SECTION V
Summary and conclusion

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SUMMARY AND CONCLUSION

Migraine is a common, chronic, incapacitating neurovascular disorder, characterized by attacks of severe headache, autonomic nervous system dysfunction, and in some patients, an aura involving neurologic symptoms. A recent survey by the World Health Organization (WHO) rates severe migraine, along with quadriplegia, psychosis, and dementia, as one of the most disabling chronic disorders. This ranking suggests that in the judgment of the WHO, a day with severe migraine is as disabling as a day with quadriplegia. It is suggested that may be a 'migraine generator', is possibly located in the brain stem Ergots and triptans(most widely used antimigraine class of drugs) act at the 5-HT_{1B}, 5-HT_{1D}, and, in part, at the 5-HT_{1F} receptors. They constrict extracerebral intracranial vessels, inhibit trigeminal neurons, and block transmission in the trigeminal nucleus. They block plasma protein extravasation by activating prejunctional trigeminal 5-HT_{1D} and 5-HT_{1F} heteroreceptors, blocking neuropeptide release. The 5-HT_{1D} receptor is located on CNS neurons and trigeminal nerve endings. 5-HT_{1F} receptors are located on trigeminal nerve endings. Cardiovascular toxicity has also been reported with use of sumatriptan as it also constricts coronary artery.

Sumatriptan (most widely used triptan for treatment of migraine) is generally given by oral or parental routes However, a substantial proportion of patients suffer severe nausea or vomiting during their migraine attack which may make oral treatment unsatisfactory Moreover, sumatriptan has previously been shown to have a low oral bioavailability in human volunteers (15%). Subcutaneous administration is an alternative, but dislike of injections or inability to self-administer by this route makes subcutaneous treatment unacceptable to some individuals. The intranasal route may be a viable alternative route of self administration, however problem associated with nasal delivery of sumatriptan solution is lower retention time of solution in nasal cavity (15minutes) resulting in lower bioavailability. Hence, to overcome all above mentioned drawbacks effective therapy for migraine call for the development of new strategies to enhance the drug concentration, particularly in intracranially located sites. As sumatriptan succinate is known to be very poorly permeable across blood brain barrier, other alternative delivery route apart from systemic delivery is needed to increase concentration of drug in the brain. Nasal drug delivery is one of potential route whereby concentration of drug in the brain region can be increased, as olfactory region is the only region in the body which has direct access to

brain. Hence formulation which would increase residence time in the nasal cavity and at the same time increase absorption of the drug across the olfactory region to intracranially located target sites would be highly beneficial in all the respects. Such formulation will also render an additional advantage of lowering cardiovascular toxicity due to its dose reduction and more specific action.

The thesis describes the development, characterization and evaluation of mucoadhesive microsphere and thermoreversible drug delivery system as carriers for sumatriptan succinate for effective intranasal therapy of migraine. The contents of the thesis are divided into four main section. Section I deals with introduction and literature review. Section II deals with the development of analytical methods for the estimation of drug and in vitro permeation mechanism of sumatriptan succinate across sheep nasal mucosa. Section III deals with the preparation, optimization, evaluation and stability of chitosan glutamate microspheres,carbopol 934P microspheres, pluronic F-127 gels, mixed gels of pluronic F-127 and carbopol 934P and mixed gels of pluronic F-127 and chitosan glutamate loaded with sumatriptan succinate. Section IV describes In vivo studies in rat model which further includes Pharmacokinetic study, Pharmacodynamic study and Heemodynamic study.

Chapter 1 gives introduction and objective of the work done.

Chapter 2 reviews the literature corresponding to migraine, nasal drug delivery system, mucoadhesive drug delivery, mucoadhesive microspheres, thermoreversible gels and profile of sumatriptan succinate used in this study.

Chapter 3 described the development and validation of analytical techniques for the estimation of sumatriptan succinate in drug loaded formulations and assessment of in vitro permeation mechanism of the drug and in vitro permeation study of various formulations across nasal mucosa. The analytical method developed was subjected to statistical analysis and the relevant statistical parameters were established. The methods were evaluated for accuracy and precision The results revealed that the analytical methods were selective and accurate with high precision. The methods could also estimate the sumatriptan succinate in presence of other constituents of the formulations.

A simple and sensitive high performance liquid chromatography method using UV-visible detector for estimation of sumatriptan succinate in rat plasma, CSF (cerebro spinal fluid) and brain tissues was developed and validated. Analytical method was validated for specificity, robustness, absolute recovery, linearity, sensitivity, precision and accuracy. The method was rapid, simple, selective, sensitive, robust, reproducible, accurate and precise This method gives an alternative method for the analysis of sumatriptan in plasma samples and novel method for the determination of sumatriptan in brain homogenate and CSF samples. This method is inexpensive, easy to run, simple and rapid, which may substitute other analytical methods for the estimation in plasma which are complex and involves instrumentation not easily available in all the laboratories and are also costly Whereas method developed for the estimation of sumatriptan succinate in CSF and brain tissue is a novel method, as previously no estimation method is reported. Analytical method for the estimation of residual glutaraldehyde in chitosan glutamate microspheres was also developed and validated.

Chapter 4 describes in vitro permeation studies involving identification of potential permeation pathway for sumatriptan succinate across sheep nasal mucosal membrane.
In general, drugs can be transported by different routes across the nasal epithelium (e.g. passive diffusion limited, transcellular or paracellular transport or carrier mediated either primary or secondary active transport or transcytosis). The preliminary knowledge about the probable permeation mechanism across nasal mucosal membrane is beneficial in developing formulations for nasal delivery of sumatriptan succinate. To characterise the transport mechanism of sumatriptan succinate across the sheep nasal mucosal membrane the influence of several parameters was investigated. The initial drug concentration was varied, sodium ions were replaced by N-methyl-D-glucamine (NMDG), and furthermore, effects of addition of 2.5 mM EGTA on nasal mucosal membrane were also investigated. The results obtained from these experiments demonstrated that the transport of sumatriptan succinate across nasal mucosal membrane was independent of initial drug concentration as well as complete or partial depletion of Na+ ions and was enhanced by pre-treatment with EGTA. Taken together, results indicate that the paracellular pathway seemed to be the main route of sumatriptan succinate transport across nasal mucosal membrane.

Chapter 5 describes preparation and evaluation of mucoadhesive microspheres incorporating sumatriptan succinate using two different mucoadhesive polymers with permeation enhancing potential i e chitosan glutamate and carbopol 934P

In case of chitosan glutamate microspheres formed by emulsification-crosslinking technique detailed study of the effect of various formulation and process parameters like stirring speed, volume ratio of water: oil phase, composition of external phase and emulsification time on microsphere formation, drug entrapment and particles size during preliminary screening is described. A 3^3 factorial design was used to investigate the combined effect of three different variables in the preparation of chitosan glutamate microspheres loaded with sumatriptan succinate. Experimental design and desirability function were applied for the optimization. As part of the optimization process, the main effect, interaction effects, and quadratic effects of amounts of concentration of chitosan glutamate, drug loading and volume of crosslinking agent on drug entrapment, particle size, mucoadhesive strength and effective permeability were investigated. Results obtained from the statistical analysis showed that the both polymer concentration and drug loading significantly affected particle size, % drug entrapment, mucoadhesive potential and effective permeability, whereas volume of crosslinking agent(glutaraldehyde) had significant effect mucoadhesive strength and effective permeability The ANOVA results also showed that the interaction between polymer concentration and drug loading had significant influence on the mucoadhesive strength , interaction between polymer concentration and volume of crosslinking agent was found to have significant influence on effective permeability Also polynomial factor polymer concentration was found to have statistically significant influence on drug entrapment, mucoadhesive strength and effective permeability. Desirability function was utilized to find out the best batch out of 27 batches Batch 25S showed the highest overall desirability of 0.933 Therefore, this batch was considered to be the best batch and the values of independent variables of this batch (2.5% w/v chitosan glutamate concentration, 50% w/w drug loading and 7.5µl of glutaraldehyde) were considered to be optimum values for the preparation of microspheres. Chitosan glutamate microspheres loaded with sumatriptan succinate indicated its usefulness in the treatment of migraine by intranasal route of administration, as it would retain the formulation for prolonged period of time at the site of absorption in the nasal cavity by reducing nasal clearance due to its high

mucoadhesive potential and also enhance absorption across nasal membrane (due to permeation enhancing effect) with enhanced delivery to intracranially located sites

Carbopol 934P microspheres were prepared by spray drying technique Microspheres prepared by spray drying is driven by a number of advantages over competing technologies including lower processing costs, ease and speed of scale-up, its ability to transform liquid feed into dry powder in a one step and continuous particle processing operation. Optimization of various spray drying process parameters is very much important for obtaining maximum yield and overcoming problems like sticking, particularly with microparticles containing mucoadhesive polymers. Hence prior to preparing drug loaded microspheres, spray drying process parameters were optimized for preparing placebo microspheres with maximum yield and particle size distribution. Influence of spray drying process parameters on formulation of mucoadhesive carbopol microspheres was evaluated by employing 2^3 factorial design, whereby particle size and yield were considered as response variables. Independent variables studied at lower and higher levels included the inlet temperatures, flow rate and aspiration volume. From the analysis of run data, the best possible combination of spray drying conditions to achieve the highest yield with desired particle size were inlet temperature of 100°C, aspiration volume of 80 cuft/min and Flow rate of 15ml/min and hence sample № 2 was found to be the most suitable. Highest yield obtained with optimum spray drying process parameters is 30%, lower yield with spray drying process for small scale batch was expected as carbopol is highly hygroscopic material with adhesive properties, which has a natural tendency of adhering to the walls of cyclone separator and other glass chambers. Lower yield accounts for the loss of the particles that are adhered and difficult to collect from the chambers, moreover batch size used for optimization of the parameters was small which resulted in % losses to be greater. However, 4 times higher volume than that used for factorial design study were used, at the optimum spray drying process parameters yield obtained was 53.7%. Thus although yield is lower, the yield is expected to increase with increase in batch size as % losses will reduced remarkably. Sumatriptan succinate loaded microspheres were prepared using optimum spray drying conditions. This chapter reports a detailed study of effect of drug to polymer ratio on % yield, drug entrapment, particle size, mucoadhesive potential and permeation enhancing effect of the microspheres. Microspheres were also characterized for its surface morphology, angle of repose, infrared spectophotometry and toxicological effect on the nasal mucosal membrane. Encapsulation efficiency ranged from 96.4% to 99.5%. Spray drying technique is

generally characterized by high drug encapsulation efficiency. The yield of production ranged from 54 to 61% The results showed that with increasing polymer ratio, the mucoadhesive strength and effective permeability increased significantly, but increase in polymer concentration beyond 50%w/v did not significantly affected mucoadhesive potential or effective permeability. Considering results obtained from above discussed characterization formulation CS-3 was considered to be the one exhibiting best possible combination of mucoadhesive strength and permeation enhancing effect. Increase in polymer concentration beyond 50 %w/w, apart from resulting in bulkier dosage form(which may not be ideal for nasal delivery system) had no significant advantage in terms of mucoadhesive potential or permeation enhancing effect. Hence, CS-3 with drug to polymer ratio (1:1) was selected for further studies. Carbopol microspheres containing sumatriptan succinate prepared by spray drying process could be one of the potential carriers for nasal drug delivery, because of their high mucoadhesive potential and permeation enhancing effect it would retain the formulation for prolonged period of time at the site of absorption in the nasal cavity by reducing nasal clearance and also enhance absorption across nasal membrane with enhanced delivery to intracranially located target sites.

Chapter 6 deals with development of effective intranasal delivery systems of sumatriptan succinate using thermoreversible polymer pluronic F127 and mixed gels prepared by addition of mucoadhesive polymer carbopol 934P or chitosan glutamate to pluronic F127 The formulations were evaluated for its gelation temperature, rheological characteristics, mucoadhesive strength, in vitro permeation across sheep nasal mucosa and in vitro toxicological effects of the vehicles on sheep nasal mucosal membrane.

Thermoreversible polymer-based liquid formulations that provide *in situ* gelling property in nasal cavity were designed to delay clearance of the formulations from the nasal cavity and enhance retention and thereby increase absorption of drug from the nasal cavity. Usually, the gelation temperatures have been considered to be suitable if it is in the range of 25–34 °C, as the temperature of the nasal cavity is 34°C. This study in the chapter also determined the lowest possible concentration of pluronic which gels below 34°C with suitable rheological and mucoadhesive potential. Pluronic F127 thermoreversible gel formulation for nasal administration was prepared by incorporating antimigraine drug sumatriptan succinate Various batches of formulations containing pluronic F127 (12% w/v to 20%w/v) were screened for its rheological behaviour and gelation temperature. It

was found that formulations containing pluronic F127 (18%w/v or greater) exhibited thermoreversible property well below physiological temperature in the nasal cavity and they also exhibited mucoadhesive properties Formulations containing pluronic (18% and above) were newtonian at 20°C and pseudoplastic at its gelation temperature, with viscosity that increased with the polymer concentration. Gelation temperature reduced with increase in pluronic F-127 concentration. Permeation enhancing effect was not observed with pluronic F-127 formulations. Thermoreversible gel containing 18%w/v pluronic F127 was the one with lowest concentration of pluronic exhibiting required gelation temperature, mucoadhesive potential and rheological property and hence was considered as optimum formulation. Although Pluronic F127 gel formulation does not have permeation enhancing effect, it appears to be promising nasal drug delivery system for antimigraine drug that would enhance nasal residence time attributed to increased viscosity and mucoadhesive characteristics.

In order to fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers have been added to the in situ-gelling vehicles of pluronic F-127, This chapter deals with development of system containing effective amount of sumatriptan as succinate salt , thermoreversible polymer pluronic F127and mucoadhesive polymer (carbopol 934P) or chitosan glutamate which has absorption enhancing property o the mucoadhesive property for enhanced delivery to intracranially located sites. Effect of concentration of carbopol 934P (0 1%w/v to 0.5%w/v) and chitosan glutamate (0.1% to 1%w/v) on viscosity, gelling temperature, mucoadhesive potential and in vitro permeation is also investigated. Addition of anionic mucoadhesive polymer C934P to pluronic F127 resulted in reduction of gelling temperature along with increased mucoadhesive potential. Incorporation of carbopol (above 0 3%) resulted in significant increase in effective permeability coefficient of the drug. Histopathological evaluation after in vitro permeation studies did not exhibited any evidence of toxicity on the nasal tissue. Thus, pluronic F127 gel formulation with 0.3% carbopol (PLC-3) was considered as optimum system for nasal drug delivery of sumatriptan succinate (as increase in concentration of carbopol 934P beyond 0.3% had no significant enhancement of mucoadhesive potential or permeation enhancing effect).This would enhance nasal residence time attributed to increased viscosity and mucoadhesive characteristics; furthermore it also exhibited permeation enhancing effect.

Mixed gels prepared by incorporating thermoreversible polymer pluronic F127 and mucoadhesive polymer chitosan glutamate, exhibited gelation temperature below 34°C, which reduced with increase in concentration of chitosan glutamate. All formulation possessed mucoadhesive property as well as permeation enhancing effect across sheep nasal mucosal membrane, which significantly increased with increase in chitosan glutamate concentration upto 0.5%w/v, further increase in concentration had no significant effect on above mentioned parameters Thus mixed gels containing 18%w/v pluronic F127 and 0.5% w/v chitosan glutamate was considered to be the best with desirable characteristics.This chapter demonstrated that the use of *in situ* gelling vehicles of pluronic F-127 incorporating mucoadhesive polymer carbopol or chitosan glutamate could effectively and safely improve the nasal residence time and absorption of sumatriptan succinate.

Chapter 7 describes the pharmacokinetic and distribution(in CSF and brain) studies of sumatriptan succinate loaded chitosan glutamate microspheres(CGM), carbopol 934P microspheres(CM), pluronic F127 gel(PLB), mixed gels of pluronic F127 and chitosan glutamate(PLCG) and mixed gel of pluronic F127 and carbopol 934P(PLC), after intranasal administration to rats. Drug levels were estimated in blood, brain and cerebro spinal fluid and compared with the levels obtained after intranasal administration of sumatriptan succinate solution and subcutaneous administration of sumatriptan succinate solution to investigate transport of drug across the nasal membrane into the CNS using rat model. The chapter also describes the influence of route of administration and formulation on the pharmacokinetics and distribution in brain and CSF. The results demonstrated that sumatriptan succinate, poorly permeable through BBB, when administered nasally has a characteristic of brain targeting; it may be helpful for both increasing the CSF and brain therapeutic levels and reducing the systemic side effects.

Relative bioavailability of drug in plasma was significantly lower for all formulations compared to s.c route, with intranasal solution exhibiting lowest availability. Possibility of existence of direct pathway for transport of sumatriptan succinate from nose to brain apart from transport of drug from systemic circulation across BBB was speculated by higher peak concentration of drug in CSF and brain after intranasal administration, which also appeared earlier compared to s.c injection thus onset of action of drug required for immediate treatment of migraine attack would be very early. Although intranasal solution

resulted in higher drug concentration in CSF and brain compared to s.c. route they were not statistically significant, this may be due to rapid clearance of the drug solution from the nasal cavity. After intranasal administration, sumatriptan succinate was able to penetrate into the brain and CSF directly from the nasal cavity, with the olfactory epithelium being path of direct transport. Nasal administration of sumatriptan succinate in form of mucoadhesive microspheres (chitosan glutamate and carbopol 934P) and thermoreversible gel (pluronic F127 gel, pluronic F127 gel with chitosan glutamate and pluronic F127 gel with carbopol934P), significantly increased relative availability of drug in CSF and brain, peak concentrations in CSF and brain compared to intranasal solution and s.c route. Drug targeting Index was significantly higher for all the formulations as compared to s.c route. It also slightly increased half life of drug in brain and CSF compartments further exploring the possibility of prolonged delivery of drug to the target sites and thereby possibly ruling out the necessity of repeated doses required during the attack. The study also shows that mucoadhesive microsphere and thermoreversible gel formulations (with or without mucoadhesive polymer) exhibited increased transfer of drug across olfactory epithelium to intracranially located sites in brain. This could be a combined effect of mucoadhesion/viscosity of the formulation resulting in enhanced retention time in nasal cavity along with permeation enhancing effect of the formulations resulting in increased permeation of drug across olfactory epithelium to CSF and brain region. Drug nose to brain direct transport percentage of all nasal formulations including intransal solution as calculated by the method of Hunt et al was found to be in the range of 75% to 93%.These results indicates that all the formulations are promising intranasal delivery systems for sumatriptan to effectively treat migraine with reduced associated risks and rapid onset of action. Thus nasally administered sumatriptan succinate in above mentioned formulations is promising to become an effective non-invasive route for treatment of migraine; however it needs to be proven clinically.

Chapter 8 describes pharmacodynamic study of the above mentioned formulation administered intranasally in comparision with solution form of sumatriptan succinate administered intranasally and subcutaneously in migraine model of rat. A high circulating plasma concentration of calcitonin gene-related peptide (CGRP) has been demonstrated during migraine headache and these concentrations can be normalized by tryptans in parallel with alleviation of headache. This chapter describes validation of a rat migraine model that would allow the study of CGRP release (induced by capsaicin) and

its inhibition by sumatriptan succinate administered by subcutaneous and intranasal route (various mucoadhesive formulation and solution form) in a manner closely related to the human situation. Administration of capsaicin was found to significantly increase CGRP levels in plasma of all the rats which remained high throughout the experimental period , suggesting the induction of migraine. Administration of sumatriptan in different forms reduced CGRP levels in plasma. When sumatriptan solution was administered subcutaneously or intranasally, CGRP levels were significantly reduced upto 90 min but after 120min CGRP levels were significantly higher as compared to control group. This increase in CGRP levels may be due to reduction in sumatriptan levels at target sites. When sumatriptan succinate is administered by subcutaneous route, its action is highly limited to prejuctional 5HT receptors as its penetration in brain is highly limited. In case of intranasal solution CGRP levels evoked after 120 min as residence time of the solution in the nasal cavity is very less due to mucociliary clearance resulting in limited permeation of sumatriptan in brain by direct olfactory pathway due to rapid clearance of the drug from the site of permeation. While administration of sumatriptan nasal formulations caused significant decrease in CGRP levels and CGRP levels remained lower for 3 hrs. PLCG formulation effectively reduced CGRP levels in rat plasma and it remained significantly lower (compared to migraine control) for 4 hours, whereas all other formulation did not showed significant reduction in CGRP levels at four hours (compared to migraine control). From the present study the efficacy of sumatriptan formulations is as follows PLCG > CGM > PLB = PLC = CM > nasal soln = s.c. As all the formulations possessed mucoadhesive potential which will retain the formulations for longer period of time in the nasal cavity and vicinity for direct permeation of sumatriptan succinate to brain via olfactory pathway will increase. This will result in longer duration of action of sumatriptan at centrally located target sites ($5HT_{1B/1D}$ receptors). Intranasal mucoadhesive formulations with permeation enhancing effect seems to be effective antimigraine therapy with more specific and prolonged central action on intracranially located target sites, however it needs to be proven clinically.

Chapter 9 investigated the cardiovascular effect of sumatriptan formulations in rat model. Sumatritpan, due to its vasoconstrictive nature, is reported to have cardiovascular side effects and is not preferred in cardiovascular patients and therefore it's necessary to study cardiovascular effects of intranasally administered sumatriptan succinate. Sumatriptan succinate administered intravenously showed 50% mortality rate within 15-20 minutes of

administration, also there was significant increase in blood pressure and QT interval and significant decrease in heart rate. However subcutaneous administration showed 0% mortality with significant increase in blood pressure and significant decrease in heart rate, but it can be considered safe as there was no increase in QT interval. Intranasal administration of solutions form and all above mentioned formulations had no significant effect on hemodynamics as compared to control group and hence it could be concluded that intranasal administration of sumatriptan succinate is safer in terms of cardiotoxicity.

In conclusion, the results of this thesis demonstrates

1. The incorporation of sumatriptan succinate in mucoadhesive microsphere, thermoreversible gel and mucoadhesive thermoreversible gel had definite advantage not only in vitro, but also in increasing CSF and brain uptake of drug after intranasal administration.
2. Increased retention and absorption of drug from the formulation in the nasal cavity due to excellent mucoadhesive properties and permeation enhancing effect.
3. Low toxicity on nasal membrane.
4. Significantly greater availability of drug with rapid onset in CSF and brain.
5. Prolonged reduction in levels of CGRP in migraine model.
6. No change in haemodynamic state suggesting its cardiovascular safety.

The above merits of these formulations makes them an ideal candidate to be considered for treatment of migraine which will probably rule out the possibility of repeated dosing during migraine attack with enhanced rate and extent of transport of sumatriptan succinate to brain and CSF. Intranasal administration of formulations may also help in decreasing the dose and frequency of dosing and associated cardiovascular complication. These drug delivery systems are expected to pave a new way for combating migraine pain and lead to the safe and effective migraine therapy.