CHAPTER V

CONCLUSIONS

Recently, biological active macromolecules have become increasingly important as a new class of therapeutic agents. Most of them are peptides and their derivatives such as insulin, calcitonin, interferons, vasopressin, growth hormone and its analogs etc. Since these peptides are mostly destroyed by the acids and enzymes in the gastrointestinal tract, they cannot be administered orally. Currently, the only effective route of administration of these drugs is by parenteral administration, e.g. intravenous, subcutaneous or intramuscular injection. However, the parenteral routes suffer several serious drawbacks such as patient compliance, high risk of overdose, infection and local thrombophlebitis as a result of its invasive nature of administration. Non-oral, non- parenteral routes of administration such as nasal, buccal, rectal, pulmonary and transdermal routes have received greater attention as possible alternatives. Since these peptides are poorly absorbed across the nasal mucosa, the use of absorption promoters seem necessary to effect significant absorption.

Salmon calcitonin (sCT) is a polypeptide hormone that plays a vital role in calcium homeostasis. This calcium-regulating hormone secreted from the thyroid gland plays an important role in the physiological regulation of calcium concentration in plasma. It is currently used in the treatment of Paget's disease, postmenopausal osteoporosis, and malignant hypercalcemia. Due to the chronic nature of the disease for which sCT is used, a nasally administered dosage form would increase the clinical utility

of sCT and improved patient compliance. The bioavailability of sCT alone following nasal administration, however, is lower than that by injection. In this study, the in vivo efficacy of chitosans as nasal absorption enhancer of sCT was assessed by measuring the hypocalcemic effect and serum sCT concentration. The results demonstrated the potential of chitosans as nasal absorption enhancer of sCT.

The results from this study can be summarized as follows :

1. The in vivo efficacy of chitosans, either in the free amine (CS J) or salt form (CS G), as nasal absorption enhancer of sCT was assessed by measuring the hypocalcemic effect and plasma sCT concentration. The results demonstrated that both chitosans possessed significant nasal absorption enhancing activity for a poorly absorbed drug like salmon calcitonin.

2. The enhancing effect of CS J appeared to depend on pH, with increasing adjuvant activity as the pH of the preparation was decreased. This is in accordance with the ability of the free amine chitosan to ionize, hydrate and dissolve better in the more acidic condition which may lead to more elongated shape and better contact with the nasal epithelium. The pH for the optimum enhancing activities of CS J was pH 4.0. Although CS J at pH 3.0 also good exhibition nasal absorption, the buffer used may be too acidic for the nasal mucosa and may have direct deleterious effects on the membrane.

3. The enhancing effect of CS G, on the other hand, appeared to be optimum at higher pH. This could be due to the nature of CS G which exists as a soluble glutamate salt, a chemical form which may facilitate swelling and enable the chitosan molecule to maintain the high charge density, a property believed to be essential for its enhancing activity, even at high pH values. The optimal pH for CS G appeared to be 6.0 since the highest absorption enhancement of sCT was observed at this pH. However, the reasons as to opposite ranking results to that of CS J are not clearly known at present.

4. At their respective optimal pH (CS J at pH 4.0 and CS G at pH 6.0), the enhancing activity of both CS J and CS G was found to be concentration-dependent, in the range of 0.25-1.0% w/v. Beyond 1.0% w/v, the enhancing effect appeared to be saturated since the sCT absorption at 1.25% enhancer concentration was similar to 1.0%. Significant absorption enhancement was observed at concentration as low as 0.25 % w/v. ($p < 0.05$) The results thus demonstrated that chitosans were very effective even at a very low concentration.

5. The ranking order of % sCT absorption, as indicated by %D in a decreasing order was 1% w/v CS J pH 4.0 (9.85 ± 1.89) .5% w/v DM-β-CD pH 7.4 (9.68 ± 0.31) > 1% w/v CS G pH 6.0 (8.43 ± 0.67) > 5% w/v HP β-CD pH 7.4 (8.05 ± 0.46). Using specific RIA, the absolute bioavailability of sCT after comparison with intravenous administration was determined to be 2.45, 1.91 and 1.22% for 1% CS J, 5% DM-β-CD and control group (intranasal sCT alone), respectively. Although absolute nasal bioavailability seemed to be low when compared to the intravenous

administration, the inclusion of 1% w/v CS J resulted two-fold increase in the AUC_{0-180} of plasma sCT relative to that of the control group (intranasal administration of sCT without enhancers). This equivalent to 201.18% relative bioavailability when compared to the control group at the same nasal dose (10 IU/Kg). Addition of 5% w/v DM-β-CD also led to the relative nasal bioavailability of 156.8% or 1.56 fold increase in absorption over the control with CS J. All the enhancers showed significant absorption enhancement ($p < 0.05$) with the highest effect observed with CS J.

6. In order to estimate further the inhibitory effect of chitosans on proteolytic enzymes, the activities of trypsin and leucine aminopeptidase, two major proteolytic enzymes found in the nasal mucosa, were investigated in vitro. The results show that both CS J and CS G did not have any pronounced inhibitory effects on these enzymes as opposed to aprotinin and bestatin, their respective specific inhibitors. As a result, the nasal absorption enhancement of chitosans may not involve protection of the peptide drug against proteolytic degradation by enzymes in the nasal cavity but it may involve the direct effect of chitosans on the mucosal permeability.

7. In conclusion, the cationic polysaccharide chitosans like CS J and CS G demonstrated significant nasal absorption enhancing activities even at very low concentration and may have promising potential for use as absorption enhancer in sCT nasal formulations.