to Nose

Brain

CHAPTER 9

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SUMMARY AND CONCLUSIONS

Despite enormous advances in brain research, brain and central nervous system (CNS) disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. Beyond loss of life, this broad category of disorders can have an overwhelming effect on the quality of life for the surviving patient and can lead to serous social and economic burdens on society. CNS disorders contribute to as much as 35% of the disease burden in the seven major pharmaceutical markets (US, Japan, France, Germany, Italy, Spain and UK) as measured in terms of daily-adjusted life years. The worldwide patient population with CNS disorders is steadily rising, both in terms of prevalence and in terms of treatment, driven by an aging population, improving diagnostic techniques, increasing physician and patient awareness and a gradual shift away from the social stigma traditionally attached to many psychiatric conditions. The CNS disorders could increase their share of the total global burden of disability and mortality from 10.5% in 1990 to 15% in 2020 (a larger proportionate increase than even cardiovascular disease) as reported by the 1990 Global Burden of Disease Study. Thus, the treatment of CNS disorders is the greatest challenge and largest potential growth sector of the pharmaceutical industries. A large number of therapeutic agents are found to be ineffective in the treatment of CNS disorders because of variety of formidable obstacles in effective drug delivery and maintenance of therapeutic concentrations in CNS for prolonged period. The delivery of drugs to CNS is a challenge in the treatment of CNS disorders. The clinical failure of most of compounds active in CNS disorders is often not due to a lack of drug efficacy but mainly due to shortcomings in the drug delivery approach. The method of delivering a drug to the CNS has an impact on the drug's commercial potential. Thus, the market of CNS drug delivery technology is directly linked to the CNS drug market. General methods that can enhance drug delivery to the brain are, therefore, of great interest. Hence, scientists are exploring the novel approaches so that delivery of the drugs can be enhanced and/or restricted to the brain and CNS. In response to the insufficiency in conventional delivery mechanisms, scientists are aggressively pursuing the research on the development of new strategies for delivering the drug molecules efficiently and effectively to brain and CNS. Many advanced and effective approaches to CNS delivery of drugs have emerged in recent years. Intranasal (i.n.) drug delivery is one of the focused delivery option for brain targeting as brain and nose compartments are connected to each other via olfactory/trigeminal route and via peripheral circulation. Realization of nose-to-brain

transport and the therapeutic viability of the route can be traced from the ancient times and has been successfully investigated for rapid and effective transport in last two decades. Intranasal drug delivery delivers the drug directly to the brain by circumventing BBB and reduces drug delivery to non targeted sites. Direct transport of drugs to the brain may lead to the administration of lower doses and in turn can reduce toxicity. Systemic dilution effect and first pass metabolism are also avoided. Direct transport also results into rapid and/or higher uptake in the brain, which provides an alternative option of selfmedication in management of emergencies. However, the development of nasal drug products for brain targeting is facing enormous challenges. The nose-to-brain transport is also dependant on various formulation variables and physicochemical factors. Better understanding in terms of properties of drug candidate, nose-to-brain transport mechanism and transport to and within the CNS is of utmost importance. High lipophilicity and preferably low molecular weight of drug are the prerequisites as it could 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 i.n. drug delivery systems. Further, the low dose/volume, especially with compounds having poor aqueous solubility makes it difficult to formulate i.n. delivery of such compounds. The other practical difficulties that have to be overcome include active degradation or alteration by enzyme, low pH of nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold. In addition to this, a few formulation factors affect the rapid on set of action and complete absorption of the drug substance from a formulation when administered via i.n. route. The whole process of determining the development of suitable dosage form, its transport to and within CNS will lead to development of products meant for CNS targeting via i.n. route which are therapeutically effective, stable and safe. 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 portal 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 bio-adhesive 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 indicated as the most promising approach for delivery of drugs to the brain/CNS in near future.

CNS disorders are one of the major issues related to aging population all over the world and account for 5 of the top ten leading causes of disability worldwide as reported by the 1990 Global Burden of Disease Study. Projections show that psychiatric and neurological conditions could increase their share of the total global burden of disability and mortality from 10.5% in 1990 to 15% in 2020, a larger proportionate increase than even cardiovascular disease. In geriatric population all over the world, dementia has emerged as a major health problem and experts currently believe that 60% of cases of dementia are due to AD. AD is now the fourth leading cause of death in adults and almost 8 million individuals world wide are suffering from it. AD is the fastest growing CNS indication in terms of prevalence and by 2010 it is forecast that AD will afflict about 15 million individuals. Unless effective methods for prevention and treatment for AD are developed, this disorder will reach epidemic proportion afflicting an estimated 22 million individuals world wide within 50 years. Over the last 3 years, the value of the AD market has grown by an average of 19% per year. Growth has been driven by increasing availability and reimbursement of Alzheimer's drugs. There are considerable opportunities within these markets due to an ageing population and the high unmet clinical need, particularly within the late stage of disease. Alzheimer's disease is a highly disabling neuropsychiatric disorder characterized by an irreversible deterioration of memory and intellectual behavior. While the etiology of AD remains unknown, evidence has been presented that the hippocampus (an essential brain structure for memory and learning) is one of the principal areas affected by AD. A specific loss of cholinergic neurons and deficits of choline acetyltransferase have been suggested to play a major role in the primary cognitive symptoms of the disease. Decreased central cholinergic activity has received major attention from investigators in search of biochemical approach that supports a pharmacotherapy for the disease. Inhibition of acetylcholinesterase is a promising

approach and the most common method under investigation for the treatment of AD. Tacrine (1,2,3,4-tetrahydro-9-aminoacridine), a potent, centrally active, reversible cholinesterase inhibitor, was the first drug approved by the USFDA in 1993 for treating the symptoms of mild to moderate AD. Presently tacrine is available in the market as oral capsule dosage forms. However, peroral administration of tacrine is associated with low bioavailability, extensive hepatic first pass effect, rapid clearance from the systemic circulation, a short elimination half life, large inter individual differences, a reversible dose dependent hepatotoxicity and peripheral cholinergic side effects. Its clinical uses have been limited due to associated cholinergic, hepatic, and gastrointestinal adverse reactions. A recent study had shown that gastrointestinal side effects, such as diarrhea, anorexia, dyspepsia, and abdominal pain, and raised serum liver enzymes were the major reasons for its withdrawal.

Donepezil hydrochloride (2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4piperidinyl]methyl]-1H-inden-1-one hydrochloride) is a specific, reversible acetyl choline esterase inhibitor used widely for the palliative treatment of mild to moderate dementia of the Alzheimer's type. Doenpezil was approved by FDA in 1996. Presently donepezil is available in the market as oral film coated or orally disintegrating tablets in the strength of 5 and 10 mg. Donepezil HCl is well absorbed from the gastrointestinal tract, maximum plasma concentrations being achieved within 3 to 4 h. It is about 95% bound to plasma proteins, mainly albumin. It undergoes partial metabolism via the cytochrome P450 isoenzyme CYP3A4, and to a lesser extent by CYP2D6, to 4 major metabolites. Over 10 days, about 57% of a single dose is recovered from the urine as metabolites, and about 15% from the faeces; 17% of the drug remains unchanged and is excreted in urine; 28% remains unrecovered suggesting accumulation. The elimination half-life is about 70 h. Steady-state concentrations are achieved within 3 weeks of the start of therapy. The most frequently reported side effects associated with donepezil include headache, generalized pain, fatigue, dizziness, nausea, vomiting, diarrhoea, loss of appetite, weight loss, muscle cramping, joint pain, insomnia, and increased frequency of urination.

In light of above facts, an alternative drug delivery system is needed which can selectively target the selected cholinesterase inhibitors (Tacrine/donepezil) to the various regions of the brain for the treatment of AD. Due to preferential transport of drugs to the brain, intranasal delivery approach may be expected to reduce the first pass metabolism and may result in 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. Previous experience with the nasal delivery of neuropeptides and neurotropic factors, and monosialoganglioside to rats has shown that the nose could be a possible administration route for these potential drugs in treating AD. Therefore, the nasal route for delivering tacrine/donepezil to the brain appears to be an attractive alternative to conventional administration route for the management of AD. However, to enhance effectiveness of the drug, a few issues should be considered by the formulator when designing i.n. drug delivery. Intranasal drug delivery system must be meticulously designed to provide rapid transport of drug across nasal mucosa and longer residence time in nasal cavity. Microemulsions have been explored widely as a delivery system by virtue of having considerable potential to enhance transport of a wide range of drug molecules across biological membranes²⁵. The addition of a mucoadhesive agent such as a polyelectrolyte polymer helps in retention of formulation in nasal cavity^{26, 27}. Evidences of i.n. drug delivery systems formulated using mucoadhesive agent and its benefits in enhancing nose-to-brain drug transport have been reported by our team as well as other scientists

The objectives of this investigation were to prepare and characterize rapid brain-targeted microemulsion/mucoadhesive microemulsions of tacrine/donepezil and to assess their pharmacokinetic performance for brain drug delivery in mice after i.n. delivery. It was an also objective to assess their role pharmacodynamically for improvement in memory in scopolamine induced amnesic mice. It was hypothesized that i.n. administration of tacrine/donepezil microemulsion/mucoadhesive microemulsion will result in to selective and effective nose-to-brain drug transport, reduce drug distribution in other parts of the body, reduce side effects, and rejuvenate their life in treatment of AD.

Tacrine drug substance was estimated using UV-Visible double beam spectrophotometry method at 326 nm. The absorbance was found linear between $2 - 20 \ \mu g/mL$ and hence, was used for estimation of tacrine. The regressed graph plotted for linearity revealed that correlation coefficient (r²) was greater than 0.999 and equation of straight line was y = 0.0635x + 0.0022. The method was validated for linearity, accuracy, precision, robustness and ruggedness. The validation parameters were found to meet the "readily pass criteria"

specified in the USP. Tacrine formulations, tacrine in diffusion media (in vitro diffusion studies) and drug retention studies samples were analyzed using validated UV-Visible double beam spectrophotometry method. Microemulsions and samples of in vitro diffusion studies were analyzed by preparing dilution in methanol and measuring the absorbance at 326 nm. The ingredients used for microemulsion preparation or diffusion media were not found to interfere with the proposed method. Donepezil drug substance was analyzed using UV-Visible double beam spectrophotometry method at 313 nm. The absorbance was found linear between $5 - 50 \,\mu\text{g/mL}$ and hence, was used for estimation of donepezil. The regressed graph plotted for linearity revealed that correlation coefficient (r^2) was greater than 0.999 and equation of straight line was y = 0.0261x + 0.0062. The method was validated for linearity, accuracy, precision, robustness and ruggedness. The validation parameters were found to meet the "readily pass criteria" specified in the USP. Donepezil formulations, donepezil in diffusion media (in vitro diffusion studies) and drug retention studies samples were analyzed using validated UV-Visible double beam spectrophotometry method. Microemulsions and samples of in vitro diffusion studies were analyzed by preparing dilution in methanol and measuring the absorbance at 313 nm. The ingredients used for microemulsion preparation or diffusion media were not found to interfere with the proposed method.

Tacrine microemulsions were successfully prepared using the titration technique followed by construction of pseudo-ternary phase diagrams. To screen out a drug vehicle suitable for i.n. delivery of tacrine, four different ME systems were prepared wherein system 1 and 2 comprise of Labrafil M 1944 CS^{\oplus} as an oil phase, Transcutol P[®] as a co-surfactant and distilled water as an aqueous phase. Cremophor RH 40[®] and Cremophor EL[®] were used as surfactant for system 1 and system 2 respectively. Similarly, system 3 and system 4 were formulated in the identical manner as system 1 and system 2 respectively by replacing Labrafil M 1944 CS^{\circledast} with Labrafac CC^{\circledast} . Microemulsion formation was spontaneous upon addition of aqueous phase to drug in oil-surfactant-co-surfactant mixture. The solubility data shown that tacrine has maximum solubility in Labrafil M 1944 CS^{\circledast} (> 35 mg/mL) and Labrafac CC^{\circledast} (> 10 mg/mL) therefore; these oils are selected to formulate ME. However, with castor oil, corn oil, sunflower oil and isopropyl myristate, the solubility of drug in oil was less than 10 mg/mL. Moreover, nasal formulations are concentrated preparations as low volumes can be administered into the nostril (< 200 µL), the ME base was selected on the merits of solubilization capacity of tacrine. 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. Phase studies were done to investigate the effect of S:CoS ratios on the existence range of stable o/w ME region. Microemulsions were formulated at different S:CoS ratio such as 1:1, 2:1 and 3:1 and plotted as the pseudo-ternary phase diagrams. The transparent ME area is presented in the phase diagrams as shaded region. No distinct conversion from w/o to o/w ME was seen; therefore, this single isotropic region is considered as a bicontinuous ME. The rest of the region on the phase diagram represents the viscous gel area or turbid and conventional emulsions based on visual identification. From these phase diagrams, the influence of relative S:CoS concentrations on the ME isotropic region can be evidently seen. The phase study revealed that with all four systems, changing S:CoS ratio from 1:1 to 3:1, the ME region increased in size with the higher surfactant concentration. This increase was toward the oil-water axis, indicating that by increasing the surfactant concentration, the maximum amount of water and oil that could be solubilized into the ME increased. From a formulation viewpoint, the increased oil content in ME may provide a greater opportunity for the solubilization of drug. The viscosities of ME were also affected by the surfactant content. With the higher weight percentage of surfactant, the viscosities of the ME formulation increased, and a gel formation was observed. For nasal delivery, a less viscous ME is preferred considering the requirement of spravability of nasal formulation by the pump device and the dispersion uniformity of the spray. The globule size and zeta potential data for selected compositions of TME (System 1 to 4) reveled that increase in concentration of oil phase, resulted in increase in globule size. This may be due to fact that part of the oil phase may not form micelles. The globule size and zeta potential were fairly reproducible within \pm 5 nm / \pm 2 mV range respectively. Comparing globule size of different formulations with varying concentrations of S:CoS mixture, it was observed that increase in the S:CoS mixture concentrations results in the increase in the globule size. Therefore, it was concluded that the concentration of S:CoS mixture may be critical for the formation of TME. Increase in the concentration and the ratio of surfactant to cosurfactant, resulted into formation of bicontinuous ME or o/w ME. It was also observed that increase in the aqueous phase concentration resulted in decrease in the zeta potential (anionic). Reports in the literature revealed that ME having zeta potential more than 25 mV absolute value exhibit moderate to best physical stability in terms of phase

separation. Therefore, ME having zeta potential close to -25 mV or less were selected for further studies. Also, ME 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 TME 1 and TME 2 or TME 3 and TME 4, the ME region obtained with Cremophor RH 40[®] was found to be wider in comparison to those obtained with Cremophor EL[®]. This may be attributed to the lower HLB value of Cremophor RH 40[®] which is responsible for more oil solubilization. Comparing TME 1 and TME 2 vs. TME 3 and TME 4, it was observed that the S:CoS systems used produced smaller ME regions when Labrafac CC[®] was used as an oil phase. The oil concentrations in excess of 30% w/w did not yielded ME like system 1 and system 2. Further, the globule sizes and zeta potentials obtained for system 3 and system 4 were more compared to system 1 and system 2. A few of the batches of TME 3 (Formulations 04, 07, 08, and 09) and TME 4 (Formulations 04, 07 and 08) 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%. It was found that with increase in the oil concentration, the globule size also increased significantly and lead to poor transmittance (less than 98%). At large, the ME 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 tacrine microemulsions. The prepared ME of tacrine having globule size less than 50 nm and zeta potential close to -25 mV or less have been further evaluated for physical stability and chemical stability.

Donepezil microemulsions were successfully prepared using titration technique and the ME regions are plotted using pseudo-ternary phase diagrams. Three different DME systems (DME 1, DME 2, and DME 3) were prepared wherein system 1 comprises of Captex 355[®] as oil phase, Tween 80 as surfactant, Capmul MCM[®] as co-surfactant and distilled water as an aqueous phase. System 2 and system 3 were prepared using Labrafil M 1944 CS[®] and Labrafac CC[®] an oil phase respectively, Cremophor RH 40[®] as surfactant, Transcutol P[®] as a co-surfactant and distilled water as an aqueous phase. The oils were selected on the merit of highest solubility of donepezil which is performed using biopharmaceutical classification system solubility studies. Microemulsion formation was found to be spontaneous upon addition of aqueous phase to drug in oil-surfactant-co-surfactant mixture and the prepared ME were visually clear and transparent. The percent transmittances were measured using spectrophotometer (Shimadzu UV-1601, Japan) at

630 nm. As seen from the results, all DME systems showed transmittance > 99% for all batches of ME prepared except for few batches of DME 3. The SD 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 3. The globule sizes were found within \pm 5 nm of the estimated globule size which indicates uniform globule size distribution and narrow distribution for all the batches. Zeta potentials were also fairly reproducible within $\pm 2 \text{ mV}$ range. Increase in the oil concentration to highest 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 ME. This may be attributed to the fact that 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 (up to medium range), absolute zeta potential increases. This may be due to increase in the concentration of co-surfactant result into formation of bicontinuous or o/w system due to higher HLB value hence, the negative charge of the system also increases. At higher concentrations of S:CoS absolute zeta potential decreases due to substantial decrease in aqueous phase. Comparing DME 2 and DME 3, it was observed that the S:CoS systems used produced smaller ME regions when Labrafac CC[®] was used as an oil phase. The oil concentrations in excess of 30% w/w did not yielded ME like DME 2. Further, the globule sizes and zeta potentials obtained for DME 3 were more compared to DME 2. A few of the batches of DME 3 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% (System 3, Formulation 04, 07, 08 and 09). It was found that with increase in the oil concentration, the globule size also increased significantly and lead to poor transmittance (less than 98%). At large donepezil microemulsions were found to be clear, transparent (transmittance > 99% at 630 nm), spontaneous formation and either of o/w or bicontinuous for all three systems. The prepared ME of donepezil having globule size less than 50 nm and zeta potential close to -25 mV have been further evaluated for physical stability and chemical stability.

The ME of both the drug substances were prepared successfully and the phase regions delineating phase boudaries were successfully plotted in a psedo-ternary phase diagrams. Selected batches of tacrine and donepezil microemulsions were subjected to accelerated

centrifugation for assessing the physical stability of the formed microemulsions. The data revealed that there was no appreciable change before and after centrifugation for 15 min at accelerated conditions. Moreover, the layers from top, middle and bottom following centrifugation were sampled and analyzed to determine homogeneity. The globule size of the TME in top, middle and bottom layer for different formulations were within ± 5 nm from the initial values. The data clearly suggested that tacrine microemulsions were found physically stable under the testing conditions. The ME were selected on the basis of globule size. All the batches of ME were having globule size less than 50 nm and zeta potential close to -25 mV or less. It was also observed that ME having zeta potential close to or less than -25 mV gives reasonably good physical stability with regards to phase separation. For donepezil microemulsions, significant increase and difference in the globule size and size distribution of a DME 1, Formulation 04 was observed. It was also observed that the total concentration of surfactant and co-surfactant mixture was less in DME 1, formulation 04 as compared to DME 1, formulation 05. Thus, lower concentration of S:CoS mixture may result into spontaneous formation of ME 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. Moreover, 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. It was concluded that physical stability assessment can be successfully performed using accelerated centrifugation technique by sampling the ME from top, middle and bottom layers. Microemulsions which are bicontinuous, w/o or o/w were found to be stable.

Drug retention study were performed on physically stable ME by subjecting tacrine and donepezil microemulsions at 30°C / 65% RH and 40°C / 75% RH. The ME were assessed for globule size, size distribution, zeta potential, percent transmittance, and drug content. When globule size was evaluated up to six months, it was found that globule size for all TME and DME formulations were within the range of \pm 5 nm from the initial values 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 and within the range \pm 5 mV from the initial values. The data clearly indicated that the formulations were physically stable at both the accelerated conditions and the systems were found to be

thermodynamically stable. Percent transmittances at 630 nm for all the selected experimental batches were found to be greater than 99% which indicated the clarity of the tested ME and indirectly gives an indication that no inversion, phase separation or cracking of the prepared TME and DME were observed. Drug content for different TME and DME formulations were found to be more than 95 % of the label claim. The data clearly demonstrated that there was no appreciable degradation at 30°C / 65% RH and 40° C/ 75% RH. The results conclusively demonstrates selected TME and DME formulations. The formulations were found to meet the general monograph of Pharmacopoeia and criteria stipulated therein for the liquid preparations.

In vitro diffusion studies were performed to evaluate relative diffusion behavior of different formulations of tacrine and donepezil. Cumulative drug diffused across sheep nasal mucosa of physically and chemically stable TME formulations up to 8 h indicated that TME 1 (formulation 05) was found to have substantially higher diffusion across the sheep nasal mucosa. Furthermore, mean kinetic flux and diffusion coefficient were calculated from the cumulative % drug diffused and concentration gradient at specific time intervals also indicated that TME 1 (formulation 05) has the highest mean kinetic flux (2.06 μ g/min) and diffusion coefficient (3.61E-09 cm²/sec) amongst the tested formulations. The mechanism of drug diffusion was also predicted by inputting the regressed data into the excel spread sheet and it was observed that all the tested formulations of tacrine follow Higuchi's kinetics whereas, the regression coefficient values were found less for zero-order and first-order compared to Higuchi's kinetic fit.

Diffusion kinetics of donepezil formulations was also studied and cumulative drug diffused up to 8 h across the sheep nasal mucosa indicated that DME 2 (formulation 05) has shown better drug diffusion across the sheep nasal mucosa. The diffusion kinetics data indicated that DME 2 (formulation 05) has the highest mean kinetic flux (2.29 μ g/min) and diffusion coefficient (4.22E-09 cm²/sec) amongst the tested formulations. The mechanism of drug diffusion predicted by inputting the regressed data into the excel spread sheet indicated that all the tested formulations of donepezil follow Higuchi's kinetics.

Following evaluation of microemulsions, mucoadhesive agents such as Carbopol 934 P (0.25%, 0.5% and 1.0%) and chitosan (0.25%, 0.5% and 1.0%) were incorporated and diffusion kinetics of the drug in solution (in propylene glycol), and mucoadhesive

microemulsions were evaluated. It was observed that all formulations follow Higuchi's kinetics. Further, it was observed that mucoadhesive microemulsions containing Carbopol 934 P shown better drug diffusion across sheep nasal mucosa. Mean flux and diffusion coefficients were also higher for Carbopol 934 P containing mucoadhesive microemulsions. This may be attributed to the fact that Carbopol 934 P may deplete calcium ions from the nasal mucosa which in turn result into channel formation due to stretching 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 donor and the recipient compartment. Amongst the different Carbopol 934 P containing mucoadhesive microemulsions, concentration beyond 0.5 % does not result into significant increase in % drug diffused, mean flux and diffusion coefficient. Hence, mucoadhesive microemulsion TCP 0.5% and DCP 0.5% was selected for further evaluation in in vivo studies. On comparing tacrine/donepezil solutions, optimized tacrine microemulsion (TME 1(05))/donepezil microemulsion (DME 2 (05)) and tacrine mucoadhesive microemulsion (TCP 0.5%)/donepezil mucoadhesive microemulsion (DCP 0.5%) it was observed that microemulsion and mucoadhesive microemulsion showed better drug diffusion compared to solution. Mucoadhesive microemulsion showed 2-fold mean flux and 3-fold diffusion coefficient compared to solutions. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa. Consequently, following in vitro evaluation, promising formulations such as microemulsions and 0.5% Carbopol 934 P containing mucoadhesive microemulsions were taken up for comparative in vivo evaluation including solutions. Prior to in vivo evaluation selected tacrine and donepezil formulations were successfully radiolabeled, using ^{99m}Tc direct labeling method (external labeling method). The radiolabeling was performed using the methods reported in literature. However, the reaction conditions were optimized to achieve maximum radiolabeling efficiency (> 95%). Radiochemical purities achieved were 96.35%, 97.66% and 98.31% for TS, TME and TMME respectively when evaluated for R/H ^{99m}Tc and free ^{99m}Tc. The optimal

SnCl₂.2H₂O concentration was found to be 200 μ g/mL at pH 6.5 ± 0.2 with an incubation time of 30 min. ^{99m}Tc-TS/TME/TMME were found to be stable in 0.90% w/v sodium chloride solution (physiological saline) and in mice serum up to 24 h (degradation < 5%w/w). Bonding strength of ^{99m}Tc-TS/TME/TMME was also investigated by the DTPA challenging test, and the percent transchelation of the labeled complex was <1.60% w/w

at 25 mM DTPA concentration, while at 100 mM, it increased up to only around 5.00% w/w. The results suggested high bonding strength and stability of ^{99m}Tc-TS/TME/TMME. DS, DME and DMME were also successfully radiolabeled with ^{99m}Tc by direct labeling method and optimized for maximum labeling efficiency and stability. The radiochemical purities achieved were 98.86%, 98.42%, and 98.02% for DS, DME, and DMME respectively when evaluated for R/H ^{99m}Tc and free ^{99m}Tc. The optimal SnCl₂.2H₂O concentration was found to be 200 µg/mL at pH 6.5 ± 0.2 and incubation time of 30 min. The ^{99m}Tc labeled formulations were found to be stable in 0.90% w/v sodium chloride solution (physiological saline) and in mice serum up to 24 h (degradation < 5% 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 <1.15% w/w at 25 mM DTPA concentration, while at 100 mM, it increased up to only around 4.65% w/w. The results suggest high bonding strength and stability ^{99m}Tc labeled donepezil formulations. Thus, these radiolabeled tacrine and donepezil formulations were found suitable for *in vivo* studies.

Biodistribution studies of ^{99m}Tc-TS following i.v. and ^{99m}Tc-TS/TME/TMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 480min. The brain/blood ratios of the drug at all time points for different formulations were calculated. The pharmacokinetic parameters of the drug were calculated from biodistribution data using WinNonlin[®] software (version 5.0.1, Pharsight Corporation, North Carolina, USA). After nasal administration, tacrine was delivered to the brain quickly compared to i.v. administration (T_{max} - 60min versus 120min). Similarly, lower T_{max} values for brain (60 min) compared to blood (120 min) were observed for all the three nasally administered formulations. This may be attributed to preferential nose to brain transport following nasal administration. The brain/blood ratios of the drug at all time points were found to be higher following i.n. administration of formulations. This further confirms direct nose to brain transport. The concentrations of drug in the brain following i.n. administration were found to be significantly higher at all sampling time points compared to i.v. administration up to 480 min. The substantially higher uptake in the brain with i.n. administration suggests a larger extent of selective transport of tacrine from nose-to brain. The T_{1/2} and K_{el} of drug in blood was found to be significantly different for i.v. and i.n. administration of different tacrine formulations, but insignificant differences in these values were observed in brain irrespective of the routes

of administration and the type of the formulations. These differences in the results may be due to more selective distribution of the drug to the brain after i.n. administration. Significantly higher C_{max} (brain) and AUC (brain) were observed when TS_{i.n.}, TME_{i.n.} and TMME_{in} were compared to TS_{iv}. The bioavailability of tacrine in brain after i.n. compared to i.v. administrations was 131.99%, 218.67% and 287.09% for TS, TME and TMME respectively. This is suggestive of direct nose to brain transport of the drug following i.n. administration. When TME_{i.n.} was compared to TS_{i.n.}, significantly higher AUC and C_{max} were observed. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa. 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 (TMME), significantly higher C_{max} and AUC were observed compared to TS_{in} and TME_{i.n.} 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. To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were also calculated, from the pharmacokinetics data, for nasally administered formulations. Amongst all the three nasally administered formulations, TMME showed highest DTE (%) and DTP (%) values followed by TME and then TS. These results demonstrated the significance of the mucoadhesive microemulsion formulation in prolonging the residence time in nasal cavity which resulted in higher uptake of the drug in the brain. The higher DTE (%) and DTP (%) demonstrated that TMME_{in} has greater brain targeting efficiency compared to TME i.n. and TS i.n., may be because of preferential nose to brain transport. In order to ascertain the brain uptake following i.v. and i.n. administrations of ^{99m}Tc-tacrine formulations, we used gamma scintigraphy imaging of rabbit 15 min post i.v. and i.n. administrations. The presence of some radioactivity in the esophagus following i.n. administration led to absorption of part quantity of formulation from gastrointestinal tract. Accumulation of significantly higher radioactivity in the rabbit brain following i.n. administration of tacrine compared with i.v. administration was observed. Amongst i.n. formulations, TMME shows higher radioactivity compared to TS and TME. Scintigraphy images are consistent with the observations of biodistribution studies.

Biodistribution studies of ^{99m}Tc-DS following i.v. and ^{99m}Tc-DS/DME/DMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at

predetermined time intervals up to 8 h. The brain/blood ratios of the drug at all time points for different formulations were calculated. The pharmacokinetic parameters of the drug were calculated from the biodistribution data using WinNonlin[®] software (version 5.0.1, Pharsight Corporation, North Carolina, USA). After nasal administration, donepezil was delivered to the brain quickly compared to i.v. administration $(T_{max} - 1 h \text{ versus } 2 h)$. Similarly, lower T_{max} values for brain (1 h) compared to blood (2 h) were observed for all the three nasally administered formulations. This may be attributed to preferential nose to brain transport following nasal administration. The brain/blood ratios of the drug at all time points were found to be higher following i.n. administration of formulations. This further confirms direct nose to brain transport. The concentrations of drug in the brain following i.n. administration were found to be significantly higher at all sampling time points compared to i.v. administration up to 8 h. The substantially higher uptake in the brain with i.n. administration suggests a larger extent of selective transport of donepezil from nose-to brain. The $T_{1/2}$ of 68.10 – 73.31 h (blood), 28.45 – 30.09 h (brain), and K_{el} of 0.0095 - 0.0102 (blood), 0.0230 - 0.0244 (brain) were observed. The T_{1/2} and K_{el} of drug in blood were found to be significantly different from the corresponding values in brain for i.v. and i.n. administration of different donepezil formulations, but insignificant differences in these values were observed in individual tissues irrespective of the routes of administration and the type of the formulations. Significantly higher Cmax (brain) and AUC (brain) were observed when DS_{i.n.}, DME_{i.n.} and DMME_{i.n.} were compared to DS_{i.v.} The bioavailability of donepezil in brain after i.n. compared to i.v. administrations was 123.23%, 185.50% and 255.21% for DS, DME and DMME respectively. This is suggestive of direct nose to brain transport of the drug following i.n. administration. When DME_{in} was compared to DS_{in}, significantly higher AUC and C_{max} were observed. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa. 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 (DMME), significantly higher C_{max} and AUC were observed compared to DS_{in} and DME_{in}. 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. To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were also calculated, from the pharmacokinetics data, for nasally administered formulations. Amongst all the three

nasally administered formulations, DMME showed highest DTE (%) and DTP (%) values followed by DME and then DS. These results demonstrated the significance of the mucoadhesive microemulsion formulation in prolonging the residence time in nasal cavity which resulted in higher uptake of the drug in the brain. The higher DTE (%) and DTP (%) demonstrated that DMME_{in} has greater brain targeting efficiency compared to DME _{in} and DS _{i.n.}, may be because of preferential nose to brain transport. In order to ascertain the brain uptake following i.v. and i.n. administrations of ^{99m}Tc-donepezil formulations, we used gamma scintigraphy imaging of rabbit 15 min post i.v. and i.n. administration led to absorption of part quantity of formulation from gastrointestinal tract. Accumulation of significantly higher radioactivity in the rabbit brain following i.n. administration of donepezil compared with i.v. administration was observed. Amongst i.n. formulations, DMME shows higher radioactivity compared to DS and DME. Scintigraphy images are consistent with the observations of biodistribution studies.

To evaluate the influence of developed formulation on learning and memory capacities, Morris water maze test was performed in scopolamine induced amnesia model in mice. Mice were evaluated once daily for 2 water maze test for 4 consecutive days. In the 1st test mice were placed on the platform for 15 seconds and then placed in the water. Escape latency (indicative of Learning and Intact Reference Memory) was measured (Figure 3). After 15 seconds on the platform the animals were placed back in the water (in previous position) and allowed to search for platform (retained in previous position). Escape latency (indicative of Short-term Working Memory i.e. 2nd test) was recorded. Salinetreated mice rapidly learned the location of the platform as indicated by a gradual decrease in escape latency. Minimum escape latency was achieved on day 3 in both of the tests and thereafter there was no significant decrease in escape latency observed. While in scopolamine-treated mice, a characteristic swimming behavior consisting of circling around the pool was observed and the latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged throughout 4 days of testing period. Intranasal and i.v. administration of different tacrine/donepezil formulations in saline-treated mice and i.n./ i.v. administration of placebo formulations in scopolamine-treated mice did not result into any noticeable improvement in learning and memory capacities. While, i.n. and i.v. administration of tacrine/donepezil formulations in scopolamine-treated mice antagonize scopolamine induced amnesia as evidenced by

significant decrease in escape latency in both of the tests. These results indicated an increase in learning and memory capacities associated with tacrine/donepezil. Following i.n. administration of TMME/DMME, mice learned to reach the platform within 3 days and exhibited behavioral pattern identical to saline-treated control mice. While, following $TS_{i,n}/DS_{i,n}$ and $TME_{i,n}/DME_{i,n}$, similar behavior was observed at the end of 4 days. In case of $TS_{i,v}/DS_{i,v}$, a noticeable decrease in the escape latency and improvement in learning and memory capacities were observed. But, it was slow compared to i.n. administrations and mice did not learn to reach the platform by end of 4 days. Thus, the results suggest fastest memory regain in scopolamine induced amnesic mice following i.n. administration of TMME/DMME and it further supports the findings of biodistribution studies.

To conclude, mucoadhesive microemulsion of cholinesterase inhibitors (tacrine and donepezil) were successfully prepared and demonstrated to deliver the drug to the brain quickly and in larger quantities following i.n. administration in mice. Studies of this investigation amply demonstrate direct nose-to-brain tacrine/donepezil delivery and regain of memory loss in scopolamine induced amnesic mice following i.n. administration and hence, suggest possible role of i.n. tacrine/donepezil delivery in treating Alzheimer's patients possibly by reducing/eliminating dose dependent side effects of otherwise abandoned drug, tacrine and enhanced benefit from established drug, donepezil. However, clinical studies with special focus on toxicity evaluation on chronic use of the developed formulations is necessary for establishing suitability in clinical practice in the treatment of AD.