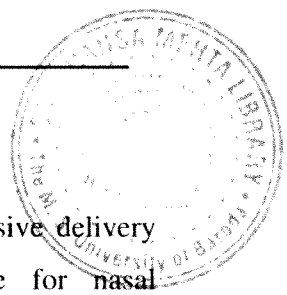
Chapter 11
Summary & Conclusion



SUMMARY AND CONCLUSION

The present investigation was aimed at the development of mucoadhesive delivery systems of the cardiovascular drugs, carvedilol and nitrendipine for nasal administration. Microparticulate drug delivery systems using chitosan and sodium alginate were prepared and optimized.

It was envisaged that the proposed delivery systems will overcome the first pass metabolism and result in increased bioavailability of the drugs. The mucoadhesive microspheres were formulated to prolong the residence time in the nasal cavity. This would permit the microspheres to adhere on the nasal mucosa and control (decrease) the rate of clearance from the nasal cavity.

11.1 Carvedilol

Chitosan and alginate microspheres of carvedilol were prepared by emulsification – cross linking method using gluteraldehyde (25% v/v) and calcium chloride as cross linking agent respectively. The microspheres were optimized for various parameters like drug : polymer ratio, concentration of cross linking agent and time of cross linking.

The microspheres were evaluated for particle size, surface morphology, flow properties, entrapment efficiency, in vitro mucoadhesion, in vitro drug release, DSC, XRD, histology and stability studies.

The **particle size** of chitosan microspheres was 20.82–49.26 µm and for alginate microspheres it was 26.36–54.32 µm, which is favorable for intranasal absorption.

The **surface morphology** revealed that the microspheres were non aggregated, free flowing powders with spherical shape and smooth surface. However, one noticeable characteristic of the cross linked chitosan microspheres was their yellow to brownish color.

The microspheres showed reasonably good **flow potential** as the values of angle of repose were in the range of 28.81^0 to 33.32^0 for chitosan microspheres and 26.38^0 to

34.68^0 for alginate microspheres. The compressibility index was 15.33 to 18.89 for chitosan microspheres and 14.39 to 19.24 for alginate microspheres.

The % **entrapment efficiency** was found to be in the range between 42.62 and 67.24 for chitosan microspheres and between 36.62 and 56.18 for alginate microspheres. The porous nature of the alginate microspheres could be responsible for the leaching of the drug during the washing process and could explain the lower encapsulation efficiencies than the chitosan microspheres. The % entrapment efficiency was found to be proportional to drug loading. The formulations loaded with higher amount of drug exhibited higher entrapment efficiencies. The entrapment efficiency, however, showed an inverse relationship with increasing amount of cross linking agent and cross-linking time. This may be due to an increase in cross – link density that will thereby reduce the free spaces within the polymer matrix and hence lead to reduced entrapment efficiency.

The **mucoadhesive property** of chitosan microspheres was assessed by mucus glycoprotein assay using the suspension of chitosan microspheres in different amounts of mucin (Type III) in aqueous solutions at room temperature. The data obtained from **adsorption studies** were fitted to Freundlich and Langmuir equations. It was found that the values of R^2 were significantly higher (P <0.05) for the Langmuir equation as compared to the Freundlich equation. This explains a more specific adsorption process where electrostatic interaction is involved. The adsorption of mucin to chitosan is expected to be dominated by the electrostatic attraction between the positively charged chitosan (containing amino groups) and negatively charged mucin (containing ionized sialic acid). As the mucin concentration was increased, the amount of mucin adsorbed increased.

The **in vitro mucoadhesion** showed that all the batches had mucoadhesive property ranging from 74.72±1.54 to 88.56±1.74 % for chitosan microspheres and 69.25 to 85.28% for alginate microspheres. The slightly higher values for chitosan microparticles indicated strong interaction between chitosan microspheres and mucous glycoprotein and/or mucosal surfaces as compared to alginate microspheres as reported earlier. The results showed that with increasing polymer ratio, higher mucoadhesion percentages were obtained. This could be attributed to the availability of higher amount of polymer for interaction with mucus. The percent in vitro

mucoadhesion was found to decrease slightly with increase in the amount of cross linking agent and cross linking time.

The **in vitro drug release** pattern in pH 6.2 phosphate buffer showed a moderate and controlled release following near zero order release for both chitosan and alginate microspheres. All batches released around 70% of drug in 8 hour for chitosan microspheres and around 80 – 85% of drug for alginate microspheres. The porous structure of alginate microspheres formed by gelation of sodium alginate with Ca^{2+} could explain this fast release pattern of encapsulated carvedilol as compared to chitosan microspheres. Another reason for faster release of carvedilol could be attributed to the removal of the cross-linker bivalent cation, calcium, from the alginate microspheres by monovalent cations, such as sodium or potassium contained in phosphate buffer. These ion exchanges could cause the erosion of the microspheres and therefore the fast release of the drug. The values of n for all the batches ranged from 0.74 to 0.84 for chitosan microspheres and from 0.60 to 0.75 for alginate microspheres with correlation coefficient close to 0.99, indicating non-Fickian or anomalous type of transport.

Differential scanning calorimetry (**DSC**) results indicated a molecular level dispersion of carvedilol in the microspheres, as no characteristic drug peak at 122 ^{0}C was observed in case of both chitosan and alginate microspheres loaded with carvedilol. X-ray diffraction (**XRD**) studies indicated that drug particles were dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drugs was observed in the drug loaded microspheres as no characteristic peaks at 2θ of 11.18^0, 12.80^0, 13.48^0, 15.06^0, 17.38^0, 18.29^0, 20.15^0, 24.18^0 and 26.02^0 were observed due to the amorphous nature of CRV.

In **histology studies** using sheep nasal mucosa, sections of sample mucosa treated with chitosan microspheres showed slight modification to the epithelial layer because of chitosan which is characterized by absorption enhancing effects, as it improves the paracellular transport by opening the tight junctions in the epithelial layer. It can also be assumed that the slight change in epithelial layer may be due to retention of drug on mucosa. Nasal mucosa treated with alginate microspheres showed negligible

changes as neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed.

Particle size and drug content values after 1, 2 and 3 months at 40^0C/75% RH showed no significant differences ($p>0.05$) indicating a stable formulation.

Performance evaluation of MIAT® nasal monodose insufflator was carried out for reproducibility of dose delivered by filling accurately weighed optimized microsphere formulations into a No. 3 gelatin capsule. The appearance of the puffs during the delivery of microsphere powder formulations from the MIAT® nasal insufflator were recorded by means of a video camera and the image analysis was carried out. We determined that after the second puffing more than 95% of the content was delivered for all the formulations with 5, 10 and 20 mg loading. After three puffs, almost the entire amount of powder was delivered for all the microsphere formulations. The images of microsphere powder clouds demonstrated that microspheres were delivered forming an elongated puff. The core of the clouds was homogeneous which can be expected to provide effective distribution pattern upon intranasal administration.

The **gamma scintigraphy** was carried out to study the nasal clearance characteristics of two microsphere drug delivery systems. Lactose powder was used as negative control. The results showed that the control lactose powder was cleared rapidly (half-life of nasal clearance was less than 1.0 h), whereas the mucoadhesive delivery systems were retained within the nasal cavity for extended periods of time (half-lives of nasal clearance were >2.5 h). Among microsphere formulations studied, the lowest clearance rate and highest mucoadhesion was shown by chitosan microspheres followed by alginate microspheres. After 4 h, 53.86% of chitosan and 61.55% of alginate microspheres have been cleared from nasal cavity while in the same time 87.36% lactose powder cleared, respectively.

Pharmacokinetic study of chitosan and alginate microspheres containing Carvedilol in blood displayed an increase in AUC and hence relative bioavailability when compared with intravenous administration of drug. The C_{max} values observed after intranasal administration of chitosan and alginate microspheres were 78.79±4.23 KCPM/gm and 64.85±4.15 KCPM/gm respectively. The AUC after intranasal administration of chitosan and alginate microspheres were about 236.59±21.68

KCPM.h/gm and 215.83±18.56 KCPM.h/gm respectively which was statistically not significant ($p>0.05$). The MRT was considerably increased following nasal administration of the mucoadhesive formulations of carvedilol (chitosan and alginate microspheres) as compared to IV administration. The average MRT values after nasal administration of chitosan and alginate were 4.31±1.02 and 4.37±1.31 h, respectively, as compared to 2.18 ± 0.78 h after IV administration of Carvedilol which were significantly different ($p<0.05$). Between the two mucoadhesive formulations i.e. chitosan and alginate microspheres, the difference was non significant ($p>0.05$) indicating that there was no formulation variation. The relative bioavailability for mucoadhesive chitosan and alginate microspheres were 74.39% and 67.87% respectively which indicate that nasal administration results in improved absorption of carvedilol.

11.2 Nitrendipine

Nitrendipine loaded chitosan and alginate microspheres were prepared by emulsification – cross linking method using gluteraldehyde (25% v/v) and calcium chloride as cross linking agent respectively. The optimization of microsphere formulations was carried out by studying different variables like drug: polymer ratio, concentration of cross linking agent and time of cross linking.

The Nitrendipine loaded chitosan and alginate microspheres were characterized for particle size, surface morphology, flow properties, entrapment efficiency, in vitro mucoadhesion, in vitro drug release, DSC, XRD, histology and stability studies.

The **particle size** of chitosan microspheres was in the range of 26.12 – 48.65 μm and for alginate microspheres it was in the range of 22.68 – 48.95 μm which is favorable for intranasal absorption. The size of the microspheres was found to increase with an increase in drug loading. Similar increase in the size of alginate microspheres was also observed with increase in calcium chloride concentration as well as cross-linking time. The addition of higher amount of Ca^{2+} will result in relatively more cross-linking of the guluronic acid units of sodium alginate, thereby leading to formation of larger microspheres.

The **surface morphology** study by SEM indicated that the chitosan and alginate microspheres were non aggregated, free flowing powders with spherical shape and smooth surface. However, one noticeable characteristic of the cross linked chitosan microspheres was their yellow to brownish color while alginate microspheres where white in colour.

The results obtained for **flow properties** of the microspheres showed reasonably good flow potential as the values of angle of repose were in the range of 25.24^0 to 34.56^0 for chitosan microspheres and 25.41^0 to 35.62^0 for alginate microspheres. The values of compressibility index which were in the range 15.24 to 19.67 for chitosan microspheres and 14.39 to 19.24 for alginate microspheres.

The % **entrapment efficiency** was found to be in the range between 46.63 and 69.37 for chitosan microspheres and between 42.12 and 63.42 for alginate microspheres. These lower entrapment efficiencies of alginate microspheres of nitrendipine could be attributed to the porous nature of the alginate microspheres. The formulations loaded with higher amount of drug exhibited higher entrapment efficiencies. The % entrapment efficiency was found to be proportional to drug loading. The entrapment efficiency, however, showed an inverse relationship with increasing amount of cross linking agent and cross-linking time. This may be due to an increase in cross - link density that will thereby reduce the free spaces within the polymer matrix and hence lead to reduced entrapment efficiency.

The **mucoadhesive property** of nitrendipine loaded chitosan microspheres was assessed by the suspension of chitosan microspheres in different amounts of mucin (Type III) in aqueous solutions at room temperature. The data obtained from **adsorption studies** were fitted to Freundlich and Langmuir equations. It was found that the values of R^2 were significantly higher ($P < 0.05$) for the Langmuir equation as compared to the Freundlich equation. This explains a more specific adsorption process where electrostatic interaction is involved. The adsorption of mucin to chitosan is expected to be dominated by the electrostatic attraction between the positively charged chitosan (containing amino groups) and negatively charged mucin (containing ionized sialic acid). As the mucin concentration was increased, the amount of mucin adsorbed increased as the chitosan microspheres have the ability to

adsorb mucin. Thus, these studies indicated that mucoadhesion may increase the residence time of the formulation in the nasal cavity.

The **in vitro mucoadhesion** showed that all the batches had mucoadhesive property ranging from 75.04 to 87.48% for chitosan microspheres and ranging from 69.24 to 83.67% for alginate microspheres. The results showed that with increasing polymer amount, higher mucoadhesion percentages were obtained. This could be attributed to the availability of higher amount of polymer for interaction with mucus. The percent in vitro mucoadhesion was found to decrease slightly with increase in the amount of cross linking agent and cross linking time.

The **in vitro drug release** in pH 6.2 phosphate buffer showed a moderate and controlled release following near zero order release for chitosan microspheres and a slow and controlled release phase resulting from the controlled diffusion of entrapped drug following zero order and Higuchi type of release. When the microspheres come in contact with the buffer solution, they swell by absorbing water into their matrix and form a gel diffusion layer. This layer hinders the outward transport of the drug producing a diffusion controlled release effect as well as for alginate microspheres. All batches released around 70 - 75% of drug in 8 hour for chitosan microsphere and around 80 – 90% of drug for alginate microspheres. This faster release of nitrendipine from alginate microspheres may be due to the porous structure formed by gelation of sodium alginate with Ca^{2+}. The removal of the cross-linker bivalent cation, calcium, from the alginate microspheres by monovalent cations, such as sodium or potassium contained in phosphate buffer and could cause the erosion of the microspheres resulting to the faster release of nitrendipine. The values of n for all the batches ranged from 0.50 to 0.58 for chitosan microspheres and from 0.56 to 0.73 for alginate microspheres with correlation coefficient close to 0.99, indicating non-Fickian or anomalous type of transport. Non- Fickian release is described by two mechanisms: a combination of drug diffusion and polymer relaxation.

Differential scanning calorimetry (**DSC**) results indicated a molecular level dispersion of nitrendipine in the microspheres, as no characteristic drug peak at 159 ^{0}C was observed in case of both chitosan and alginate microspheres loaded with nitrendipine. X-ray diffraction (**XRD**) studies indicated that drug is dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drugs was

observed in the drug loaded chitosan and alginate microspheres as no characteristic peaks at 2θ of 9.99^0, 11.33^0, 13.09^0, 23.80^0, 24.30^0, 27.45^0 and 28.70^0 were observed due to the amorphous nature of NTD.

In **histology studies** using sheep nasal mucosa, a slight modification to the epithelial layer was observed in sections of sample mucosa treated with nitrendipine loaded chitosan microspheres because of chitosan which is characterized by absorption enhancing effects, as it improves the paracellular transport by opening the tight junctions in the epithelial layer. It can also be assumed that the slight change in epithelial layer was may be due to retention of drug on mucosa. Nasal mucosa treated with alginate microspheres showed no changes as neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed.

Particle size and drug content values after 1, 2 and 3 months at 40^0C/75% RH showed no significant differences ($p>0.05$) indicating a stable formulation.

Performance evaluation of MIAT® nasal monodose insufflator was carried out for reproducibility of dose delivered by filling accurately weighed optimized microsphere formulations into a No. 3 gelatin capsule similar as carvedilol formulations. After three puffs, almost the entire amount of powder was delivered for all the microsphere formulations. The images of microsphere powder clouds demonstrated that microspheres were delivered forming an elongated puff. The core of the clouds was homogeneous which can be expected to provide effective distribution pattern upon intranasal administration.

The **gamma scintigraphy** was carried out to study the nasal clearance characteristics of two microsphere drug delivery systems of nitrendipine. Lactose powder was used as negative control. The results showed that the control lactose powder was cleared rapidly (half-life of nasal clearance was less than 1.0 h), whereas the mucoadhesive delivery systems were retained within the nasal cavity for extended periods of time (half-lives of nasal clearance were >2.5 h). Among microsphere formulations studied, the lowest clearance rate and highest mucoadhesion was shown by chitosan microspheres followed by alginate microspheres. After 4 h, 56.08% of chitosan and 64.76% of alginate microspheres have been cleared from nasal cavity while in the same time 87.36% lactose powder cleared, respectively.

Pharmacokinetic study of nitrendipine loaded chitosan and alginate microspheres in blood of rabbits displayed an increase in AUC after nasal administration and hence relative bioavailability when compared with intravenous administration of drug. The C_{max} values observed after intranasal administration of CHNT and ALNT were 86.49±5.27 KCPM/gm and 76.85±4.95 KCPM/gm respectively. The AUC after intranasal administration of CHNT and ALNT were about 337.05±34.81 KCPM.h/gm and 314.41±29.22 KCPM.h/gm respectively which was statistically non significant ($p>0.05$). T_{max} values were 1.0 and 2.0 h for nasal administration of CHNT and ALNT. The average $T_{1/2}$ values were 3.34±1.18 and 3.01±0.86 h for CHNT and ALNT, respectively, as compared to 2.60±0.54 h following IV administration of NTD. The MRT was considerably increased following nasal administration of the mucoadhesive formulations of nitrendipine (CHNT and ALNT) as compared to IV administration. The average MRT values after nasal administration of CHNT and ALNT were 5.27±0.93 and 5.29±1.24 h, respectively, as compared to 3.36±0.96 h after IV administration of NTD and they were significantly different ($p<0.05$) from IV. Between the two mucoadhesive formulations i.e. CHNT and ALNT, the difference was non significant ($p>0.05$) indicating that there was no formulation variation. The relative bioavailability for mucoadhesive chitosan and alginate microspheres were 68.94% and 64.31% respectively which indicate that nasal administration results in improved absorption of nitrendipine.

Conclusion

In the present investigation, mucoadhesive microparticles of two cardiovascular drugs, carvedilol and nitrendipine were prepared using chitosan and sodium alginate for nasal delivery. In the in vivo study, the relative bioavailability of carvedilol loaded chitosan and alginate microspheres was 74.39% and 67.87% respectively. Similarly, the relative bioavailability of nitrendipine loaded chitosan and alginate microspheres was 68.94% and 64.31% respectively. The gamma scintigraphy images showed that the microsphere formulations were spread over a wide area within the nasal cavity of rabbits and the results indicated that the microspheres cleared slowly and were retained for longer time in the nasal cavity, thereby providing sustained and enhanced drug absorption from the nasal mucosa, as confirmed from pharmacokinetic studies.

Histology studies revealed that the microsphere formulations seem to be safe with respect to nasal administration.

The results of the present investigations conclusively indicate the enhancement in bioavailability of the drugs, Carvedilol and Nitrendipine, when administered as microspheres through nasal route. Hence the developed mucoadhesive microspheres of Carvedilol and Nitrendipine can be potentially useful in clinical treatment of hypertension and angina pectoris. Thus, these formulations hold promise as better alternative to the conventional dosage forms. However, further investigations in human beings under clinical conditions are necessary before they can be fully exploited.