CHAPTER 8 SUMMARY AND CONCLUSIONS

Summary and Conclusions

Many drugs are not being effectively and efficiently delivered using conventional drug delivery approach to the brain and to the central nervous system due to its complexity. Brain and central nervous system have limited accessibility to blood compartment due to number of barriers. Many advanced and effective approaches for delivering drugs to brain have emerged in recent years. Intranasal drug delivery is one of the focused delivery options for brain-targeting as the brain and the nose are connected to each other via the olfactory and the trigeminal pathways, and via peripheral circulation. Realization of nose-to-brain transport and therapeutic viability of this route can be traced from the ancient times and has been scientifically investigated for rapid and effective transport of drugs in last two decades. Various models have been designed and studied by the scientists to establish the qualitative and quantitative transport through nasal mucosa to the brain. However, presently, development of nasal drug products for brain-targeting is still faced with enormous challenges. A better understanding in terms of properties of drug candidate, nose-to-brain transport mechanism and transport to and within the brain is of utmost importance. There are some pertinent practical issues and challenges which must be considered when formulating brain-targeted intranasal drug delivery.

Intranasal drug delivery delivers the drug directly to the brain circumventing the brain-barriers and limits drug delivery to non-targeted sites. Direct transport of drugs to the brain may result in administration of low dose and in turn can reduce toxicity. Also, systemic dilution effect and first pass metabolism are avoided. Direct transport could result rapid and higher uptake in the brain, which provides an alternative option of self-medication in management of emergencies. However, a few limitations of intranasal delivery such as low dose/volume especially when compounds have less aqueous solubility are difficult to formulate. High lipophilicity and preferably low molecular weight of drug are the prerequisite as it can greatly influence the uptake across nasal mucosa. Drug compounds devoid of offensive/pungent odor/aroma and non-irritant nature are highly desirable to facilitate dosage form design for intranasal drug delivery systems. Many scientists have reported unique connection between the nose and the brain which result in direct nose-to-brain transport. Lian Li et al reported rapid onset intranasal delivery of diazepam using ethyl-laurate-based microemulsion. At 2 mg/kg dose, the
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maximum drug plasma concentration was arrived within 2 to 3 min, and the bioavailability (0-2 h) after nasal spray compared with i.v injection was about 50%. They inferred that this approach may be helpful during emergency treatment of status epileptics. Illum et al have studied the effect of chitosan-morphine nasal formulation vis-à-vis slow intravenous (i.v.) infusion of morphine in healthy volunteers who reported sedation at the earliest time point after nasal administration compared with i.v. administration. They concluded that following nasal administration, morphine reaches more rapidly to CNS following intranasal administration compared to intravenous administration.

Many sophisticated and effective approaches to CNS drug delivery have emerged in recent years. Direct transport of drugs through the olfactory pathway to the CNS has generated immense interest in devising strategies and methodologies to exploit this approach as a portico for CNS drug delivery. However, numerous factors work in tandem which determines the effectiveness of drug delivery. The problems arise due to the physiological status in terms of nasal function and accompanying pathologies, and pharmaceutical challenges with respect to CNS drug delivery, for instance, low bioavailability, local irritation and toxicity upon long-term usage. Synthesis of more lipophilic analogues, enzyme inhibitors, permeation enhancers, colloidal and bioadhesive novel drug delivery modalities could help to eliminate few of the problems to some extent. Few formulations have already been successfully on the market and many are under phase I/II/III clinical stages. Furthermore, the emergence of peptide and protein moieties in the therapeutic scene has certainly heightened the scientific and industrial attention to rediscover the potential of this route of drug delivery. It is needless to say that the nasal route with all its inherent advantages has been heralded as the most promising approach for delivery of drugs to the brain/CNS in near future.

Status epileptics, a neurological disorder, require quick management of seizures to avoid permanent damage to the brain. Clonazepam, a benzodiazepine derivative is used widely in the treatment of Status epileptics. Clonazepam is preferred over other benzodiazepines due to its longer duration of action (24 h). Clonazepam is the drug of choice in suppression of the myoclonic seizures and it acts by increasing the effectiveness of the
inhibitory neurotransmitter, gamma amino butyric acid, within the central nervous system. Presently, clonazepam is available in tablet and injectable dosage forms (Revotril, Roche, USA). These formulations release clonazepam in peripheral circulation which limits the drug uptake across the brain-barriers and result in drug distribution to non-targeted sites. Although intravenous administration provides rapid seizure suppression, an alternative route of drug delivery is needed since oral and intravenous routes for delivering drugs are sometimes impractical and inconvenient, for instance, because of a delay in hospitalization of the patient, lack of an available hospital facility or a patient condition incompatible with oral ingestion of a tablet dosage form.

Migraine attack is a troublesome physiological condition associated with throbbing, intense headache in one-half of the head. During an attack, the blood vessels in the brain dilate and then draw together with stimulation of nerve endings near the affected region. These changes to the blood vessels are probably what cause the pain, although migraine is still a poorly understood condition or phenomenon. Sumatriptan and zolmitriptan, triptan derivatives are serotonin (5-hydroxytryptamine) agonist available in the market in form of oral tablets and subcutaneous injection for the treatment of migraine. These drugs are also available in rectal suppository dosage form for the treatment of migraine attacks. Substantial proportion of the migraine patients not only suffer from gastric stasis but have also been associated with severe nausea and vomiting, at large. These circumstances may lead to erratic absorption of triptan from the gastrointestinal tract and may result in ineffective treatment. Moreover, the situation will lead to incompatible or inconveniency to the patient in oral ingestion of the dosage form. Hence, triptan delivered by the conventional routes may result in ineffective treatment of migraine. Reports in the literature on animal studies (rat and rabbits) revealed that orally ingested triptan undergoes first pass metabolism which results in poor bioavailability for instance less than 40% whereas in humans it was found less than 14%. In animal species, circulating sumatriptan gets cleared rapidly from plasma by metabolism and renal clearance with a half life of 1-2 h. Moreover, the passage of triptan across the blood-brain barrier is not appreciable although evidence of detection of some drug in cerebrospinal fluid following high intravenous doses can be cited in the literature. In light of above
facts, an alternative drug delivery system is needed which can selectively target the drug to the site of action (brain). Due to preferential transport of drugs to the target, intranasal delivery approach may be expected to reduce the first pass metabolism due to poor and/or restricted distribution of the drug to the non-targeted sites such as systemic/peripheral circulation. Intranasal drug delivery also offers the advantages that drugs can be administered simply, cost effectively and conveniently. Direct transport of drugs to the brain circumventing the brain-barriers following intranasal administration provides a unique feature and better option to target drugs to the brain. However, to enhance effectiveness of drug, a few issues should be carefully considered when designing intranasal drug delivery. The formulation should be designed so as to provide rapid transport of drug across nasal mucosa and longer residence time in nasal cavity. Microemulsions, by virtue of their lipophilic nature and having low globule size, are widely explored as a delivery system to enhance uptake across mucosa. Addition of a mucoadhesive agent such as a polyelectrolyte polymer helps in retention of the formulation on the nasal mucosa. Evidences of intranasal drug delivery systems formulated using mucoadhesive agent and its benefits in enhancing nose-to-brain drug transport have been reported by many scientists.

The objectives of this investigation were to prepare and optimize rapid brain-targeted microemulsions/mucoadhesive microemulsions of CL, ST and ZT, and evaluate its performance in vitro and in vivo in rats. Studies were focused on the preparation of microemulsions/ mucoadhesive microemulsions, characterization, optimization, biodistribution, elucidation of nose-to-brain transport mechanisms, and justification of role of developed formulations in the treatment of acute status epileptics and migraine. It was hypothesized that microemulsion of selected drugs will promote direct nose-to-brain transport by olfactory and trigeminal pathways and drugs will also cross BBB by systemic route greater drug transport and distribution into and within the brain. Hence, this can help to maximize the therapeutic index of the drug, reduce side effects, reduce the dose and frequency of dosing and perhaps even the cost of the therapy.

Clonazepam drug substance was estimated using spectrophotometric method at 245 nm and 310 nm. The absorbance was found linear between 0.50 mcg/mL - 10 mcg/mL hence,
was used for estimation of clonazepam. The method was validated for accuracy, precision, linearity, primary stock solution and standard (QC) solution stability, and robustness/ruggedness of the method was found to meet USP criteria. The validation parameters were found meeting the USP criteria. The regressed graph plotted for linearity revealed that correlation coefficient ($r^2$) was greater than 0.999. The dilution integrity was established up to 100 mcg/mL concentration. Clonazepam formulations, clonazepam in diffusion media (in vitro permeation studies) and drug retention studies samples were analyzed using HPLC method. The method was validated for accuracy, inter- and intra-day precision, solution stability and linearity. The method was found to be linear over a concentration range between 0.10 µg/mL and 10 µg/mL. The regressed graph plotted ($n=6$) and the correlation coefficient for 6 different sets were found greater than 0.999. The statistical parameters such as CV (%) and SD were found to meet the USP criteria. Microemulsions were analyzed by preparing dilution in methanol and final dilution was made using the mobile phase. The ingredients used for microemulsion preparation or diffusion media were not found to interfere with the proposed method. The validation parameters were found to meet the “readily pass criteria” specified in the USFDA guidance and CV (%) were found less than 3% for clonazepam formulation, clonazepam estimation in diffusion media and clonazepam estimation during drug retention studies. Regression analysis data ($r^2 > 0.999$) and stability (long and short term) at ambient temperature and 2°C to 8°C were found satisfactory meeting the RSD/SD criteria. Clonazepam in blood and brain homogenate samples were analyzed using LC/MS/MS method. The method was validated as stated in the “USFDA bioanalytical method validation guidance for industry”. The method was found accurate and precise (CV <15% for LLOQ, LQC, MQC and HQC samples) over a range to 0.10 ng/mL to 10 mg/mL. The inter- and intra-day precision was found meeting the criteria at all three QC sample concentrations and the injection repeatability precision was less than 10% (CV). The recovery study was also found satisfactory (CV < 15%). Stability of primary stock solution and standard (QC samples) at 2°C to 8°C (long term and short term) were found satisfactory. Freeze and thaw cycles for plasma and brain homogenate samples were within the stated norms of USFDA guidance and the results were reproducible up to three
cycles. Sumatriptan was analyzed using HPLC method equipped with C8 column and detector at 245 nm. The linearity results of drug substance, formulation, diffusion media and drug retention studies samples revealed that correlation coefficient ($r^2$) was greater than 0.999. The method was found to be linear at 0.50 mcg/mL to 20 mcg/mL concentration range. Zolmitriptan was analyzed using HPLC method equipped with C18 column (shimadzu) and detector at 245 nm. The linearity results of drug substance, formulation, diffusion media and drug retention studies samples revealed that correlation coefficient ($r^2$) was greater than 0.999. The method was found to be linear at 0.20 mcg/mL to 20 mcg/mL concentration range. The excipients used for preparation of microemulsions were not found to be interfering with the method for sumatriptan and zolmitriptan. The SD and CV for inter- and intra-day precision, and accuracy were within the USP criteria (<5%).

Clonazepam microemulsions were prepared using the titration technique followed by construction of pseudo-ternary phase diagrams. Four different systems were prepared wherein system 1 and 2 comprise of medium chain triglyceride as an oil phase and propylene glycol as anhydrous aqueous phase. Surfactant and co-surfactant were caprylocaproyl macrogolglyceride and propylene glycol laurate respectively for system 1, and poloxyl ethylene-35-ricinoleate and polyethylene glycol (PEG) monooleate respectively for system 2. Microemulsions formation was spontaneous upon addition of aqueous phase to drug in oil-surfactant-cosurfactant mixture. The solubility data shown that clonazepam has maximum solubility in medium chain triglyceride and sunflower oil (> 5 mg/mL) therefore; these oils are selected to formulate microemulsions. Clear solution of drug in oil-surfactant was obtained on application of heat above 50°C and upon cooling no precipitation was observed. However, with castor oil, corn oil and isopropyl myristate, the solubility of drug in oil-surfactant mixture was less than 5 mg/mL. Moreover, nasal formulations are concentrated preparations as low volumes can be administered into the nostril (< 200 μL), the microemulsion base was selected on the merits of solubilization capacity of clonazepam. Similarly, system 3 and system 4 were formulated in the identical manner as system 1 and system 2 respectively by replacing medium chain triglyceride with sunflower oil. The selection of surfactant and co-
surfactant mixture was on the basis of HLB values. The mixtures reported in literature and which can provide HLB value between 9 and 12 were selected. Microemulsions were formulated at different surfactant: co-surfactant ratio such as 1:1, 2:1 and 4:1. The percent transmissions (at 630 nm) of the formulated microemulsions were greater than 99% and such microemulsions were represented in pseudo-ternary phase diagram. These points were plotted and connected in a clock-wise manner to obtain microemulsion region. It was observed that the microemulsion formation was spontaneous having transmission value greater than 99%. However, at S: CoS ratio (2:1), the percent transmission was approximately 95%. This may be attributed to clear gel formation. The gel formation may be accredited to higher oil concentration (approximately 40% w/w). It is evident from the data that percent transmission is greater than 99.5% where the oil concentration is less than 20% w/w. Upon repetition of the experiments, it was observed from the standard error of mean of the percent transmission value that formation was spontaneous and process was reproducible. At large, it was observed from the data that increase in concentration of oil phase, resulted in increase in globule size and zeta potential. The globule size was fairly reproducible within the SEM ± 25 nm range whereas for zeta potential SEM was found to be within ± 2 mV. Comparing globule size of S:CoS ratios 1:1, 2:2 and 4:1, it was observed that increase in the S:CoS ratio does not appreciably reduce globule size. Therefore, it was concluded that the concentration of S:CoS may be critical for the formation of clonazepam microemulsions rather than increase in the HLB value by increasing surfactant concentration. It was also observed that increase in the aqueous phase concentration resulted in increase in the zeta potential (anionic). Reports in the literature revealed that microemulsions having zeta potential between -30 mV to -60 mV exhibit moderate to best physical stability in terms of phase separation. Therefore, microemulsions having zeta potential less than -30 mV were selected for further studies. Also, microemulsions with less globule size may have larger surface area and better permeation across the mucosal interstitial spaces. Therefore, globule size of 50 nm was identified as a filter for the selection criteria for further studies. Comparing system 1 and system 2 for the microemulsion region, the microemulsion region obtained with polyoxyethylene-35-ricinoleate and PEG monooleate was found to
be wider. This may be attributed to the fact that polyoxyethylene-35-ricinoleate have less HLB value compared to caprylocaproyl macrogol glyceride and therefore, can accommodate larger concentrations of oil phase in the formulations. Increase in the concentration and the ratio of surfactant to co-surfactant, resulted into formation of bicontinuous microemulsions or o/w microemulsions. Comparing system 1 and system 2 vs. system 3 and system 4, it was observed that the S:CoS systems used for MCT produced smaller microemulsion regions when sunflower oil was used. The oil concentrations in excess of 30% w/w did not yielded microemulsions like system 1 and system 2. This may be attributed to low HLB requirement for sunflower oil based microemulsion systems (<9). However, the globule sizes obtained for system 3 and system 4 were less compared to system 1 and system 2 at all three S:CoS ratios (1, 2 and 4). Zeta potential was also found to reduce compared to medium chain triglycerides with increase in the oil concentration. It is also evident from the data that increase in the concentration of aqueous phase contributed negatively to the system and found to reduce the net negative charge of the system (anionic potential). The surfactant ratio and concentration also found to contribute negatively to the system and found to reduce zeta potential of the system with increase in the concentration. At large, the microemulsions were found to be clear, transparent (transmittance > 99% at 630 nm), spontaneous formation and either of o/w or bicontinuous for all four systems of clonazepam microemulsions. The prepared microemulsions of clonazepam having globule size less than 50 nm and zeta potential less than -30 mV have been further evaluated for physical stability and chemical stability. Sumatriptan microemulsions were prepared using titration technique and the microemulsion regions are plotted using pseudo-ternary phase diagrams. Four different systems (system 1, 2, 3 and 4) of sumatriptan microemulsions were prepared wherein system 1 and system 2 consist of medium chain triglyceride as oil phase whereas system 3 and system 4 were prepared using corn oil. The oils were selected on the merit of highest solubility of sumatriptan which is performed using biopharmaceutical classification system solubility studies. System 1 and system 3 were prepared using caprylocaproyl macrogolglyceride and fatty acid esters of polyglycerol as surfactant and co-surfactant respectively whereas system 2 and system 4 were prepared
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using caprylocaproyl macrogol glyceride and plurol oleique as surfactant and co-
surfactant respectively. Microemulsions formation was found to be spontaneous and the
prepared microemulsions were visually clear and transparent. The percent transmissions
were measured using spectrophotometer at 630 nm. The results indicated that percent
transmission for system 1 and system 2 were greater than 99% for all the batches of
microemulsions which were transparent. The SEM data for transmittance for six different
batches indicated that process was reproducible and all the values are within the range of
±1 %. Microemulsions for all the systems were prepared at S:CoS ratios 1, 2 and 4. A
few of the batches show globule size greater than 200 nm which were visually hazy in
appearance and in addition to that, it showed percent transmittance less than 98%
(Formulation 3 at S:CoS ratio 2:1). It was found that with increase in the oil
concentration, the globule size also increased significantly and lead to poor transmittance
(98% to 99.2%). However, system 2 showed less globule size compared to system 1 at
same S:CoS concentration and ratios. The globule sizes were found within ± 25 nm of the
estimated globule size which indicates uniform globule size distribution and narrow
distribution for all the batches. Increase in the oil concentration and reduction in the
water concentration resulted in increase in cationic charge and the system shows net
positive charge compared to other batches. However, apparently no size separation was
noticed immediately after formation of microemulsions. As the ratio of S:CoS was
increased from 1:1 to 2:1 and 4:1, no appreciable change in the globule size was noticed
although, the zeta potential were found to increase in 4:1 ratio as compared to 1:1 ratio of
S:CoS. This may be attributed to the fact that increase in the surfactant favors formation
of o/w system, and increase in the oil concentration may lead to reduction in the
concentration of aqueous phase (water) which may result in poor conductivity and
increased zeta potential. Moreover, it was also found that increase in the total
concentration of S:CoS, the globule size decreases and zeta potential increases. This may
be due to increase in the concentration of co-surfactant result in formation of
bicontinuous or o/w system due to higher HLB value hence, the negative charge of the
system also increases and globule size decreases due to less concentration of oil phase.
Comparing system 1 and 3, it was found that corn oil increase possibility of formation of
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O/w microemulsions whereas medium chain triglyceride facilitates formation of w/o microemulsions. It was found that the microemulsion region for corn oil gets shifted towards aqueous vertices (higher concentration) and the microemulsion region also expands in size. In addition, the globule size data were also found to be consistent with the observations. It was observed that, change in the oil phase from MCT to corn oil, the globule size at same concentration and S:CoS ratio were found to be decreasing significantly. Increase in the total concentration of S:CoS resulted in minimum globule size and maximum zeta potential (SME 3, batch no. 05) and the percent transmission was found to be greater than 99.9%. Comparing formulation 05 of SME 3 (1:1) and formulation 01 (4:1), it was observed that increase in the co-surfactant concentration does not appreciably affect microemulsion formation, globule size and zeta potential. Therefore, globule size and zeta potential is mainly the function of surfactant concentration for sumatriptan microemulsions. Comparing system 3 and system 4, the globule size and zeta potential were found to be less in system 4 compared to system 3. This may be due to combination of corn oil with pluronic oleique which may reduce the total energy of the microemulsion system at the time of formation and lead to narrow globule size distribution and least zeta potential. The sumatriptan microemulsions were found to be clear, transparent (transmittance > 99% at 630 nm), spontaneous formation and either of o/w or bicontinuous for all four systems. The prepared microemulsions of sumatriptan having globule size less than 50 nm and zeta potential less than -30 mV have been further evaluated for physical stability and chemical stability. Sumatriptan succinate microemulsions were also prepared using the same systems as mentioned under sumatriptan microemulsions. Total four systems were prepared in the identical manner as sumatriptan. However, the solubility of drug in oil was not kept at the criteria. The drug was dissolved in the water phase and added to oil containing surfactant and co-surfactant mixture which spontaneously resulted in clear, transparent, homogenous microemulsions. Comparing sumatriptan succinate with sumatriptan microemulsions, the globule size were found to be higher at S:CoS ratios and concentrations. This may be attributed to the fact that sumatriptan succinate being more water soluble may compete for solubilization and in turn may result in larger oil globules and high energy system
compare to sumatriptan. The zeta potential was found unaffected and close to sumatriptan microemulsions. This may be due to the fact that drug do not impact much on the surface charges and hence, no change in the zeta potential. However, increase in surfactant or oil concentration within the sumatriptan microemulsion systems, contributed negatively and zeta potential and globule size distribution was found to be increased. The clarity and percent transmission was greater than 99% for all the batches which were prepared. However, the microemulsion regions were having wide area, indicated formation of o/w microemulsions. The zeta potentials were found to be between -40 mV to -50 mV for the experimental batches where the aqueous phase concentrations are more than 40% w/w. This may be attributed to the fact that increase in water concentration may result in better conductivity and in turn contributed negatively to the system (anionic potential). The microemulsion regions are wide and spread over the large area in the pseudo-ternary phase diagrams. However, no appreciable changes in the microemulsion regions were noticed with increase in the ration and S:CoS concentration. When sumatriptan microemulsions were compared to sumatriptan succinate microemulsions with regards to microemulsion area within the four systems, system 4 was fond to better of other three systems and larger microemulsion region was found. Surfactant concentration between 25% and 35% was found to be optimum and below or above these concentrations, the micelle size and size distribution was found to increase. This may be attributed to the fact that sumatriptan succinate may remain and have high affinity for aqueous phase and a part of water phase may remain non-emulsified resulting into non formation of micelles. Zolmitriptan microemulsions were prepared using titration technique followed by construction pseudo-ternary phase diagrams. The phase boundaries are identified using titration and microemulsion regions were identified and plotted using pseudo-ternary phase diagram. Four different systems were prepared using MCT as oil phase (system 1 and system 2) whereas corn oil was used for the preparation of system 3 and system 4. Caprylocaproyl macrogol glyceride and fatty acid ester of polyglycerol were used as surfactant and co-surfactant (system 1 and system 2) and caprylocaproyl macrogol glyceride and mixture of plurol oleique with transcutol (1:1) were used as co-surfactant. The ratios of surfactant: co-surfactant was 1, 2 and 4. Percent transmissions for all the
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clear microemulsions were found greater than 99% with SEM within ± 0.9. However, for the experimental batches where the total concentration of surfactant and co-surfactant was less than 30%, microemulsions were found hazy and percent transmissions values were less than 99%. It was observed that increase in the oil concentration resulted in increase in the globule size, may be due to part of the oil phase may not form micelles. However, at large, increase in the surfactant and co-surfactant concentration from 30% to 50% resulted in reduction in the globule size and size distribution. This is indicative of formation of micelles of oil phase with water phase and, surfactant: co-surfactant mixture. Increase in the concentration of water phase and reduction in the oil phase shown increase in the net negative charge of the system and reduction in zeta potential. The zeta potential for system 1 was between -18.20 mV to -49.88 mV. The highest zeta potential was found in the system containing 10% of oil phase and 50% of aqueous phase (water). Increase in the ratio of concentration of surfactant: co-surfactant does not appreciably affect the globule size and size distribution of the microemulsions however, 30% to 50% concentration of surfactant: co-surfactant was found optimum leads to formation of microemulsions having globule size less than 50 nm. Comparing system 1 with system 2, the microemulsion region was found to increase in system 2. It was also observed that system 2 can be formulated with aqueous phase concentration as high as 70%. This may be attributed to the fact that, zolmitriptan being more water soluble at neutral pH (> 20 mg/mL) may interact and get uniformly distributed in water and oil phase hence, non formation of micelles was not observed even at higher aqueous phase concentrations. Pseudo-ternary phase diagrams also revealed that, there was appreciable change in the phase boundaries for system 1 at S: CoS ratios 1, 2 and 4 however, insignificant changes in the phase boundaries of microemulsion regions were noticed for system 2. Comparing system 3 with system 4, the globule size and size distribution of system 3 was smaller and narrow compared to system 4 for all the experiments when compared using same concentration levels of oil and aqueous phases. The least globule size for system 3 was found to be 19.70 nm ± 11.03 nm whereas for system 4, it was found to be 31.56 nm ± 14.19 nm. In system 3 as well as system 4, zeta potential was found to reduce (anionic) with increase in the ratio and concentration of S: CoS. It was

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seen from the data that zeta potential appreciably changes with increase in the concentration of water phase in addition to s: CoS. In most experimental batches for all four systems of zolmitriptan microemulsions, it was found that optimum surfactant: cosurfactant concentration (total) between 30% and 50% resulted in formation of microemulsions with globule size less than 50 nm whereas optimum oil concentration was between 10% and 20%, and increase in the aqueous phase concentration resulted in decrease in the zeta potential (increase in anionic charge) of the system. This may be attributed to the fact that increase in surfactant level resulted in a decrease in surface tension and surface free energy of the formed micelles. Therefore, net negative charge (anionic) of the microemulsion increased (Nash et al., 2002). The prepared experimental batches of microemulsions were expected to have good physical stability with respect to phase separation and/or flocculation when zeta potential is less than -30 mV. Comparing anhydrous microemulsions to aqueous microemulsions, it was observed that the globule size was less than 30 nm for most of the batches of anhydrous microemulsions of clonazepam compared to sumatriptan, sumatriptan succinate and zolmitriptan microemulsions. Moreover, lipophilic drugs require lower HLB values of the system (<10) for formation of microemulsion whereas, highly water soluble drug compounds such as sumatriptan succinate and zolmitriptan requires higher HLB value (>10) compared to clonazepam and sumatriptan microemulsions. The microemulsion formation was indirectly assessed by measuring the globule size and size distribution and it was found that clear, transparent and homogenous microemulsions were obtained having percent transmittance > 99%. The microemulsions of all the drug substances were prepared successfully and the microemulsion regions delineating phase boundaries were successfully plotted in a pseudo-ternary phase diagrams. Microemulsions were prepared using pseudo-ternary phase diagram technique although, since the construction of pseudo-ternary phase diagrams is time consuming and laborious, novel optimization technique using numerical simulation was also studied for the prediction of phase behaviour of the microemulsions at different ratios other than included in the experiments.
Numerical simulations with computer aided mathematical modeling was used for optimization of microemulsions of clonazepam at S: CoS ratios 1, 2 and 4. Three models viz. Quadratic, quadratic-linear and exponential fits were applied to study the phase behaviour predicted by each of the model and evaluated against the experimental data. Quadratic and exponential fits were selected since ratio 1, 2 and 4 as three different vertexes and hence, can be inter- and extra-polated using parabolus fits. The models were fitted by inputting the experimental data to MATLAB software. The mathematical modeling for system 2 was carried out in a similar fashion. For system 2, the ME regions of polygon shape were found to be appropriate. The theoretical values for different components of ME obtained from Quadratic, Quadratic-linear and Exponential models. These ME were prepared six times at different S: CoS ratios using the method described under section preparation of clonazepam microemulsions. The transmittance values (%) for quadratic-linear and exponential fits at different S: CoS ratios (1.50, 3.00 and 6.00) were found greater than 99% however transmittance was less than 21% at vertex-1 and vertex-3 for the quadratic fit. Experimentally prepared ME were visually clear for quadratic-linear and exponential fits at different S: CoS ratios (1.50, 3.00 and 6.00) and for quadratic fit (S: CoS ratio 6.00), milky and gel formation were obtained at vertex-1 and vertex-3 respectively. Therefore, quadratic-linear fit and exponential fits were more appropriate to predict ME regions at different S: CoS ratios for clonazepam microemulsions. Mathematical modeling and numerical simulation techniques were found to be useful tools for identification of ME region in pseudo-ternary phase diagrams. The results of this investigation suggested that the quadratic-linear fit and exponential curve fit are more appropriate techniques for optimization and theoretical prediction of clonazepam ME compositions at different S: CoS ratios. The mathematical model and numerical simulation may be extrapolated for the prediction of globule size and zeta potential if it can be applied and extrapolated in the similar fashion.

Microemulsions of clonazepam, sumatriptan, sumatriptan succinate and zolmitriptan were subjected to accelerated centrifugation for assessing the stability of the formed micelles. Four different batches of clonazepam were subjected for the assessment of physical stability. The data revealed no appreciable change before and after following
accelerated centrifugation for 15 minutes. Moreover, the layers from top, middle and bottom following centrifugation were sampled and analyzed to determine homogeneity. The drug content of the clonazepam in top, middle and bottom layer for formulation 05 was within ± 5 nm, for formulation 02 within ± 8 nm, for formulation 03 within ± 10 nm and for formulation 01, within ± 9 nm from the initial values. The data clearly suggested that clonazepam microemulsions were found physically stable under the testing conditions. The microemulsions were selected on the basis of globule size. All the batches of microemulsions were having globule size less than 50 nm and zeta potential less than -30 mV. It was also observed that microemulsions having zeta potential less than -30 mV gives reasonably good physical stability with regards to phase separation. For sumatriptan microemulsions, eight different batches having particle size less than 50 nm and zeta potential less than -30 mV were selected for the studies. Furthermore, significant increase and difference in the globule size and size distribution of a few of the batches were observed. It was also observed that the total concentration of surfactant and co-surfactant mixture was less than 40% for the batches where physical separation was observed. This may be attributed to the fact that the lower concentration of S:CoS mixture may result in spontaneous formation of microemulsion. However, it is indicative of phase separation on aging. The top layer showed higher globule size compared to middle and bottom layer, this may be due to separation of oil and floating on the top layer due to low bulk density compare to aqueous phase. Also, the globule sizes in the bottom layer was found similar to the initial values, this is indicative that the part quantity of oil phase gets separated and remaining oil phase gets emulsified by the surfactant: co-surfactant used in the formulation. Most of the experimental batches of sumatriptan succinate microemulsions did not show appreciable phase separation and the globule size values were found to be similar to the initial values. However, experimental batch with S:CoS concentration (70% w/w) showed significant separation and globule size in the top, middle and bottom layer was found to be 112 nm to 145 nm compared to initial values less than 50 nm. This may be attributed to the higher concentration of S:CoS and low concentration of oil and aqueous phase (10% w/w) which may lead to selective separation of oil phase/aqueous phase from the S:CoS mixture due to alike nature of
surfactant and co-surfactant molecules. The data clearly suggest that no appreciable separation was noticed in the experimental batches having S: CoS concentration 40% w/w and aqueous phase concentration up to 60% w/w. The globule size was observed within ± 15 nm compared to initial values and the size distribution following centrifugation were also found similar to the initial observations. Following accelerated centrifugation of zolmitriptan microemulsions, no appreciable change in globule size and size distribution were observed in any of the experimental batches. It was concluded that zolmitriptan microemulsions showed ± 15 nm compared to the initial values. It was concluded that zolmitriptan microemulsions were physically stable with oil phase concentration between 10 and 20%, S:CoS concentration 40% and aqueous phase concentration between 30 and 50% w/w. It was concluded that physical stability assessment can be successfully performed using accelerated centrifugation technique by sampling the microemulsions from top, middle and bottom layers. Microemulsions which are bicontinuous, w/o or o/w were found to be stable between oil phase concentration between 10 to 30% w/w, S:CoS concentration 40 to 60% w/w and aqueous phase concentration between 30 to 60% w/w.

Drug retention study were performed on physically stable microemulsions by subjecting clonazepam, sumatriptan, sumatriptan succinate and zolmitriptan microemulsions at 30°C / 65% R.H. and 40°C / 75% R.H. Clonazepam microemulsions were assessed for globule size, size distribution, zeta potential and percent transmittance. When globule size was evaluated up to six months, it was found that globule size was within the range of 18.23 nm to 22.76 nm (CME 1) and no abnormal changes in the globule size were noticed at both the accelerated testing conditions. The zeta potential values were also found to be consistent with the initial values. Zeta potential values for CME 1 were between -31 mV and -36 mV; for CME 2 between -30 mV to -35 mV; for CME 3 between -33 mV to -37 mV; and for CME 4 between -31 mV to -37 mV. The data clearly indicated that the formulations were physically stable at 30°C / 65% R.H. and 40°C / 75% R.H. without noticeable change in the zeta potential values. Percent transmittances for all the experimental batches were found to be greater than 99% which indicated the clarity of the tested microemulsions and indirectly gives an indication that
no separation was observed in the clonazepam microemulsions. Drug content for CME 1, CME 2, CME 3 and CME 4 were found between 97.88 and 99.45; 96.98 and 99; 96.22 and 100.29; and 95.99 and 99.78 % of the label claim (5 mg/mL) respectively. The data clearly demonstrated that there was no appreciable degradation at 40° C/75% R.H. The results of clonazepam microemulsions demonstrated that the formulations are physically and chemically sable at accelerated stability conditions. The formulations were found to meet the general monograph of Pharmacopoeia and criteria stipulated therein for the liquid preparations. Drug retention studies for sumatriptan was also performed as mentioned under ICH guidance. It was evident from the data that the globule size and size distribution remain unchanged after 6 months at both the testing conditions for all the formulations of sumatriptan. The globule size for SME 1, SME 2, SME 3 and SME 4 were found between 16 and 20 nm; 32 and 42 nm; 15 and 19 nm; and 12 to 18 nm respectively. No appreciable change was noticed when compared with the initial results. Zeta potential also was found to be consistent with the initial observations. The zeta potential values were within ± 5 mV compared to the initial observations indicated the physical stability of the oil/S:CoS/aqueous interface of the prepared microemulsions. The percent transmittance at 630 nm was found to be greater than 99% indicated the clarity of the emulsions. Values greater than 99% also suggest that there was no inversion, phase separation or cracking of the prepared microemulsions of sumatriptan. The globule size data and zeta potential values clearly pointed-out that the prepared microemulsions of sumatriptan were physically stable at both the accelerated conditions and the systems were found to be thermodynamically stable. It is also evident from the dug retention data that the degradation of drug substance was well within the criteria stipulated by the Indian Pharmacopeia. The content of the drug (sumatriptan) was found to be within ± 5% of the labeled claim (20 mg/mL). Similarly the stability data for sumatriptan succinate and zolmitriptan succinate demonstrated good physical and chemical stability. The globule size and zeta potential including drug content were found to be satisfactory and meet the stipulated criteria.

*In vitro* diffusion studies were performed to evaluate relative diffusion behavior of different formulations of clonazepam, sumatriptan, sumatriptan succinate and
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Zolmitriptan. Cumulative drug release across sheep nasal mucosa of clonazepam formulations, up to 8 h indicated that CME 2 (formulation 02) was found to have substantially higher diffusion across the sheep nasal mucosa. Furthermore, the rate and extent of diffusion, mean kinetic flux and diffusion coefficient were calculated from the cumulative drug release and concentration gradient at specific time intervals. The data clearly indicated that CME 2 (formulation 02) has the highest mean kinetic flux (7.86 µg/min) and diffusion coefficient (1.87E-08 cm²/min) amongst the tested formulations. The mechanism of drug release was also predicted by inputting the regressed data into the excel spread sheet and the result demonstrated that all the tested formulations of clonazepam follow Highuchi’s drug release kinetics whereas, the regression coefficient values were found less for zero-order and first-order compared to Highuchi’s kinetic curve-fit. Release kinetics of sumatriptan formulations was studied and cumulative drug releases up to 8 h across the sheep nasal mucosa. As evident from the data, SME 2 (formulation 01) showed more drug. The diffusion kinetics data indicated that SME 2 (formulation 01) has the highest mean kinetic flux (33.47 µg/min) and diffusion coefficient (1.28E-08 cm²/min) amongst the tested formulations. The mechanism of drug release was also predicted by inputting the regressed data into the excel spread sheet and the result suggested that all the tested formulations of sumatriptan follow Highuchi’s drug release kinetics. Sumatriptan succinate formulations drug diffusion studies have been performed and cumulative drug release up to 8 h. The data revealed that SSME 1 (Formulation 01) shown the highest diffusion across the sheep nasal mucosa. Also, SSME 1 (formulation 01), was found to have mean kinetic flux (43.72 µg/min) and diffusion coefficient (2.42E-08 cm²/min) amongst the tested formulations. The diffusion rate and coefficient were higher compared to sumatriptan microemulsions. The release kinetics indicated that all the tested formulations of sumatriptan succinate follow Highuchi’s drug release kinetics. Zolmitriptan formulations drug diffusion studies have been performed and cumulative drug release up to 8 h. The data indicated that ZME 4 (Formulation 01) showed the highest diffusion across the sheep nasal mucosa. Furthermore, ZME 4 (formulation 01), was found to have mean kinetic flux (16.97 µg/min) and diffusion coefficient (1.36E-08 cm²/min) amongst the tested formulations.
The diffusion rate and coefficient were comparable to sumatriptan microemulsions. The release kinetics (Table 5.16) indicated that all the tested formulations of zolmitriptan follow Highuchi's drug release kinetics. Following evaluation of microemulsions, mucoadhesive agents such as polycarbophil P and chitosan were incorporated and release kinetics of the drug in solution, microemulsions and mucoadhesive microemulsions were compared. Clonazepam mucoadhesive microemulsions diffusion parameters indicated that polycarbophil P containing microemulsions showed two-fold mean flux and 10-fold rate of diffusion. Similarly, sumatriptan, sumatriptan succinate and zolmitriptan mucoadhesive microemulsions were evaluated for rate and extent of diffusion across the sheep nasal mucosa and it was observed that polycarbophil P containing microemulsions were found to have maximum rate and diffusion. This may be attributed to the fact that polycarbophil P may deplete calcium ions from the nasal mucosa which in turn result in opening of the tight junctions. Moreover, higher viscosity of the formulations may facilitate interaction formulation with nasal mucosa due to close proximity and hence, more concentration gradient between the reservoir and the recipient compartment.

Clonazepam formulations, sumatriptan and sumatriptan succinate formulations and zolmitriptan formulations were successfully radiolabeled using \(^{99m}\)Tc direct labeling method (external labeling method). Furthermore, sumatriptan and zolmitriptan were radiolabeled using \(^{99m}\)Tc and drug labeled formulations were prepared to study biodistribution. The radiolabeling was performed using the methods reported in literature. However, the reaction conditions were optimized to achieve maximum radiolabeling efficiency (> 95%). Radiochemical purity achieved was 96.35%, 96.21% and 96.17% for CS, CME and CMME respectively when evaluated for reduced/hydrolyzed (R/H) \(^{99m}\)Tc and free \(^{99m}\)Tc. The optimal SnCl\(_2\)2H\(_2\)O concentration was found to be 100 µg/mL at pH 7.0 with an incubation time of 30 minutes. \(^{99m}\)Tc-CS/CME/CMME were found to be stable in normal saline solution and in rat serum up to 24 h (degradation < 5%w/w). Bonding strength of \(^{99m}\)Tc-CS/CME/CMME was also investigated by the DTPA challenging test, and the percent transchelation of the labeled complex was 1.82% w/w at 25 mM DTPA concentration, while at 100 mM, it increased to 4.03% w/w. The results
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suggested high bonding strength and stability of $^{99m}$Tc-CS/CME/CMME. Thus these formulations were found suitable for biodistribution studies of the drug in rats.

SSS, SME, SMME, SSME and SSMME formulations were radiolabeled with Technetium-99m, optimized for maximum labeling efficiency and stability. The radiochemical purity achieved was 95.96%, 97.69%, 98.22%, 96.54% and 98.74% for SSS, SME, SMME, SSME and SSMME respectively. The optimum concentration of SnCl$_2$.2H$_2$O was found to be 100 μg/mL at pH 6.80 ± 0.20 and incubation time of 30 minutes. The $^{99m}$Tc labeled formulations were found to be stable in 0.90% (w/v) sodium chloride solution (saline) and in rat serum up to 24 hours (degradation < 4% w/w). Bonding strength of all $^{99m}$Tc labeled formulations were also investigated by DTPA challenging test and the percent transchelation of the labeled complex was 1.69% w/w at 25 mM DTPA concentration, while at 100 mM, it increased up to 3.47% w/w. The results suggest high bonding strength and stability $^{99m}$Tc labeled formulations and hence, were found suitable to study biodistribution of the drug in rats. ZT was effectively labeled using $^{99m}$Tc and radiolabeled-drug formulations of ZS, ZME and ZMME were optimized for maximum labeling efficiency and stability and radiochemical purity achieved was 98.77%, 96.30% and 96.99% for ZS, ZME and ZMME respectively when evaluated for reduced/hydrolyzed (R/H) $^{99m}$Tc and free $^{99m}$Tc. Optimum SnCl$_2$.2H$_2$O concentration was found to be 100 μg/mL at pH 6.60 ± 0.20 with an incubation time of 15 minutes. The $^{99m}$Tc- ZS/ZME/ZMME were found to be stable in 0.90% (w/v) sodium chloride solution (saline) and in rat serum up to 24 hours (degradation<3% w/w). Bonding strength of the $^{99m}$Tc- ZS/ZME/ZMME were also investigated using DTPA challenging test and the percent transchelation of the labeled complex was 1.35% w/w at 25 mM DTPA concentration, while at 100 mM it increased to 2.95% w/w. The results suggested high bonding strength and stability $^{99m}$Tc- ZS/ZME/ZMME and hence, were used to study biodistribution of the drug in rats.

Biodistribution studies of $^{99m}$Tc-CL formulations following i.v. administration (CME) and intranasal (CS, CME and CMME) administration on Swiss albino rats were performed and the radioactivity was estimated at predetermined time intervals up to 8 h. After nasal administration of formulations, lower $T_{max}$ values for brain (1-2 h) compared
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to blood (2-4 h) were observed. This may be attributed to preferential nose-to-brain transport following nasal administration. Moreover, following nasal administration of formulations, the drug concentrations in the brain were sustained for 2-3 h as evident from the plateau-like curve. The brain/blood ratios of the drug were found to be higher for formulations when administered intranasally. This further confirms direct nose-to-brain transport. The concentrations of the drug in brain following intranasal administration of CME and CMME were found to be significantly higher at all sampling time points compared to CME (intravenous) up to 8 h after nasal administration.

The substantially higher uptake in the brain with intranasal administration suggests a larger extent of selective transport of CL from nose-to-brain. Many researchers have reported a unique connection between the nose and the brain and intranasal delivery of drugs to the brain bypassing the blood-brain-barrier. The $T_{1/2}$ (brain) and $K_{el}$ (brain) were non significant irrespective of the routes of administration and the type of the formulations. When CME i.v. was compared to CME nasal and CS nasal, significantly lower $C_{max}$ and AUC were observed. The mucociliary clearance under normal circumstances rapidly clears the instilled formulation. However, when mucoadhesive agent was incorporated in the formulation (CMME), significant improvement in $C_{max}$ and AUC was observed. Comparable AUC to CME i.v. was achieved with CMME nasal. This demonstrates the value of the mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa. Significantly higher AUC and $C_{max}$ for CME nasal compared to CS nasal are attributed to microemulsion formulation. The drug targeting efficiency (DTE (%)) and brain drug direct transport percentage (DTP (%)) were also calculated for nasally administered formulations. The CMME showed the highest DTE (%) and DTP (%) values among all the three formulations followed by CME and then CS. The two-fold higher DTE (%) and two-fold higher DTP (%) for CMME compared to CS show the benefit of the mucoadhesive microemulsion formulation. The higher DTE (%) and DTP (%) suggest that CMME has better brain targeting efficiency mainly because of substantial direct nose-to-brain transport. These findings are in congruence with the observations reported by Qizhi et al. that microemulsion increases nose-to-brain uptake of the drugs. In order to visualize brain uptake following intranasal
and intravenous administrations of $^{99m}$Tc-clonazepam microemulsion, we used a gamma scintigraphy camera to derive comprehensive biodistribution information. The gamma scintigraphy images were captured in rabbits' after 0.50 h post intravenous injection and intranasal administrations. The presence of some radioactivity in the esophagus following i.n. administration could lead to absorption of a part of the formulation from gastrointestinal tract. The scintigraphy images were consistent with the biodistribution results obtained, and high uptake of CMME into the brain was observed. Electron micrographs of normal, formulation treated and washed human nasal mucosa were taken to investigate the mechanism of transnasal transport. The electron micrographs of nasal mucosa treated with various formulations revealed that CS treated nasal mucosa showed presence of unaltered tight junctions which is similar to untreated nasal mucosa. However, higher uptake of CME (Figure 3C) was found compared to CS. Significant accretion of CMME compared to CME and presence of formulation was noticed within the junctions of nasal mucosae cells. The nasal mucosa washed after formulation treatment was found to restore the innate cellular structure, and normal endoplasmic reticulum, mitochondria and nuclei were observed. The electron micrographs revealed that the innate structure of mucosa is restored after formulation treatment and washing suggesting reversal of dilation of tight junctions. Moreover, the results also demonstrated the presence of a high quantity of CMME within the interstitial spaces of tight junctions of nasal mucosae cells indicating paracellular mode of transport of CMME. These findings corroborate observations reported by Gavini E and coworkers that on exposure of nasal mucosa to formulation containing mucoadhesive agent showed opened tight junctions.

Biodistribution studies of $^{99m}$Tc-ST/SS formulations following i.v. administration (SME) and intranasal (SSS, SME, SMME, SSME, SSMMME and SMP) on Swiss albino rats were performed and the radioactivity was estimated at predetermined time intervals up to 8 h and the brain/blood ratio at different time points for different formulations were also calculated. The pharmacokinetic parameters were also calculated to comprehensively study the drug distribution and brain-targeting. The percent drug targeting efficiency (%DTE) and percent drug direct nose-to-brain transport (%DTP) were also calculated.
The drug concentrations in brain following i.n. administrations of SME and SMME were found to be significantly higher at all sampling time points compared to i.v. administration of SME. The brain/blood ratio at 0.50 h for SME (i.n.) and SMME (i.n.) was found to be 2.50-fold to 3-fold higher as compared to SME (i.v.). This may be attributed to direct drug nose-to-brain transport. Nose-to-brain transport occur due to unique connection of nose and the CNS, the intranasal route can deliver therapeutic agents to the brain bypassing the blood brain barrier as reported by the scientists. The SME (i.n.) shows significantly higher brain/blood ratio at 0.50 h compared to SSS and SMP (i.n.), and shown rapid nose-to-brain transport of ST from microemulsion. SME and SMME show 2-fold higher Cmax and 8-fold higher AUC compare to SMP. Also, significantly higher % DTP and % DTE values were observed for SMME compared to SME proved the role of mucoadhesive agent. This may be attributed to longer residence time of mucoadhesive microemulsion in the nasal cavity. This observation corroborate the findings that higher and rapid nose-to-brain transport of drug when incorporated in microemulsion. SSMME and SSME showed comparable direct nose-to-brain transport (% DTP) to that of SSS and SMP. The difference in DTE (%) for SSMME and SSME was found non significant compared to SSS and SMP. However, SSMME and SSME show approx. 2-fold higher Cmax and 2-fold higher AUC compare to SSS and SMP. $T_{1/2}$ was also extended to 3 h from 1.5 h for SSMME compared to SSS and SMP. Significantly higher Cmax and $T_{1/2}$ for SSMME/SSME (i.n.) compared to SSS/SMP (i.n.) may be attributed to longer residence time of microemulsion formulation due to higher viscosity and mucoadhesion. The drug concentrations in brain following i.n. administrations of SSME and SSMME were found to be significantly higher at all sampling time points compared to i.v. administration of SME. SMME and SME showed significantly higher brain concentrations compared to SSMME and SSME. The SMME showed 2-fold higher uptake ($C_{max}$) of ST in brain compared to SSMME is suggestive of higher ST nose-to-brain transport compared to SS. The substantially higher uptake of SMME compared to SSMME in the brain compartment at all sampling points is suggestive of larger extent of selective transport of ST to brain. This may be attributed to the higher partition coefficient (lipophilicity) of the ST compared to that of SS, resulted
into higher drug uptake. The significantly higher DTP of SMME compared to SSMME also proved more uptake of ST compared to SS from mucoadhesive microemulsions. Significantly longer T1/2 of SMME compared to SSMME, suggest role of microemulsion in delaying the mucociliary clearance of lipophilic molecule (ST) and lesser extent to hydrophilic molecule. The mucociliary clearance may rapidly clear out hydrophilic molecules (SS). The higher DTP suggest that SMME has better brain targeting efficiency mainly because of substantial direct nose-to-brain transport. The findings are in congruence with the observations reported by Qizhi Zhang et al. and Lianli et al. In addition, microemulsion of liphophilic and hydrophilic drug will have different mucociliary clearance due to their presence in lipophilic and hydrophilic phase in the microemulsion. Significantly high radioactivity was noticed in the rat brain for SMME (i.n.) compared to SME (i.v.) and SME (i.n.). The scintigraphy images endorsed the biodistribution data. The electron micrographs of human nasal mucosa following formulation treatment and washing with various formulations revealed that SSS treated nasal mucosa showed presence of unaltered tight junctions, which are identical to untreated nasal mucosa. However, higher uptake of SME was found as compared to SSS. Significant accrual of SMME as compare to SME was noticed within the junctions of nasal mucosae cells. The nasal mucosa washed after formulation treatment was found to restore the innate cellular structure when compared with the normal nasal mucosa. The electron micrographs revealed that the innate structure of mucosa is retained after formulation treatment and washing suggesting reversal of dilation of tight junctions. It also showed noticeable presence of SMME within the interstitial spaces of tight junctions of nasal mucosa cells indicating paracellular mode of transport of SMME.

Biodistribution studies of $^{99m}$Tc-ZT formulations following i.v. administration (ZME) and intranasal (ZS, ZME and ZMME) administration on Swiss albino rats were performed and the radioactivity was estimated at predetermined time intervals up to 8. The brain/blood ratio of the drug at all time points for different formulations were also calculated and the pharmacokinetic parameters were also calculated. Biodistribution studies of $^{99m}$Tc-ZT formulations following i.v. administration (ZME) and intranasal (ZS, ZME and ZMME) administration on Swiss albino rats were performed. The brain/blood
ratios of the drug at all time points for different formulations were also calculated. Lower $T_{\text{max}}$ values (brain) for nasally administered formulations (0.50 h) compared to ZME (i.v.) (1 h) is indicative of direct nose-to-brain transport. The brain/blood ratios of the drug were found to be higher for formulations administered intranasally. The results suggest direct nose-to-brain transport. The concentrations of drug in the brain following intranasal administration of ZME and ZMME were found to be significantly higher at all sampling time points compared to ZME (intravenous) up to 8 h. The substantial higher uptake in the brain is suggestive of a larger extent of preferential nose-to-brain transport of ZT. Many scientists have reported unique connection between the nose and the brain, and intranasal delivery of drug circumvents the blood-brain-barrier resulting into enhanced rate and extent of transport of drugs to the brain. The $T_{1/2}$ was and $K_{e1}$ (blood and brain) were found insignificant irrespective of the routes of administration and the type of the formulations. Significantly higher $C_{\text{max}}$ (brain) and AUC (brain) were observed when ZME nasal and ZMME nasal were compared to ZME i.v. This may be attributed to preferential nose-to-brain transport of the drug following intranasal administration. Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance. However, when mucoadhesive agent was incorporated in the formulation (ZMME), significantly higher $C_{\text{max}}$ and AUC were observed compared to ZS and ZME (nasal). The results demonstrated the importance of mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of the drug. When ZME nasal was compared to ZS nasal, significantly higher AUC and $C_{\text{max}}$ were observed. This may be attributed to the fact that microemulsion enhances transport of drug across nasal mucosa. These findings are in congruence with the observations reported by Qizhi et al. that microemulsion enhances transport of drug across nasal mucosa resulting in direct nose-to-brain transport of the drugs. Drug targeting efficiency (DTE (%)) and brain drug-direct-transport percentage (DTP (%)) were also calculated from the pharmacokinetics data. Amongst all nasally administered formulations, ZMME showed highest DTE (%) and DTP (%) values followed by ZME and then ZS. Three-fold higher DTE (%) and two-fold higher DTP (%) for ZMME
compared to ZS demonstrated the significance of the mucoadhesive microemulsion formulation. The higher DTE (%) and DTP (%) demonstrated that ZMME (nasal) has greater brain-targeting efficiency compared to ZME and ZS, may be because of preferential nose-to-brain transport. In order to ascertain the brain uptake following intranasal and intravenous administrations of $^{99m}$Tc-zolmitriptan microemulsion, we performed gamma scintigraphy and scintigrams of rats at 0.50 h post-intravenous injection and intranasal administrations. The major radioactivity accumulation was seen in the abdominal region, which is in conformity with the results of biodistribution studies. In case of formulations administered intranasally, a part of radioactivity was also noticed in the esophagus. The scintigrams clearly demonstrate the accumulation of formulations within the brain. However, the accumulation of radioactivity was higher following intranasal administration of ZMME compared to intravenous administration ZME.

In this study, mucoadhesive microemulsion of clonazepam was successfully prepared and demonstrated in rats to deliver clonazepam to the brain rapidly and more effectively following intranasal administration. Accumulation of formulation within interstitial spaces and transport of drug to the brain may be due to stretching of tight junctions between the cells of nasal mucosa. The studies suggested intranasal delivery of clonazepam to be promising. The studies also demonstrated rapid and larger extent of selective ST nose-to-brain transport compared to SS and SMP in rats. Larger extent of drug transport and wider distribution of the drug in the brain is expected to maximize therapeutic index of the drug. Mucoadhesive microemulsion of zolmitriptan was effectively prepared and demonstrated in rats to deliver zolmitriptan with enhanced rate and extent, quickly and effectively to the brain following intranasal administration. These studies aptly demonstrated the effectiveness of intranasal delivery of clonazepam, sumatriptan and zolmitriptan. However, the ratio of benefits vs. the risks must be evaluated and clinical intricacies must be scientifically established for its efficacy in clinical practice, in the treatment of acute status epileptics and acute attacks of migraine.