Chapter 11

Summary and Conclusion

11.1. Summary

Basic and clinical research during the past 50 years has confirmed that there are many mechanisms involved in nociception. Pain can be "acute" or "chronic. nociceptive pain is believed to be caused by the ongoing activation of pain receptors in either the surface or deep tissues of the body. There are two types: somatic pain and visceral pain.

Migraine headache is an episodic headache disorder. It is a common condition with a prevalence of 17.6% in females and 5.7% in males. An American Migraine Study estimated that 23 million persons older than 12 years of age have severe migraine headaches; however, this condition is under-treated and under-diagnosed worldwide. The treatment of migraine has not only medical but also serious economic and social implications. While there are many variations, there are two main types of Migraines

• Migraine without aura (previously called common migraine). Almost 80 percent of migraine sufferers have this type of migraine.

• Migraine with aura (previously called classic migraine).

Opioids are the first-line drugs for the treatment of moderate to severe pain but opioids must be administered with care. Unfortunately, opioid-induced adverse side effects are common and can be serious. Common side effects of opioids include nausea, vomiting, sedation, and pruritus. Less common but more serious side effects include respiratory depression and sometimes cardiac arrest. Clearly, patient care includes appropriate monitoring and therapy for these events. In an effort to improve pain control and decrease the incidence and severity of drug-induced adverse side effects, many clinicians have introduced the use of non-opioids drugs like tizanidine HCl and cyclobenzaprine HCl for the management of pain.

Tizanidine HCl is a centrally acting skeletal muscle relaxant, used for the symptomatic treatment of painful muscle spasms and spasticity. Furthermore, tizanidine HCl has analgesic properties and is used for the treatment of chronic headache, back pain and post operative pain. The problem associated with oral dosage form is tizanidine HCl having oral bioavailability about 21% mainly due to extensive first-pass metabolism and its mean elimination half-life is approximately 3 h. Cyclobenzaprine HCl is a muscle relaxant. It works by blocking nerve impulses (or pain sensations) that are sent to your brain. Cyclobenzaprine HCl is thought to act

primarily at brain stem (and to a lesser extent at spinal cord level) to relieve skeletal muscle spasms of local origin without altering muscle function. Cyclobenzaprine HCl is used together with rest and physical therapy to treat skeletal muscle conditions such as pain or injury. Although centrally acting muscle relaxants like cyclobenzaprine HCl are also helpful in aborting a migraine headache. Estimates of mean oral bioavailability of Cyclobenzaprine HCl range from 33% to 55% and Cyclobenzaprine HCl is eliminated quite slowly, with an effective half-life of 18 hours by oral administration.

In recent years, the nasal mucosa has been considered as an administration route to achieve faster and higher level of drug absorption. The richly supplied vascular nature of the nasal mucosa coupled with its high drug permeation makes the nasal route of administration attractive for centrally acting drugs, which are not being effectively and efficiently delivered using conventional drug delivery approach to brain or central nervous system (CNS) due to its complexity. Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation. Absorption of drug across the olfactory region of the nose provides a unique feature and superior option to target drugs to brain. Scientists have also focused their research toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as pain, epilepsy, migraine, emesis, depression and erectile dysfunction. Intranasal mucoadhesive nanoparticulate systems improve mucosal absorption, because they strongly attach to the mucosa and increase the viscosity of mucin. Thereby they significantly decrease the nasal mucociliary clearance rate and thus increase the residence time of the formulation in the nasal cavity. Additionally, nanoparticles cross the mucosal epithelium better than microparticles do, since not only microfold (M) cells overlaying the mucosal associated lymphoid tissue (MALT) but also the epithelial cells are involved in the transport of NPs.

In recent years, the polysaccharide material, chitosan has attracted much interest as a nasal delivery system that is able to efficiently deliver polar drugs (including peptides) to the systemic circulation and provide therapeutically relevant bioavailability. Chitosan (poly [β -(1-4)-2-amino-2-deoxy-D-glucopyranose]) is a biodegradable cationic polysaccharide produced by partial deacetylation of chitin

derived from naturally occurring crustacean shells. The polymer is comprised of copolymers of glucosamine and N-acetyl glucosamine. Since chitosan displays mucoadhesive properties, strong permeation enhancing capabilities for hydrophilic compounds and a safe toxicity profile. Despite its biocompatibility, the uses of chitosan in biomedical fields are limited by its poor solubility in physiological media. Chitosan has an apparent pKa value between 5.5 and 6.5 and upon dissolution in acid media the amino groups of the polymer are protonated rendering the molecule positively charged. At neutral and alkaline pH, most chitosan molecules loose their charge and precipitate from solution. To improve the poor water-solubility of chitosan at physiological pH, several derivatives have been studied, for example, the modification of chitosan by methylation and thiolation. Thiolation of chitosan provide much higher mucoadhesive properties than polymers and the enhancement of mucoadhesion can be explained by the formation of covalent bonds between the polymer and the mucus layer which are stronger than non-covalent bonds such as ionic interactions of chitosan with anionic substructures of the mucus glycoproteins via disulfide exchange reactions or via simple oxidation process. These thiolated chitosans have several advantageous features in comparison to chitosan, such as significantly improved mucoadhesive and permeation enhancing properties. *N*-trimethyl chitosan chloride (TMC), a partially quaternized chitosan derivative, shows good water solubility over a wide pH range. Hence, soluble TMC has mucoadhesive properties and excellent absorption enhancing effects even at neutral pH. The absorption promoting effect of chitosan and chitosan derivatives have been found to be a combination of mucoadhesion and a transient opening of the tight junctions in the mucosal cell membrane.

There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force.

The modified chitosan an unmodified chitosan were characterized by FTIR and ^{1}HNMR. The nanoparticles were also characterized for particle size, zeta potential, surface morphology, *in- vitro* drug release. The amount of immobilized thiol groups in thiolated chitosan was determined via Ellman's reagent. RPMI 2650

cells were used to assess the *in vitro* cytotoxicity study of the modified and unmodified chitosan and its formulations. The stability studies of modified and unmodified chitosan NPs were conducted to determine the particle size, zeta potential, % EE, *in- vitro* drug release and the physical changes like caking and discoloration. *In vivo* studies in animals include the tissue biodistribution in the different body organs or tissues. The biodistribution studies were carried out after radiolabeling of the drug and the nanoparticles formulation with ^{99m}Tc. The drug delivery to the brain was confirmed by gamma scintigraphy technique. Estimation of tizanidine HCl and cyclobenzaprine HCl in nanoparticles formulations were carried out using UV-visible spectrophotometry at 320nm and 290nm respectively. The calibration curve of Tizanidine HCl by U.V visible spectrophotometry was established in both distilled water and phosphate buffer pH 5. The linearity of Tizanidine HCl was found to be 5 to 17.5 µg/ml (R^2 =0.995) and 2.5 to 17.5 µg/ml (R^2 =0.996) in distilled water and Phosphate buffer pH 5 respectively. The calibration curve of Cyclobenzaprine HCl by U.V visible spectrophotometry was established in both distilled water and phosphate buffer pH 5. The linearity of Cyclobenzaprine HCl was found to be 5 to 25 µg/ml (R^2 =0.993) and 5 to 25 µg/ml (R^2 =0.993) in distilled water and Phosphate buffer pH 5 respectively. Drug content in nanoparticles for both drugs was measured by the U.V visible spectrophotometry in supernant and nanoparticles both.

Drug in the *in-vitro* release medium was estimated using HPLC method for both drugs. The drug was estimated using a Shimadzu HPLC system (Shimadzu, Japan). The HPLC system was composed of a pump (LC-20AT prominence, Shimadzu), a sample 20-µl loop injector (Rheodyne 7725) and a UV-visible spectrophotometric detector (SPD-20A prominence, Shimadzu). The separation was carried out on a Phenomenex C_{18} 250 x 4.6 mm HPLC column (Phenomenex) having particle size of 5µm. The calibration curve of Tizanidine HCl and Cyclobenzaprine HCl was also established in acetonitrile: water (80:20) and acetonitrile: methanol: water (50:30:20) system by HPLC at 241nm and 290nm respectively. The linearity of Tizanidine HCl and Cyclobenzaprine HCl was found to be 0.25 to 10µg/ml (R^2=0.997) and 0.25 to 20µg/ml (R^2=0.991). The *in-vitro* release study was performed using Franz diffusion cell. At different time intervals, the samples were removed and diluted with mobile phase and analyzed for the drug content in diffusion medium using HPLC method. Rhodamine B was estimated by UV-visible spectrophotometry.

The estimation of Rhodamine B was carried out using U.V visible spectrophotometry at 553 nm. The calibration curve was established at 1 to 5 µg/ml (R^2=0.995).

To determine the amount of Tizanidine HCl and Cyclobenzaprine HCl, drug loaded nanoparticles suspension diluted with 1% (v/v) acetic acid solution, being ultrasound damaged for 30 min, filtrated through 0.22 µm microporous membrane. The entrapment efficiency was determined upon separation of NPs from the aqueous suspension containing non-entrapped drug by centrifugation at 18000 rpm 4 °C for 30 min. The amount of free drug in the supernatant was measured by U.V spectroscopy at 320 nm and 290 nm for Tizanidine HCl and Cyclobenzaprine HCl respectively.

Thiolation of chitosan was carried out using carbodiimide method and thiol groups in thiolated chitosan were analysed quantitatively by 'Ellman's method' and qualitatively by FTIR. The carboxylic acid moieties of TGA were activated by EDC that forms an intermediates *O*-acylurea derivative, which reacts with the primary amino groups of chitosan. In order to optimize the synthesis of thiolated chitosan, the infuence of the pH (pH 3 to 5) and the amount of chitosan to TGA during the coupling reaction on the amount of polymer-immobilized thiol groups was evaluated. Results of optimization studies were demonstrated that the pH at the coupling reaction has a great impact on the amount of TGA bound to chitosan. At pH 3 the amounts of covalently attached thiol groups were comparatively low, as EDC could not gain its full reactive potential at this pH-value. Hence, at a pH-value of 4 the amount of attached thiol groups was significantly higher than the pH value 3 and lower than the pH value 5. Thiolation was maximum at pH 5 and above pH 5 the yield of polymer-bound thiol groups decreased again because of the oxidation of sulfhydryl groups during the coupling reaction, which is favoured at higher pH-values. In order to achieve a further improvement in the amount of thiol group covalently bound to the polymer, the influence of the polymer to TGA amount during the coupling reaction was evaluated at pH 5. Results of this study demonstrated the highest coupling was found at 500 mg of polymer and 30 ml of TGA. At less or more than 30 ml of TGA with 500 mg of polymer led to lower yields of immobilized thiol group on the polymer. The results indicate that the amount of covalently bound thiol groups cannot be increased by raising the share of TGA during the coupling reaction. The thiolated chitosan produced by carbodiimide exhibited 42.33 mmol and 125.5 mmol immobilized free thiol groups per gram of low and medium molecular weight polymer

respectively. The presence of thiol groups on the thiolated chitosan surface at high concentration increased the mucoadhesion capacity of NPs by forming covalent bonds with the cysteine residues of the mucus glycoproteins. The spectra from thiolated samples contained a signal peak within the thiol group range at 2550-2600 cm^{-1}, that indicated the thiol group attached to the surface of chitosan, a peak that is not found in the spectrum of the unmodified chitosan.

Methylation of amino groups in chitosan can be achieved using methyl iodide at elevated temperature in strong alkaline environment to bind the acid being generated during the reaction taking place and to avoid protonation of the unreacted primary amino groups. The degree of quaternization (DQ) can be changed by increasing the number of reaction steps or by increasing the reaction time. TMC with a different degree of quaternization (DQ) was synthesized by methylation of chitosan using CH_3I in the presence of a strong base (NaOH). The purified TMC was analyzed by ^{1}HNMR spectroscopy. The NMR spectrum of the TMC in D_2O at 80 °C was recorded with a NMR spectrometer for determination of the degree of quaternization (DQ). This quaternized derivative of chitosan possesses a positive charge and is soluble over a wide range of pH. TMC shows better mucoadhesive and permeation enhancement properties than the chitosan.

The IR spectrum of chitosan and TMC was obtained by dispersing the sample in KBr disc. The IR spectrum of TMC was provided the evidence for the occurrence of methylation especially in the region 1,700–1,200 cm^{-1}. ^{1}H NMR spectra were measured with a 300 or 600 MHz spectrometer by dissolving chitosan and TMC samples in D_20 at 80°C. Degree of quaternization was calculated using equation:

$$\mathbf{DQ\ (\%) = \{[(CH3)3]/[H]*1/9\}*100}$$

where DQ (%) is the degree of quaternization as percentage, $[(CH3)_3]$ is the integral of the chemical shift of the N-trimethyl amino group at 3.3 ppm attributed to the nine hydrogen atoms of the methyl groups pertaining to trimethylated amino groups. [H] is the integral of the ^{1}H peaks between 4.7 – 5.7 ppm (reference signals) representing the protons attached to the carbon of the glucosamine unit of the glucopyranose ring. Degree of quaternization of low molecular weight trimethyl chitosan (39 kDA) and medium molecular weight chitosan (242 kDA) were found to be 38.78 and 44.33 % respectively.

Mucoadhesive properties of modified chitosan (thiolated chitosan and trimethyl chitosan) were evaluated using the mucin particle method based on the change in surface properties of mucin particle, particle size and zeta potential, by the mucoadhesion of the polymer with the mucin particle. It was observed that the suspension of ss-mucin particles when mixed with a different volume of polymer solution would induce the ss-mucin particles to aggregate if the polymer had a strong affinity to them. Commercially available procine gastric mucin type III mucin was used for the study and the interaction was determined at pH 6.8 in Tris buffer where chitosan was insoluble and lost mucoadhesive properties.

By referring to the results of ss-mucin–polymer interaction studies, it can be deduced that exhibited mucoadhesive characteristic and the rank order of mucoadhesive bond strength of polymers was MMTC/MMTMC > LMTC/LMTMC > MMC > LMC. Furthermore, it was observed that the interaction between ss-mucin particles and polymer was molecular weight-dependent. The interaction decreased with decreased molecular weight.

Ionotropic gelation method was easy and reproducible for preparation of drug loaded chitosan thiolated chitosan and trimethyl chitosan NPs. There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force. Quantitative aspects of the effects and relationships among various formulation parameters of high therapeutics payload nanoparticles produced by Ionotropic gelation method were investigated using Response Surface Methodology (RSM). To study this, we performed, "Box-Behnken" design (BBD) on critical formulation factors known to affect their results. The BBD is a popular template for RSM because it requires only three-levels of each formulation factor and only a fraction of all the possible combinations. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the centre of this multidimensional cube. This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models.

Chitosan NPs with different molecular weight were prepared by ionotropic gelation of chitosan with sodium alginate, which involves the mixing of two aqueous solutions at ambient temperature while stirring without using sonication or organic solvents. Various formulations were made with different initial concentrations of chitosan (1, 1.5, 2 mg/ml) and sodium alginate (0.5, 1, 1.5 mg/ml) to establish preparation conditions at which NPs are formed. Smaller particles generally show a higher uptake by nasal epithelia than larger ones. The criteria like size, size distribution, colloidal stability and reproducibility of NPs production were used to select the best formulation parameters to prepare NPs. The optimal chitosan NPs were formed when the chitosan solution (1 mg/ml) to sodium alginate solution (0.5 mg/ml) ratio was 2:1. At very low or high chitosan to sodium alginate ratio either a clear solution or large NPs with a low colloidal stability were obtained. When sodium alginate concentration was 1.5 mg/ml, too high chitosan concentration (2 mg/ml) made encapsulation extremely difficult, and too low chitosan concentration (0.5 mg/ml) resulted in some aggregates with large diameter. The formation of nanoparticles is only possible within some moderate concentrations of chitosan and sodium alginate. As for gelation between sodium alginate solution of 0.5 mg/ml and chitosan solution of 1–2 mg/ml, we usually observed that some opalescent suspension of nanoparticles. Increase in chitosan concentration led to decrease encapsulation efficiency of drug. With increase in the concentration of drug in optimal formulation (1, 2.5, 4 mg/ml) drug entrapment was increased and decreased above the 2.5 mg/ml.

Impact of chitosan solution pH (4 and 5) and sodium alginate pH (8, 9, 10 and 11) was seen on the effect of particle size and drug entrapment on optimal formulations. Higher entrapment and lower size of NPs was found at the pH 5 of chitosan solution and pH 8 of sodium alginate solution. One can argue that in this method when the pH of the polymers is adjusted to 5 the NH_2^- groups of the chitosan are mostly protonated and are better accessible for interaction. As with increase in the pH of sodium alginate 8 to 11, sizes of NPs and drug entrapment was increased up to pH 10 and decreased at above pH 10. To elucidate the influence of chitosan molecular weight on the particle size, as the molecular weight increases the particle size was increase and this trend may be explained by the fact that a medium molecular weight chitosan can more interact and associate drug more efficiently than a lower molecular weight chitosan. The factor is out-weighted by the fact that medium molecular weight

chitosan is less soluble compared to low molecular weight chitosan and as a result, an increase in particle diameter or even aggregation may be obtained. Chitosan free amino groups were responsible for the positive zeta potential values obtained for all formulations, which might ensure the electrostatic interactions with the anionic groups of the mucus. Above results shows, the effect of sodium alginate concentration and drug concentration was higher than the effect chitosan concentration on nanoparticles formation because chitosan and drug both are cationic in nature and sodium alginate was polyanions so its effect was more prominent toward the cationic drug. Drug entrapment and particle size both was greatly affected by sodium alginate concentration.

Nanoparticles from thiolated chitosan (TC) with different moleculer weight were prepared by ionic gelation of thiolated chitosan using sodium alginate with slight modification in above method. Various formulations were made with different initial concentrations of thiolated chitosan (1, 2 and 3 mg/ml) and sodium alginate solutions (0.25, 0.5 and 0.75 mg/ml) to establish preparation conditions at which NPs are formed. The effect of low or high thiolated chitosan to sodium alginate ratio was found to be similar as of chitosan NPs. The impact of pH change on drug entrapment was negligible as thiolation increases positive charge and improves solubility of chitosan at physiological pH. Moreover, tizanidine HCl and cyclobenzaprine HCl were highly cationic hydrophilic drugs which in terms affects the drug entrapment with highly positively charged polymer. The formation of thiolated chitosan NPs includes the preparation of thiolated chitosan (2 mg/ml) in water and drug solution (2mg/ml) in water with prior incubation with anionic sodium deoxycholate (10 mg/ml) for 30 seconds separately. The resulting drug-SD complex was added to thiolated chitosan solution. The addition of sodium alginate solution (0.05 mg/ml) with stirring to the above mixture with ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate 7:1:1:1 led to the immediate formation of NPs. Further with increases in the concentration of drug (1 to 3 mg/ml) in optimal formulation, drug entrapment was increases up to 2 mg/ml and decreases above the 2 mg/ml concentration. Moreover, with increase in the concentration of anionic surfactant up to 10 mg/ml, the drug entrapment was increases because of the formation of neutral drug-sodium deoxycholate complexes, which adsorbed more on the positively charged thiolated chitosan than the highly cationic drug. As we increase the

concentration of sodium deoxycholate solution, the size was also decrease and surface area was increased. Hence, more drug-sodium deoxycholate complexes adsorbed on the surface of thiolated chitosan leads to high drug entrapment. The mean hydrodynamic diameter of these types of nanoparticles showed a clear increased with the molecular weight of thiolated chitosan. Over all conclusions based on the above results the concentration of sodium deoxycholate and sodium alginate was affect more than the other factors on particle size and %EE. . This result may be due to the strong interactions between anions and TC because TC has a higher positive charge than chitosan.

Trimethyl chitosan NPs with difference moleculer weight were prepared by ionic gelation of TMC using sodium alginate with slight modification same as Thiolated chitosan. Various formulations were made with different initial concentrations of chitosan (1, 1.5 and 2 mg/ml) and sodium alginate solutions (0.5, 1.5, 2 mg/ml) to establish preparation conditions at which NPs are formed. The effect of low or high TMC to sodium alginate ratio was found to be similar as of chitosan NPs. The impact of pH change on drug entrapment was negligible as methylation increases positive charge and improves solubility of chitosan at physiological pH. Moreover, tizanidine HCl and cyclobenzaprine HCl were the highly cationic hydrophilic drugs that also affect on the drug entrapment with highly positively charged polymer. The optimal TMC NPs were formed when the TMC (1 mg/ml) was dissolved in distilled water. Drug solution (2mg/ml) was incubated with anionic sodium deoxycholate (10 mg/ml) for 30 seconds. The resulting complex was added to TMC solution. The addition of sodium alginate solution (2 mg/ml) with stirring to the above mixture with ratio of TMC: sodium alginate: drug: sodium deoxycholate 7.9:0.7:0.7:0.7 led to the immediate formation of NPs. Further with increases in the concentration of drug solution (1, 2 and 3 mg/ml) in optimal formulation, drug entrapment was increases up to 2 mg/ml drug concentration but above the 2 mg/ml further entrapment was decreases. Moreover, with increase in the concentration of anionic surfactant up to 10 mg/ml, the drug entrapment was increases because of the formation of drug-sodium deoxycholate complexes, which adsorbed more on the positively charged TMC than the highly cationic drug. With increase in the concentration of sodium deoxycholate solution, the size was decreased and surface area was increased. Hence, more drug-sodium deoxycholate complexes adsorbed on

the surface of TMC leads to high drug entrapment. The mean hydrodynamic diameter of these types of nanoparticles showed a clear increase with the molecular weight of TMC. Over all conclusions based on the above results the concentration of sodium deoxycholate and sodium alginate was affect more than the other factors on particle size and %EE. . This result may be due to the strong interactions between anions and TMC because TMC has a higher positive charge than chitosan.

In aqueous suspensions, the chemical and physical stability of nanoparticles has been reported to be poor. Freeze-drying has been the most utilized drying method of nanoparticles suspensions. Because the freeze-drying process is highly stressful for nanoparticles, addition of cryoprotectants becomes essential. For nanoparticles carbohydrates have been perceived to be suitable freeze-drying protectants. There are considerable differences in the cryoprotective abilities of different carbohydrates. The optimized batch of nanoparticles was lyophilized using sucrose, mannitol and trehalose (at 1:1, 1:2 and 1:3 NPs: cryoprotectant) to select suitable cryoprotectant and its concentration. When sucrose was used as a cryoprotectant, at all concentrations studied, the redispersion of freeze-dried NPs was difficult due to the formation of flakes or aggregates and also there was substantial increase in particle size after lyophilization. The Sf/Si values were higher than the 1. The increase in the particle size could have been due to the cohesive nature of the sucrose. Further, it was observed that the lyophilized nanoparticles with sucrose had tendency to absorb moisture very quickly. With mannitol, the nanoparticle formulation showed free flowing ability, however the redispersion was difficult and possible only after vigorous shaking. The Sf/Si values were slightly greater than the 1. With trehalose as cryoprotectant, the lyophilized nanoparticles were redispersed easily and the increase in particle size was not significant as indicated by S_f/S_i values which were almost nearer to 1. The redispersion of the nanoparticles depends on the hydrophilicity of the surface. The easy redispersibility is probably due to the higher solubility of trehalose in water i.e. 0.7 parts in 1 part of water. The cryoprotective effect may be attributed to the ability of trehalose to form a glassy amorphous matrix around the particles, preventing the particles from sticking together during removal of water. Furthermore, trehalose, a non-reducing disaccharide of glucose, has previously exhibited satisfactory cryoprotective effects for pharmaceutical and biological materials.

The particle size, zeta potential and drug entrapment of drug loaded and Rhodamine B loaded chitosan nanoparticles were found to be within the range of 400-700nm, 21-25mV and 40-65 % respectively. The particle size, zeta potential and drug entrapment of drug loaded and Rhodamine B loaded thiolated chitosan nanoparticles were found to be within the range of 262-283nm, 12-27mV and 65-81 % respectively. The particle size, zeta potential and drug entrapment of drug loaded and Rhodamine B loaded trimethyl chitosan nanoparticles were found to be within the range of 168-288nm, 12-17mV and 62-73 % respectively.

In vitro release of drug from nanoparticles was carried out in PBS (pH 5) using both dialysis bag (to see the release of drug from the formulation) and nasal mucosa (to see the diffusion of drug from the biological membrane). The maximum 51 to 55 % of drug release was found in 2-3 hrs for Tizanidine HCl and cyclobenzaprine HCl solution. Tizanidine HCl loaded and cyclobenzaprine HCL loaded chitosan NPs shows higher drug release around 65-72 % in 2-3 hrs. Tizanidine HCl loaded and Cyclobenzaprine HCl loaded thiolated chitosan NPs and trimethyl chitosan NPs shows the comparative faster and higher release around 88-96 % in 60 minutes. The significant change in results may due to the improved solubility of thiolated chitosan and trimethyl chitosan at physiological pH than the chitosan. Thiolated chitosan and trimethyl chitosan having higher mucoadhesive strength and higher water solubility than the chitosan so the more drug was absorbed and permeate faster through on the nasal mucosa.

The TEM images of drug loaded chitosan, thiolated chitosan and trimethyl chitosan showed uniform with size in nanometer range.

For In vitro release, freshly excised sheep nasal mucosa was mounted on Franz diffusion cells. One mucosa was treated with phosphate buffer (pH 5); the other mucosa was with Isopropyl alcohol (IPA) and the remaining with NPs formulations; after one hr the mucosa rinsed with phosphate buffer (pH 5). Nasal mucosa was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope to detect any damage to the mucosa during in vitro permeation by a pathologist blinded to the study. The sheep nasal mucosa was treated with phosphate buffer (pH 5) and isopropyl alcohol as positive and negative control respectively. The microscopic observations indicate that

the optimized formulations have no significant effect on the microscopic structure of mucosa. Neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of NPs formulations. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared with phosphate buffer (pH 5)-treated mucosa. Thus, the developed formulations seem to be safe with respect to nasal administration.

As observed by confocal laser scanning microscopy, the positively charged chitosan NPs, thiolated NPs and trimethyl NPs performed stronger interaction with the nasal mucosa than the Rhodamine B solution. This bioadhesive effect resulted in an enhanced contact time of the thiolated chitosan NPs and trimethyl chitosan NPs on the negatively charged nasal mucosa than the chitosan NPs and Rhodamine B solution. The findings may be explained in case of thiolated chitosan by the formation of covalent bonds between the thiol groups of thiolated chitosan and cysteine residue; and in case of methylated chitosan by the formation of ionic bond between positive charges of TMC and negatively charged sialic groups on the mucus protein structure. This effect leads to high concentration of drug on the penetration site to promote an effective permeation of drug through the nasal mucosa. Images after the Z sectioning of nasal mucosa showed that the higher penetration of thiolated NPs and trimethyl NPs than the chitosan NPs and rhodamine B solution.

The stability studies of the formulations were performed in order to study the influence of varying environmental conditions on the parameters of the formulation influencing the therapeutic response. The stability studies were carried out in accordance with the ICH guidelines for drug substances intended to be stored in a refrigerator. The stability of the nanoparticles were assessed for physical observation, particle size, zeta potential and the drug content (with respect to the initial) at 5°C ± 3°C for 6M and 25°C ± 2°C/60% RH ± 5% RH for 6M. The drug content in the initial sample was considered as 100 percent. For accelerated condition (i.e. 25°C ± 2°C/60% ± 5 %RH) the sampling was done at 1, 2, 3, 6 months and for 5°C ± 3°C the sampling was done at 1, 3, 6 months. It was observed that Tizanidine HCl loaded and Cyclobenzaprine HCL loaded chitosan, thiolated chitosan and trimethyl chitosan NPs have no significant change ($P>0.05$) observed in particle size, zeta potential and drug content at 5°C ± 3°C for 6M. The storage of the Tizanidine HCl loaded and Cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs

at 25°C ± 2°C/60% ± 5% RH, led to increase in the particle size. The increase in the particle size was not significant during the first month, however became significant and more prominent after 2, 3 and 6 months. During our analysis of samples, the polydispersity index of the nanoparticles stored at 25°C ± 2°C/60% ± 5% RH was found to increase as compared to the initial. The increase in the particle size may be due to the absorption of the moisture by the nanoparticles resulting in the coalescence of the small nanoparticles forming particles larger in size. The nanoparticles were also observed for physical appearance. After 3 and 6 months the physical appearance was also changed, with loss of the free flowing property followed by the difficulty in redispersibility. Also, the thiolated chitosan nanoparticles demonstrated difference in the color than the initial powder. At 6 months the color of the powder was yellow. This could be indicative of the oxidation of thiol group of the surface. At 25°C ± 2°C/60% ± 5% RH, the zeta potential of the nanoparticles shifted towards the zero for Tizanidine HCl loaded and Cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs. The lowered zeta potential values also might have contributed toward the aggregation of particles. The drug content of the Tizanidine HCl loaded and Cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs nanoparticles was not altered up to 6M at 5°C ± 3°C. However, the drug content was reduced after 6M storage at 25°C ± 2°C/60% RH ± 5% RH. This impact could be due to the moisture absorbed by the nanoparticles upon storage at 25°C ± 2°C/60% RH ± 5% RH, possibly resulting in the degradation of the drug. The release profile of the drug from the nanoparticles was not affected upon storage. The similarity factor calculated for the between the initial and the 6M samples show values greater than 80, indicating high similarity between the initial and 6M.

The cytotoxicity assay was conducted using MTT assay and the cytotoxicity of the chitosan, thiolated chitosan, trimethyl chitosan and drug loaded chitosan, thiolated chitosan, trimethyl chitosan NPs was performing on the differentiated RPMI 2650 cells (human nasal septum squamous cell carcinoma). The toxicity of TC NPs suspension (40 mg/ml), TMC NPs suspension (40mg/ml), soluble TC (20 mg/ml) and soluble TMC (20 mg/ml) were found to be non significant ($p < 0.05$); which may be due the covalent and reversible binding of polymer with the nasal epithelial cells. We observed a decrease in cell viability when cells were incubated with soluble TC and

soluble TMC at relatively high concentration ($p < 0.001$). The Triton X 100 (50 microlitere/ml) was choosen as positive control. To illustrate the safety of TC and TMC, its effect on the cell viability was directly compared with that of Triton X 100. A substantial decrease of the cell viability was observed after incubation with Triton X 100 compared to HBSS-HEPES ($p < 0.001$). Chitosan NPs suspension (10 mg/ml) and soluble chitosan (5 mg/ml) showed less cell viability compared to TC NPs suspension (40 mg/ml), TMC NPs suspension (40mg/ml), soluble TC (20 mg/ml) and soluble TMC (20 mg/ml). The possible explanation is the non-covalent and irreversible binding of polymer with the nasal epithelial cells; which leads to more cytotoxicity of chitosan and chitosan NPs compared to thiolated chitosan and its NPs. Soluble chitosan shows the less cell viability compared to the chitosan suspension. The high cell viability of TC NPs and TMC NPs may be explained by increase solubility of chitosan after thiolation at physiological pH resulting in to quicker removal from the site of application but at the same time forming intimate contact with the mucosae due to covalent linkage. Hence, trans-mucosal drug delivery will be improved due to required retention of thiolated NPs on nasal mucosae but due to complete removal from the mucosae through mucociliary clearance improves safety which is otherwise problem with the chitosan and is a contributing factor to its nasal mucosa toxicity.

Transepitheial electrical resistance was measured for TZ/CBZ formulations at different time intervals. In TZ/CBZ solution sample showed negligible change in the initial TEER value during the incubation of cells up to 150 minutes. In case of TZ/CBZ loaded chitosan NPs, TZ/CBZ loaded thiolated chitosan NPs and TZ/CBZ loaded trimethyl chitosan NPs, the TEER value was decrease in short time (30 minutes) and further decrease more at 150 minutes. TZ/CBZ loaded thiolated chitosan NPs and trimethyl chitosan NPs showed a significant ($p < 0.05$) reduction in TEER values than the TZ/CBZ solution and TZ/CBZ loaded chitosan NPs. Permeation of TZ/CBZ across the RPMI 2650 cell monolayer determines in AP to BL direction. P_{app} of TZ and CBZ solution was found to be $0.674 \pm 0.032 \times 10^{6}$ cm/second and $0.596 \pm 0.029 \times 10^{6}$ cm/second respectively. TZ/CBZ loaded thiolated chitosan NPs and trimethyl chitosan NPs shows the significant ($p < .05$) highest P_{app} value than TZ/CBZ loaded chitosan NPs and TZ/CBZ solution across the RPMI 2650 cell monolayer. P_{app} of TZ loaded thiolated chitosan NPs, trimethyl chitosan NPs and

chitosan NPs was found to be 29-fold, 31-fold and 13-fold higher than the higher than the TZ solution respectively. P_{app} of CBZ loaded thiolated chitosan NPs, trimethyl chitosan NPs and chitosan NPs was found to be 33-fold, 37-fold and 18-fold higher than the higher than the CBZ solution respectively.

Recovery after 150 min of incubation of TZ/CBZ loaded chitosan NPs formulation shows the irreversible decrease in TEER due to destruction of the tight junctions of the RPMI 2650 cell monolayer. These results might be associated with the higher cytotoxicity of chitosan NPs than the thiolated chitosan NPs and trimethyl chitosan NPs. Thiolated chitosan/Trimethyl chitosan NPs have an ability to decrease the initial TEER value that can be explain by the possible interactions between the positive surface charge with the anionic components of the glycoproteins on the surface of the nasal epithelial or to the negative charges of the interior of the tight junction.

Tizanidine HCl and cyclobenzaprine HCl formulations were effectively radiolabeled with Technetium-99m (^{99m}Tc), optimized for maximum labelling efficiency and stability. The quantity of stannous chloride to reduce ^{99m}Tc plays an important role in the labelling efficiency. Lower quantity of stannous chloride leads to low labelling efficiency where as higher amount of stannous chloride leads to formation of undesirable radiocolloids. The optimum quantity of stannous chloride for high labelling efficiency and low free and reduced/hydrolyzed ^{99m}Tc, was found to be 250µg for all preparations. The incubation time was optimized at 30minutes. The pH for all the formulations was kept at around 6.5. The labelling efficiency and the stability of labelled complex were ascertained by ascending TLC using ITLC strips. Radiochemical purity of ^{99m}Tc labelled TZ solution, LMC-TZ NPs, MMC-TZ NPs, LMTC-TZ NPs, MMTC-TZ NPs, LMTMC-TZ NPs and MMTMC-TZ achieved was 97%, 97.5%, 97.9%, 99.2%, 98.5%, 99.1%, 98.89% respectively. Radiochemical purity of ^{99m}Tc labeled CBZ solution, LMC- CBZ NPs, MMC- CBZ NPs, LMTC-CBZ NPs, MMTC- CBZ NPs, LMTMC- CBZ NPs, and MMTMC- CBZ achieved was 98.12%, 98.89%, 99.32%, 99.59%, 97.99 %, 99.21%, 98.99 % respectively. The results suggested high stability of ^{99m}Tc labelled tizanidine HCl formulations and cyclobenzaprine HCl formulations. The stability studies of ^{99m}Tc labelled tizanidine HCl and cyclobenzaprine HCl were carried out *in-vitro* using normal saline and mice serum by ascending ITLC. The bonding strength of ^{99m}Tc labelled tizanidine HCl and

cyclobenzaprine HCl formulations was assessed by DTPA (Diethylene triamine penta acetic acid) challenging test. The effect of different molar concentration of DTPA on ^{99m}Tc labelled tizanidine HCl and cyclobenzaprine HCl formulations and percentage transchelation were studied. The percent transchelation of the labelled complex was below 4%w/w at highest concentration tested (50mM). The results suggested high bonding strength and stability of tizanidine HCl and cyclobenzaprine HCl formulations, thus these formulations were found suitable for biodistribution studies of the drug in mice.

Biodistribution studies of ^{99m}Tc-TZ/^{99m}Tc-CBZ following intravenous, oral and intranasal (^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC-CBZ NPs, MMTMC- CBZ NPs) administration on Swiss albino mice were performed and the radioactivity was estimated at predetermined time intervals up to 8 h. After nasal administration of ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs, lower the T_{max} values in brain (around 1 h) compared to blood (around 2 h) were observed. The brain/blood ratios of the drug were found to be higher for formulations when administered intranasally. This result further confirms direct nose-to-brain transport. Concentration of TZ/CBZ (solution/ NPs formulation) in brain following intranasal administration were found to be significantly higher at all sampling time points compared to TZ/CBZ solution (intravenous) and TZ/CBZ solution (oral) up to 8 h. This results may recognized by the higher mucoadhesion capacity of chitosan, thiolated chitosan and trimethyl chitosan NPs on nasal mucosa that significantly improves the drug transport by enhancing the residence time of formulations on nasal mucosa compared to the TZ solution (intravenous) and TZ solution (oral). Smaller size of NPs improves the penetration of formulations through the mucosal barrier. Oral TZ/CBZ in solution form, drug transport to the brain was also significantly less than the intranasal TZ/CBZ in solution, chitosan NPs, thiolated chitosan NPs and trimethyl chitosan NPs. These results may be attributed due to the first pass metabolism of drug by oral route. Higher brain uptake of TZ/CBZ was found in thiolated chitosan NPs/Trimethyl

chitosan NPs via intranasal route than the chitosan NPs. These results may be due to higher mucoadhesive strength of thiolated chitosan than the chitosan. These results may be associated with increase in solubility at physiological pH leads quicker absorption at site of administration. The substantially higher uptake of nanoparticles formulations in the brain with intranasal administration suggests a larger extent of selective transport of TZ/CBZ from nose-to brain. ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs formulations were observed $T_{1/2}$ of 2.25–3.08 h (blood), 1.75–6.38 h (brain) irrespective of the routes of administration and the type of the formulations. ^{99m}Tc-CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs formulations were observed $T_{1/2}$ of 17.71–24.52 h (blood), 16.89–21.94 h (brain) irrespective of the routes of administration and the type of the formulations. Lower C_{max} and AUC values were observed with chitosan NPs and TZ/CBZ drug solution. The mucociliary clearance under normal circumstances rapidly clears the instilled formulation. However, the drug was incorporated in higher mucoadhesive thiolated chitosan NPs/trimethyl chitosan NPs which significantly improved C_{max} and AUC. This demonstrates the value obtained with thiolated chitosan NPs/trimethyl chitosan NPs in prolonging the contact time of the formulation with the nasal mucosa. The drug targeting Index (DTI) and brain drug direct transport percentage (DTP (%)) were also calculated for nasally administered formulations. Both the drug loaded thiolated chitosan NPs/trimethyl chitosan NPs showed the highest DTI and DTP (%) values than the chitosan NPs and TZ/CBZ solution. These higher values of DTI and DTP (%) show the benefit of the thiolated chitosan NPs/trimethyl chitosan NPs for intranasal administration. The higher DTI and DTP (%) advocate that improved brain targeting efficiency of thiolated chitosan NPs mainly because of considerable direct nose-to-brain transport.

Gamma scintigraphy studies were performed on swiss albino mice for ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC-CBZ NPs formulations in order to visualize the drug localization in the Brain. In order to visualize the brain uptake following intranasal, oral and intravenous administrations of ^{99m}Tc-TZ/CBZ solution/chitosan NPs/thiolated chitosan

NPs/trimethyl chitosan NPs, we used a gamma scintigraphy camera for to derive complete biodistribution of TZ/CBZ. The gamma scintigraphy images in mice were taken after 15 minutes post intravenous injection, oral and intranasal administrations. High brain uptake of TZ/CBZ loaded thiolated chitosan NPs and trimethyl chitosan NPs into the brain was observed than the chitosan NPs and TZ/CBZ solution when administered via intranasal route. The scintigraphy images were consistent with the findings of the biodistribution studies.

Pharmacodynamic study was performed on swiss albino mice. NMDA induced hyperalgesia and antinociceptive activity was evaluated using hot plate method. Hyper activity of the excitatory amino acids (central neuronal hyper excitability) leads the migraine state, in which the higher concentrations of excitatory amino (such as glutamic and aspartic acid) were found in plasma, saliva, cerebrospinal fluid. An excitatory neurotransmitter such as glutamate, act on N-methyl-D-aspartate (NMDA) receptors that transmitted the nociceptive within the spinal cord. This was proven by the intrathecal injection of NMDA in mice induced the short duration hyperalgesia. CBZ solution, CBZ loaded chitosan/thiolated chitosan/trimethyl chitosan NPs were able to reverse the reduced licking latency observed by intrathecal administration of NMDA via intranasal route. CBZ loaded thiolated chitosan/trimethyl chitosan shows significantly ($p<0.01$) higher capability to reverse the NMDA-induced hyperalgesia, when administered 15 and 30 minutes before the hot plate test than the CBZ loaded chitosan NPs and CBZ solution through the intranasal route. All the CBZ formulations have higher ability to reverse the NMDA-induced hyperalgesia via intranasal route than the oral route when administered 15 and 30 minutes before the hot plate test. These findings may be associated with the improvement of mucoadhesion and permeability of the chitosan by thiolation and methylation. Thiolation was also inhibiting the metabolism of CBZ by the cytochrome P450 enzymes present in the nasal cavity that reduces the breakdown of the drugs administered in the nasal cavity.

Antinociceptive effect of intranasal administered TZ formulations was significant higher ($p<0.05$) than the oral administration of TZ solution after 30 minutes. TZ loaded thiolated chitosan/trimethyl chitosan shows significantly high antinociceptive effects than the TZ loaded chitosan NPs. These findings may be due to the high mucoadhesion and permeation capacity of trimethyl chitosan and thiolated

chitosan than the chitosan. High permeation of thiolated chitosan and trimethyl chitosan was due to improvement of solubility at physiological pH after thiolation and methylation respectively. Intranasal TZ solution shows significantly lower antinociceptive effect than the NPs formulations. Theses results may be explained by the reversibly opening of the paracellular route for hydrophilic molecules so more drug can absorbed through nasal mucosa.

11.2. Conclusions

Thiolated and trimethyl derivatives of chitosan were successfully prepared by carbodiimide and quaternization methods respectively. The derivatization of chitosan was confirmed by IR and NMR spectroscopy. Comparative mucoadhesive behaviours of derivatized chitosan and chitosan were measured by mucin particle method. Thiolated chitosan and trimethyl chitosan were found to have higher mucoadhesive strength than the chitosan. Improved mucoadhesion of derivatized chitosan may be due to formation of covalent bonds between the thiol groups and cysteine residue of mucin glycoproteins on mucosal surface and formation of ionic bond between positive charges of trimethyl chitosan and negatively charged sialic groups on the mucus protein structure respectively.

Thiolated chitosan and trimethyl chitosan nanoparticles containing drugs were prepared under mild conditions using sodium alginate as cross-linker to avoid the organic solvent and complicated process parameters difficult to optimize. The nanoparticles formed were in nanometer size with narrow size distribution and positive charge. The positive charge was found to be beneficial in mucoadhesion with anionic nasal mucosal layer. The size obtained for thiolated chitosan and trimethyl chitosan nanoparticles (200-300nm) were much smaller than the chitosan nanoparticles (400-700nm). The higher positive charge on the thiolated chitosan and trimethyl chitosan nanoparticles may be due to the smaller size of nanoparticles. It may also resulted into reduction in drug entrapment. Hence, sodium deoxycholate was used to enhance the entrapment of positively charged tizanidine HCl/cyclobenzaprine HCl by complexation with negatively charged sodium deoxycholate. This complex formation diminished the repulsion between the positively charged thiolated chitosan/trimethyl chitosan and drug so more amount of drug adsorbed on the surface of thiolated chitosan/trimethyl chitosan.

The transepithelial drug permeation found to increase significantly after thiolation and methylation of chitosan, when evaluated across the RPMI 2650 cell monolayer, compared to tizanidine HCl/cyclobenzaprine HCl solutions. Significant reduction in cytotoxicity (RPMI 2650 cells) was observed after derivatizion of chitosan. It may prove safer mucoadhesive agents for intranasal drug administration compared to chitosan probably due to the formation of sturdy reversible covalent bond.

Cytotoxicity of chitosan nanoparticles is due to the sharp decrease and slow recovery of transepithelial electrical resistance across cell monolayer. chitosan was thiolated/methylated to increase solubility at physiological pH and to get a polymer having rapid and more intimate contact with the mucosae at the same time quicker removal from the site of application i.e. recovery of complete transepithelial barrier before administration of subsequent dose. The findings of our cytotoxicological evaluation supports our hypothesis and strengthens our argument of quicker and improved trans-mucosal drug delivery due to required retention of thiolated chitosan/trimethyl chitosan nanoparticles on nasal mucosae and removal from the mucosae through mucociliary clearance completely due to enhance solubility which is otherwise problem with the chitosan and is a contributing factor to its nasal mucosal toxicity.

Confocal studies demonstrated that the rhodamine B permeation across the sheep nasal mucosa increased significantly from thiolated chitosan/trimethyl chitosan nanoparticles. Biodistribution studies of radiolabel drug in swiss albino mice confirmed the rapid and enhanced delivery of tizanidine HCl/cyclobenzaprine HCl in brain after intranasal administration of thiolated chitosan/trimethyl chitosan nanoparticles. Gamma scintigraphy studies confirmed the high drug localization in to the brain for thiolated chitosan/trimethyl chitosan nanoparticles.

Pharmacodynamic studies demonstrated significantly improved antinociceptive activity of tizanidine HCl nanoparticles prepared from thiolated chitosan/trimethyl chitosan than the chitosan and drug solution. Cyclobenzaprine HCl loaded thiolated chitosan/trimethyl chitosan nanoparticles found to reverse the N-methyl-D-aspartate induced hyperalgesia in mice demonstrating its role in treatment migraine and confirmed increase in therapeutics value in treatment of migraine compared to drug chitosan nanoparticles or drug solution.

The findings of this investigation accrued into interesting and therapeutically beneficial chitosan derivatives for nose to brain drug delivery. Nanoparticles prepared using thiolated/methylated chitosan were in nanometer size, with high drug entrapment, less toxicity, quick and strong mucoadhesion and significantly improved drug brain uptake and pain alleviation activity of a centrally acting highly water soluble drugs, tizanidine HCl and cyclobenzaprine HCl. To conclude, mucoadhesive agents prepared after derivatization of chitosan may used in development of more useful product for nose to brain drug delivery to treat many CNS or CNS related disorders (otherwise difficult to treat) even for hydrophilic drugs, such as tizanidine HCl and cyclobenzaprine HCl, having limited ability across blood-brain barrier once administered systemically. However, benefits to risk ratio and clinical intricacies need to be scientifically established for its suitability in human.