and macropinocytosis (37).Endocytosis classification based on endocytosis proteins involving the initial entry of particles and solutes shown in Figure 1.6.

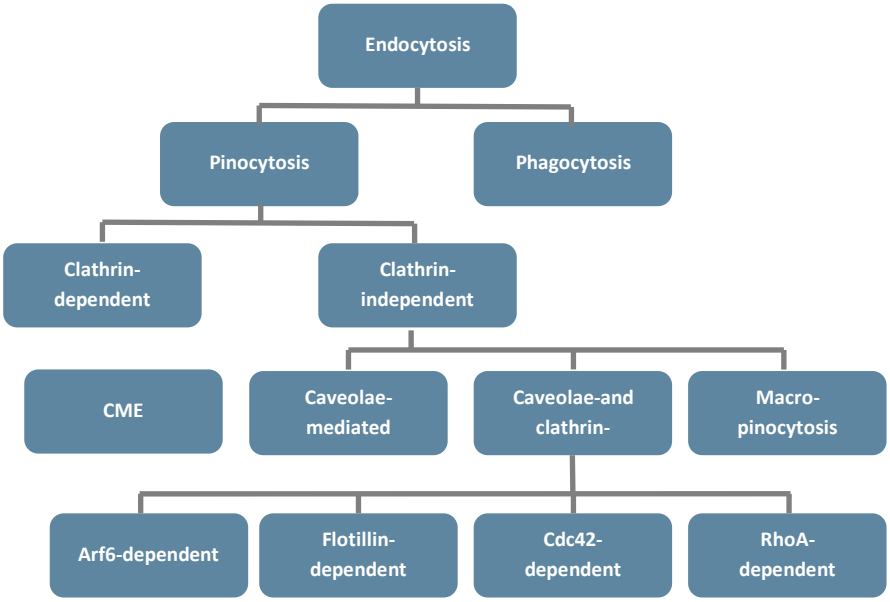

Figure 1.6:Classification of endocytosis based on endocytosis proteins that are involved in the initial entry of particles and solutes

Receptor-mediated endocytosis through clathrin-coated fossae is the most common pathway of endocytosis. Alternatively, clathrin-independent endocytosis may occur via the caveolae or lipid raft. Caveolae flask-shaped membrane invaginations on cell surfaces that have high amounts of cholesterol and sphingomyelin. Caveolae are abundant in muscle, endothelial cells, fibroblasts and adipocytes and absent in neurons and leukocytes (34, 37). At first sight, it is possible to internalize large particles with submicron and larger sizes in the cells, which lack phagocytosis (34).

1.16 SLN PREPARATION METHODS

SLN is used to control the release of the encapsulated drug, with average particle size of the colloidal system is 10 to 1000 nm, using natural or synthetic lipid lipids. The drug is trapped or incorporated into the lipid matrix to form the colloidal solid drug system.

1.16.1 GENERAL INGREDIENTS

General ingredients include solid lipids, emulsifier (s) and water. The term "lipid" is used in a broader sense and includes triglycerides (e.g.tristearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes. All classes

of emulsifiers (with respect to filler and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers can prevent particle agglomeration more efficiently.

A clear advantage of SLN is the fact that the lipid matrix is formed by physiological lipids which reduce the danger of acute and chronic toxicity. The choice of emulsifier depends on the route of administration and is more limited for parenteral administration.

1.16.2 METHODS

A) High shear homogenization and ultrasound

High shear homogenization techniques and ultrasound are used as dispersions for the production of solid lipid nano-dispersions. Both methods are scaleable. However, the quality of dispersion is often compromised by the presence of microparticles. Optimization of the various process parameters, including emulsification time, stirring speed and cooling conditions on particle size and zeta potential determines the encapsulation efficiency (38). In most cases, the average particle size was obtained in the 100-200 nm range of this study.

B) High-pressure homogenization

High-Pressure Homogenization (HPH) has become a reliable and robust SLN manufacturing technique. High-pressure homogenizerpumps the fluidic component under high pressure (100-2000 bar) through narrow space (within a few micrometers). The fluid accelerates to high speeds (more than 1000 km/h) thus creating cutting stresses and cavitation forces which result inthe creation of particles into the submicron range. Typical lipids are in the range of 5-10% and do not present any problem for the homogenizer. At even higher lipid concentrations (up to 40%) Lipid nanodispersions were homogenized (39). Two general approaches to the homogenization, hot and cold homogenization techniques, for SLN production (40) can be used. In both cases, the preparatory stage involves the introduction of a drug in the dissolution of the lipid mass or dispersion of the drug in the lipid melt.

C) Hot homogenization

The hot homogenization is carried out at temperatures above the melting point of the lipid and therefore can be treated as homogenization of the emulsion. The precipitate of the saturated fatty liquid and the aqueous phase of the emulsifier atthe same temperature

is obtained by means of a high shear mixing apparatus (Ultra-Turrax). The quality of the pre-emulsion significantly influences the quality of the final product and it is desirable to obtain droplets within the range of few micrometers. HPH in the pre-emulsion is carried out at temperatures above the melting point of the lipid. Generally, higher temperatures cause smaller particle sizes because of the reduced viscosity of the internal phase (41). However, high temperatures can also increase the degradation rate of the drug and vehicle. It is often observed that homogenization at high pressure increases the sample temperature (approximately 10°Cto500 bar) (42). In most cases, 3-5 cycles of homogenization are required at 500-1500 bar. Solid particles are expected to form by cooling the sample to room temperature or below. Due to the small particle size and the presence of emulsifiers, the lipid crystallization can be delayed and the sample can remain stable for several months (43).

D) Cold homogenization.

In contrast, the cold homogenization is carried out with the solid lipid and therefore represents a high-pressure milling of a slurry. Effective temperature control and regulation are required to ensure the non-milled state of the lipid due to the increase in temperature during homogenization(42). Homogenization has been developed to overcome the following problems of the hot homogenization technique:

1. Drug-induced temperature degradation
2. Distribution of the drug in the aqueous phase during homogenization
3. Complexity of the crystallization step of the nanoemulsion leading to various modifications and/orsuperfusion of the melts.

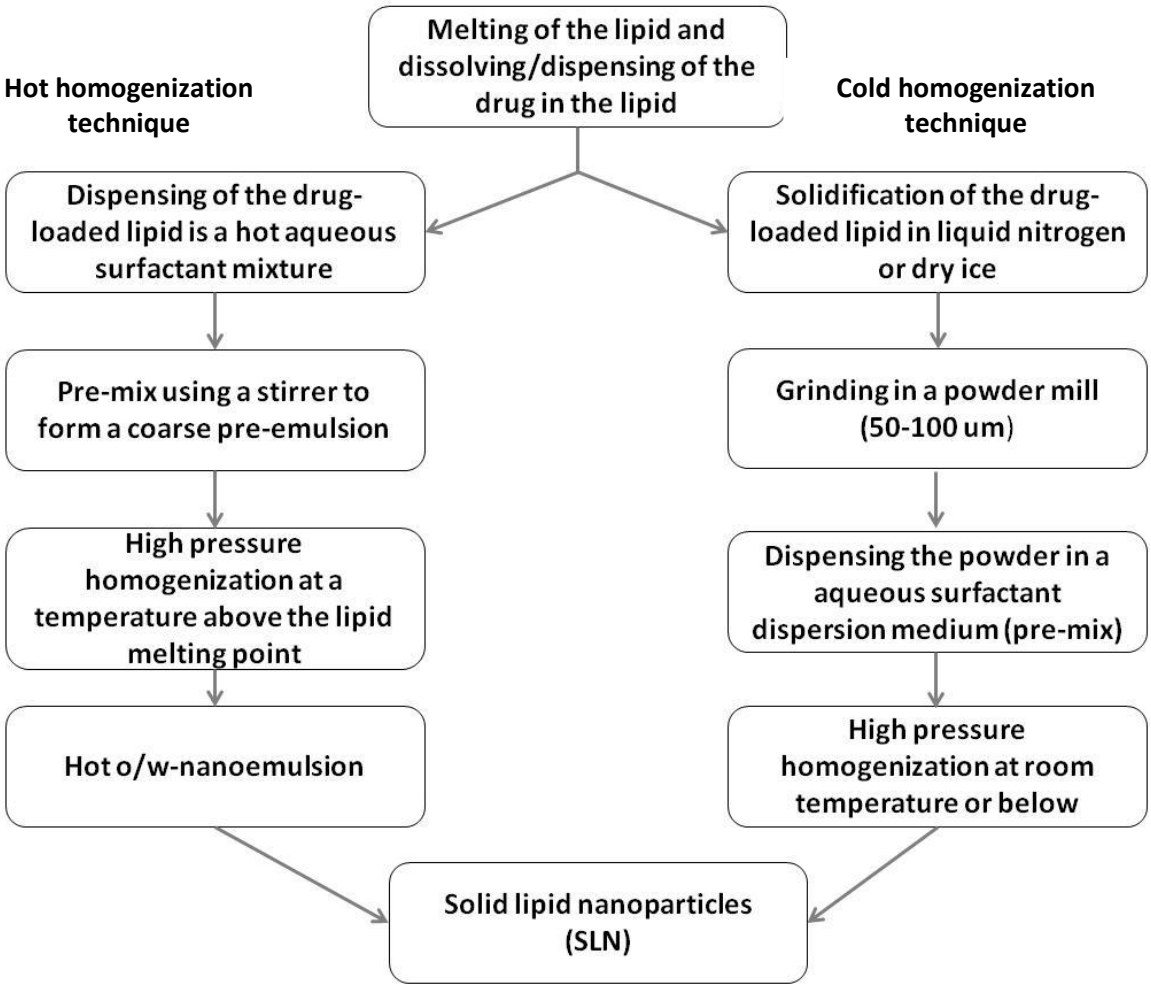

Figure 1.7: Schematic procedure of hot and cold homogenization techniques for SLN

Typical particle sizes obtained by means of beads or mortar are in the range of 50-100 micrometers. The low temperatures increase the lipid fragility and therefore favor the fragmentation of particles. The solid lipid microparticles are dispersed in a cooled emulsifier solution. The pre-suspension is subjected to high-pressure homogenization at or below ambient temperature. In general, in comparison with hot homogenization, larger particle sizes and a larger size distribution are observed in cold homogenized samples (40).

E) SLN prepared by solvent emulsification/evaporation

SjöströmandBergenståhl described a production method for preparing nanoparticle dispersions by precipitation in o / w emulsions (44). The lipophilic material is dissolved in a water immiscible organic solvent (for example cyclohexane) which is emulsified in an aqueous phase. After evaporation of the solvent, a dispersion of nanoparticles is formed by precipitation of the lipid in the aqueous medium. The mean particle diameter

obtained was 25 nm with cholesterol acetate as the model drug and using a lecithin/sodiumglycocholate mixture as the emulsifier (45). The advantage of this process over the above described cold homogenization process is the avoidance of any thermal stress. A clear disadvantage is the use of organic solvents.

F) Microemulsion based SLN preparations

Gasco and collaborators developed SLN preparation techniques that are based on the dilution of micro-emulsions. It is worth mentioning that there are different opinions in the scientific community on the structure and dynamics of the micro-emulsion. Recently, Moulik and Paul published an extensive review (46). Gasco and other scientists comprise micro-emulsions as two-phase systems composed of an inner and outer phase (e.g. o / w micro-emulsions). They are prepared by stirring an optically clear mixture at 65-70 ° C which is typically composed of a low melting point fatty acid (e.g., stearic acid), an emulsifier (e.g. polysorbate 20, polysorbate 60, soy phosphatidylcholine, salt Sodium taurodeoxycholic acid) Co-emulsifiers (e.g. butanol, sodiummonooctylphosphate) and water. The hot micro-emulsion isindispersed cold water (2-3 ° C) under stirring. Typical volume ratios of the hot micro-emulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the micro-emulsion. According to the literature (47, 48), the droplet structure is already contained in the micro-emulsion and therefore, energy is not required to achieve submicron particle sizes. Taking into accountmicro-emulsions, the temperature gradient and the pH value set the product quality in addition to the micro-emulsion composition. High-temperature gradients facilitate rapid crystallization of lipids and prevent aggregation (49, 50). Due to the dilution step, the achievable lipid contents are considerably lower compared to formulations based on HPH.

1.17 EVALUATION OF SOLID LIPID NANOPARTICLES

1.17.1 Defining the "goals"

An adequate characterization of the solid lipid nanoparticles is a necessity for the control of the quality of the product. Characterization methods must be sensitive to key SLN performance parameters and must avoid artifacts. A Laggner statement on lipids should always be taken into account (51): "Lipids and fats, as soft condensed material in general, are very complex systems, not only in their static structures but also with respect to their kinetics of supra-molecular formation hysteresis phenomena or overcooling can

seriously complicate the task of defining underlying structures and limits in a phase diagram. This is especially true for lipids in the colloidal size range. Many analytical tools do not allow direct measurement in the undiluted SLN dispersion. For example, exposure of an SLN dispersion to a syringe needle may result in a spontaneous transformation of the low viscosity SLN dispersion into a viscous gel. In this case, the artifact caused by the preparation of the sample is clearly visible, in other cases it will not be. The key factors that have a direct impact on stability and release kinetics are

a. Particle size and Zeta potential
b. The degree of crystallinity and lipid modification
c. Coexistence of additional colloidal structures (micelles, liposomes, super-cooled fusion, drug nanoparticles) and time scale of the distribution processes

1.17.2 Measurement of particle size and zeta potential

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine particle size measurements. The Coulter Counter method is rarely used to measure SLN particle size due to difficulties in evaluating small nanoparticles and the need for electrolytes that may destabilize colloidal dispersions. The development of PIDS (Polarization Intensity Differential Scattering) technology greatly increased the sensitivity of LD to smaller particles. However, despite this progress, it is recommended to use PCS and LD simultaneously. It should be noted that both methods do not measure particle size. Instead, they detect the light scattering effects that are used to calculate the particle size. For example, uncertainties may result from forms of non-spherical particles. Platelet structures commonly occur during lipid crystallization and have also been suggested in the SLN literature (52). In addition, difficulties may arise in both PCS and LD measurements for samples containing several populations of different sizes. Therefore, additional techniques may be useful. For example, optical microscopy is recommended, although it is not sensitive to the nanometer size range. It gives a quick indication of the presence and character of micro-particles (unit-shaped micro-particles or micro-particles consisting of smaller particle aggregates). Electron microscopy provides, in contrast to PCS and LD, direct information on the shape of the particles artifacts that may be caused by the preparation of the sample. For example, removal of the solvent may cause modifications that will influence the shape of the particle.

ZurMühlen demonstrated the ability of the AFM - especially operating in contactless mode - to visualize the morphological structure of solid lipid nanoparticles (38). The particle size displayed was of the same magnitude compared to the results of the PCS measurements. Dingler investigated Cetyl palmitate SLN (stabilized by polyglycerol methylglucosestearate, Tego Care 450® by electron microscopy and AFM (53, 54). Both methods suggest an almost spherical form of the particles. Westesen reported different forms of SLN, such as cubic or platelet-like patterns, for SLNs made from well defined, high purity lipids (e.g., pure triglycerides). The chemically homogeneous lipid tends to form more or less perfect crystals with the typical platelet-type pattern of β-modification. The use of chemically heterogeneous lipids in combination with heterogeneous surfactants favors the formation of ideally spherical lipid nanoparticles.

Measurement of the zeta potential predicts the storage stability of the colloidal dispersion. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electrical repulsion. However, this rule cannot be applied strictly to systems containing steric stabilizers, since the adsorption of steric stabilizers will decrease the zeta potential due to the change in the shear plane of the particle (55).

1.17.3 Measurement of crystallinity, lipid modification and assessment of alternative colloidal structures including the time scale of distribution processes

Characterization of the degree of lipid crystallinity and lipid modification is critical to estimatingrelease rates as well asto predict stability in long term storage conditions.It has been observed that polymorphic transitions can occur very slowly and that Dynasan® 112 SLN - if not artificially induced crystallization,can remain as a super-cooled melt for several months (41).

Differential scanning calorimetry (DSC) and X-ray diffraction are widely used to investigate lipid state. Infrared and Raman spectroscopy are useful tools for investigating the structural properties of lipids. Its potential to characterize SLN dispersions has not yet been explored.

1.18 AIMS AND OBJECTIVES OF THE CURRENT RESEARCH WORK

Tapentadol (used as HCl salt) is a centrally acting oral analgesic and widely used in the acute and chronic pain of diverse etiologies. Tapentadol when given orally, it undergoes extensive first pass metabolism, the main confoundingfactor for its low bioavailability (32%). About 97% of the parent compound, in the system, is metabolized. None of the metabolites contribute to the analgesic activity. Lipid solubility of Tapentadol is highwith logP value as 2.8, which indicates good BBB permeability. The maximum serum concentration of Tapentadol is typically observed at around 1.25 hours after oral dosing. The aim of the present study is to design formulations for improvement in systemic as well as brain availability with early $T_{max}$and higher C_{max}as compared to oral formulation. Thus, it was planned to design formulations for nasal delivery and do in vitro evaluation. Based on results of in vitro evaluation, pharmacokinetic and systemic availability studies were carried out inan animal model for selected two formulations and compared with oral route.

This work is a part of an industry sponsored project forthe designof anovel nasal formulation of suitable CNS selective drug for improved delivery tothe brain. In another work, development of liposomal and solid lipid nanoparticles (SLN) was prepared for direct delivery of dopamine to thebrain. The work suggested that SLN and liposomal formulations of dopamine made very high delivery of dopamine tothe brain. Based on those findings, it is planned to prepare novel systems for better delivery of Tapentadol tothe brain. (Ph.D. thesis of VibhuNagpal titled "Studies on Liposomal Delivery of Dopamine to brain via nasal route" 168, 2015, BITS, Pilani).

1.19 SCIENTIFIC HYPOTHESIS

Addressed problem: The main problems with nasal administration are rapid muco-cilliary clearance, which reduces the residence time of nasally applied dosage forms and the poor nasal permeability of many drugs.

Several alternative strategies have been employed to overcome these limitations. Bio-adhesive polymers, for example, can be used to achieve a long residence time in the nasal mucosa resulting in a higher concentration gradient and subsequent increase in drug absorption. As a part of the scope of the industry sponsored project,the same

research group studied liposomal delivery of candidate drug Dopamine for direct delivery to the brain via nasal route with positive outcome.

For analgesic drug, higher and early $C_{max}$and early T_{max} are desired but extended Plasma exposure is to be maintained to produce sufficient and extended pharmacodynamic effect. Therefore, to address this,the scope of current work is based on extending the plasma exposure of highly soluble analgesic drug through transient absorption or slow absorption via trans-mucosal absorption fromthe intranasal route for systemic availability and extended action.

Suitable formulations via nasal route using Tapentadol base, instead of the salt form was hypothesized to offerdistinctivebenefit in pain management owing to its moderate solubility as compared to the hydrochloride salt. In the present research, Tapentadol base in aqueous Gellan gum solution and Solid lipid nanoparticles nasal drug delivery systems were developed and evaluated for different physicochemical propertieslikeparticle size, shape,morphology,zeta-potential,in-vitro-release,encapsulation, efficiency,loadingefficiency.Further to evaluate the scientific objectives, the designed formulations were evaluated in-vivo bythe intranasal administration in NZ rabbits and the pharmacokinetic parameters were compared tothe oral solution.

1.20 NEED FOR A TAPENTADOL NASAL SPRAY

- Intravenous administration, in general, provides rapid action; Howsoever, the maximum effect of morphine with T_{max}ranging between 5 to10 minwith i.vNSAIDs, the peak effect is slow, with T_{max}ranging between 15 to 30 min. Rectal administration usually provides a faster, but unreliableaction, when compared to the oral route.
- Alternatively,marketed intranasal Fentanyl offers T_{max}ranging between 5 to 16 min with avery short half-life of 65 mins but treatment modalities are often complicated due to therisk of respiratory depression, hence cannot be administered without the healthcare staff.
- Thus, the use of semi-opioid analgesics with established safety and proven efficacy such as Tapentadol may be a beneficial alternative and amenable treatment option for acute pain management.The desirable attributes of the current work include:
    - Fast onset of action (early T_{max}) as compared to Oral solution.
    - Higher or comparable C_{max} and AUC to Oral solution.

- Non-invasive; No needle stick risks.
- No infection risk.
- Appropriate for chronic as well as acute break-through pain management.

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CHAPTER 2

DRUG PROFILE