# CHAPTER I

# INTRODUCTION

The systemic delivery of therapeutic agents such as peptide and protein drugs through the transmucosal route has several potential advantages including rapid absorption and avoidance of first-pass gastrointestinal and hepatic metabolism. Recently, the intranasal route has received a great deal of attention due to its added advantages of ease of administration and good patient acceptability.(Chandler, Illum and Thomas,1991a).

Nevertheless, the bioavailability of intranasal administration of peptide-based pharmaceuticals is often low because of their high molecular weight and hydrophilicity. For example, it has been reported that the average bioavailability of salmon calcitonin (sCT) in human obtained after intranasal administration was about 1.6 % (Lee et al., 1994).

Accordingly, nasal peptide delivery usually needs an absorption enhancer which facilitates the drugs to reach an effective absorption level from the nasal mucosa without causing local irritation. Unfortunately, little is known about potential side effects of these enhancers, which is a drawback for the clinical application .

The influence of the enhancers on the absorption of the drug across the nasal membrane is related to a direct effect on the drug such as solubilization and / or influence on the nasal membrane. The enhancers may act by alteration of the properties of the nasal membrane by opening the tight junctions between epithelial

cells or by increasing membrane fluidity, either by creating disorders in the phospholipid domain in the membrane or by facilitating the leaching of proteins and lipids from the membrane (Merkus et al., 1991). These mechanisms may somehow lead to the nasal mucosal irritation that may be evaluated by determining the amount of membrane proteins, phospholipids, intracellular enzymes (e.g. lactate dehydrogenase (LDH)) and membrane-bound enzymes (e.g. nucleotidase (5'ND)) that are released from the nasal epithelium (Shao, Krishnamoorthy and Mitra,1992 ; Marttin et al.,1995 ; Aspden , Illum and Skaugrud , 1996 ; Pujara et al .,1995).

Since, many enhancers may be associated with side effects such as irreversible changes in the nasal membrane, these materials are consequently unacceptable for chronic use in humans. Nevertheless, since the potential therapeutic benefits of nasal peptide drug delivery are enormous, there is considerable interest in finding novel nasal absorption enhancers that may be effective without evidence of topical or systemic toxicity following nasal administration.

The absorption enhancers that have been recently investigated included hydroxypropyl-β-cyclodextrin (HP-β-CD), dimethyl-β-cyclodextrin (DM-β-CD) lauroylcarnitine chloride (LCC) (Irie et al., 1992; Kagatani et al.,1996). Cyclodextrins are biocompatible cyclic oligosaccharides containing a minimum of 6 D-glycopyranose units attached by α-1-4 linkages and they were found able to increase the permeability of nasal mucosa and reduce proteolysis (Verhoef et al., 1994). Coadministration of 5% DM-β-CD resulted in considerable increase in nasal insulin absorption in rat, thus making DM-β-CD one of the most effective nasal absorption enhancers ever found (Shao, Krishnamoorthy and Mitra, 1992). During clinical studies up to 6 months no side effects in the women using these nasal DM-β-CD – estradiol / progesterone formulations twice daily have been reported

(Hermans et al., 1991). HP-β-CD is generally less effective than DM-β-CD. However, it was also found to be a very safe nasal permeation enhancer. It was found to result in only minimal release of epithelial membrane proteins and total absence of LDH activity (Shao and Mitra, 1992) Also, it has been reported recently that LCC, the surfactant, had a very strong enhancing effect on sCT nasal absorption in rat at the concentration of 0.1 % w/v(Kagatani et al., 1996). In the preliminary study of this thesis, it was found that 0.5%w/v of both chitosans and 0.1%w/v LCC caused cellular damage less than caused by 5%w/v DM-β-CD.

Chitosan, one of the interesting novel enhancers, is a cationic polysaccharide derived from naturally occurring chitin in crab and shrimps shells by deacetylation. The chitosans, especially the free amine form (CS J pH 4.0) and (CS G pH 6.0), were recently shown to greatly enhance the absorption of dipeptide ( L-Tyr-D-Arg ) across the rat nasal mucosa (Sahamethapat, 1996). At 1% w/v, CS J and CS G could increase the nasal bioavailability of salmon calcitonin in rat by two folds relative to that of the control group (Sinswat, 1997). Further, the chitosans caused no membrane or cellular damages in a rat nasal perfusion model (Aspden et al., 1996) , with only a transient decrease in mucociliary transport velocity as observed in frog palate model (Aspden et al., 1995). The 0.25 %w/v solution of the chitosan applied to human nasal tissues for 7 days had no effect on mucociliary transport rates or nasal histology in healthy human volunteers (Aspden et al., 1997) and it was much less toxic to the nasal membrane than 1% w/v Laureth 9, a nonionic surfactant (Aspden et al., 1996). Therefore, chitosans appear to have a very strong potential for further testing in humans.

Since the large interspecies differences appear to exist in the nasal absorption of drugs. For example, insulin/ DM-β-CD solution was ineffective in enhancing nasal

insulin absorption in rabbits and men, whereas studies in rats showed a very good nasal bioavailability of about 70-100% (Verhoef et al., 1994). thus, it is difficult to extrapolate the nasal absorption results obtained from a particular animal study to man, and it is advised to perform human experiment at an early to confirm that the particular enhancer is, in fact, safe and effective for human application. Animal studies will then remain useful for the fast screening of various efficacy of nasal drug formulations as well as for the subsequent optimization of the formulations once the effectiveness in man and appropriate correlation between human and animal data have been established (Verhoef et al., 1994).

The chitosans (CS J and CS G), cyclodextrins (DM-β-CD and HP-β-CD) and alkylcarnitine (LCC) used in this study have been separately evaluated for their safety and efficacy (Sahamethapat, 1996; Shoa and Mitra, 1992; Kagatani et al., 1996). However, they have never been compared together in the same study with respect to their effects on the nasal mucosal membrane. Thus, it would be interesting to compare them together in an attempt to evaluate their relative safety and absorption enhancing efficacy based on their effects on the reversibility of the rat nasal mucosa. Chitosan and one of the above absorption enhancers which had demonstrated reassuring safety and efficacy from the first part would then be selected for further study to confirm their absorption enhancing effects in human subjects.

Therefore, the objectives of this thesis were as follow.

1. To compare the relative safety and efficacy among three different types of novel nasal absorption enhancers, i.e. chitosans (CS G, CS J), cyclodextrins (HP-β-CD, DM-β-CD) and alkylcarnitine ( LCC) by the reversibility evaluation of rat nasal membrane permeability using *in situ* nasal perfusion technique.

2. To confirm the efficacy of the chitosans as nasal absorption enhancer of peptide in humans using salmon calcitonin as a model and compare the results with one of the above enhancers selected from 1 based on their relative safety and efficacy profiles.