12 SUMMARY

The study was planned to develop the intranasal drug delivery system to target the brain to treat the migraine disorder. As the insitu gels are thermosensitive they are liquid at room temperature and change the phase at nasal physiological temperature (30-34°C). The design of formulating the drug in the form of ethomes and loading it into the gel was highlight of the research, which was supposed to deliver the drug in sustained manner to avoid repetitive dosing and to improve the patient compliance. Eletriptan hydrobromide (EH), Zolmitriptan (ZMT) and Naratriptan Hydrochloride (NH) (All drugs approved by US FDA) from the group of triptans which are basically used in the treatment of migraine were considered for the investigation. Considering the approach various polymers (thermoreversible and mucoadhesive agents) and excipients were identified from the literature and were considered for the preformulation and compatibility studies. The initial part of the study was to develop the analytical method for each drug candidate. UV-Vis Spectroscopy was the analytical tool for the development of the method.

Initially NH a selective 5-HT1 agonist was considered for the development of analytical method. A simple, precise, accurate and economical UV-Spectrophotometric method was developed for the determination of NH in bulk and pharmaceutical dosage form. Absorption for NH was measured at maximum wavelength of 281nm. The percentage recovery of NH in pharmaceutical dosage form was found to be 98.53 to 99.43%. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. Beers law was obeyed in the concentration range of 2-10 μg/ml having line equation \( y = 0.019x \) with correlation coefficient of 0.99. Results of the analysis were validated according to the ICH guidelines. The results obtained were statistically evaluated and were found to be accurate and reproducible. The proposed method can be successfully applied for the estimation of EH in bulk and pharmaceutical dosage form.
In the further segment analytical method was developed for the drug Eletriptan Hydrbromide (EH). Here linear, reproducible and economical spectrophotometric method was developed and validated for the determination of EH in bulk drugs and pharmaceutical dosage form. UV Spectrophotometric method which is basically depend on the measurement of absorption recorded the absorbance of EH at 221 and 272 nm maximum wavelengths. Beer-Lambert’s law was obeyed in the concentration range of 2-10 μg/ml and is described by the regression equation $y = 0.081x$ with a regression coefficient ($r^2$) = 0.999. The value of molar absorptivity and Sandell’s sensitivity for EH estimation were $3.098 \times 10^3$ L/mol/cm and 0.123 μg/cm$^2$ respectively. Whereas, LOD and LOQ were found to be 0.619 and 1.877 μg/ml, respectively. The developed method was validated with respect to linearity, accuracy (recovery), precision, percentage recovery and specificity according to the ICH guidelines. Where, the developed method was found to be accurate and precise. The proposed method can be successfully applied for the estimation of EH in bulk and pharmaceutical dosage form.

The analytical method was then evaluated for the ZMT which is a selective 5-HT$_1$ agonist. The absorption was measured at maximum wavelength of 281 nm. The percentage recovery of ZMT in pharmaceutical dosage form was found to be 98.5 ± 99.5%. The developed method was validated with respect to linearity, accuracy, precision and specificity. Beers law was obeyed in the concentration range of 2-10μg/ml having line equation $y = 0.0346x + 0.0566$ with correlation coefficient of 0.9999. Results of the analysis were validated according to the ICH guidelines. The results obtained were statistically evaluated and were found to be accurate and reproducible. The proposed method can be successfully applied for the estimation of ZMT in bulk and pharmaceutical dosage form.

The compatibility studies were confirmed with the help of Differential scanning calorimetry (DSC) and Fourier Transformed Infrared spectroscopy.
Liposome based drug delivery system for brain targeting through intranasal route (FTIR). The individual samples of the drug and the excipients and the combination of physical mixtures of the drug and excipients were prepared for the analysis. All the prepared samples were stored according to the ICH guidelines and were analysed for any interactions between drug and excipients. The thermographs of DSC and the FTIR spectra revealed weak interactions between the drug and the excipients indicating its use for the development of the designed formulations.

The actual formulation was dealt for treating the migraine. Intractable migraine presents a significant treatment challenge due to associated pulsating headache which affects one half of the head and lasts from 2 to 72 hours. Naratriptan hydrochloride is an approved drug molecule for migraine headache but, its use is limited due to its poor bioavailability, reoccurrence of migraine and lesser half life requiring frequent dosing. Hence, in the present investigation ato formulate thermoreversible intranasal gel with an objective for targeting drug directly to brain via olfactory lobe pathway thereby improving bioavailability and reducing dosing frequency. Gels were formulated by using poloxamer 407 as thermoreversible polymer and carbopol 934P as mucoadhesive polymer. Formulated gels were characterized for gelation temperature, gel strength, mucoadhesive strength, viscosity, in vitro drug release and ex-vivo permeation study using sheep nasal mucosa. In vitro and ex-vivo drug release studies suggests that release rate was directly proportional to the concentration of carbopol 934P whereas poloxamer 407 reduced the rate of drug release. Histopathology study of sheep nasal mucosa showed no signs of damage to columnar epithelial cells confirming non-toxic nature of gels. Stability studies were performed as per ICH guidelines {Q1A (R2)}, and it was found that the gels were stable till three months.

Once the In-situ gel of NH was developed research was focused to develop the ethosomal thermoreversible mucoadhesive in-situ intranasal gel. Hence present study was focused on formulation and characterization of thermoreversible ethosomal gel of (NH) for brain targeting via intranasal route. Ethosomes were prepared by $3^2$ factorial design with two independent variables.
Summary

(a concentration of soya lecithin and ethanol) and two response variables (percent entrapment efficiency and vesicle size (nm)) using ethanol injection method. Formulated ethosomes were evaluated for preliminary microscopic examination followed by percent entrapment efficiency, vesicle size analysis, Zeta potential, polydispersibility index and TEM. TEM confirms spherical morphology of ethosomes, whereas Malvern zeta sizer confirms the vesicle size where formulation NE6 was reported to be optimized with the average vesicle size of 161.33 ±6.35 percent and the entrapment efficiency of 46.7 ± 2.23 nm. Ethosomes were incorporated in gel form using thermoreversible polymer (poloxamer 407) and mucoadhesive agent (carbopol 934P and PVP-K30) with varying concentrations. Ethosomal gels were evaluated for their pH, viscosity, mucoadhesive strength, in-vitro drug release and ex-vivo drug permeation through sheep nasal mucosa. Mucoadhesive strength and pH was found to be 1873 ± 75 to 3736 ± 110 dynes/cm² and 6.0 to 6.1 respectively. In-vitro drug release from optimized ethosomal gel formulation was found to be 1504 ± 2187 and 2116 µg/ml ± 1.75 percent and ex-vivo drug permeation was 2187µg/ml for the formulations of NPG, NG3 and NG6 respectively. Histopathological study of the nasal mucosa confirmed non-toxic nature of ethosomal gels. Formulated NH loaded ethosomal thermoreversible gel could serve as the better alternative for brain targeting via intranasal route which in turn could subsequently improve its bioavailability.

Further study was planned to formulate and characterize thermoreversible ethosomal gel of Eletriptan Hydrobromide (EH) for brain targeting via intranasal route. Here ethosomes were prepared by $3^2$ factorial design with two independent variables and two response variables (percent entrapment efficiency and vesicle size (nm)) using ethanol injection method. Formulated ethosomes were characterized and evaluated with various parameters. TEM and Malvern zeta sizer were used to study the surface morphology and particle size of ethosomes which recorded vesicle size was in the range of 191±6.55 nm to 381.3 ± 61 nm and
percent entrapment efficiency from 48 to 66 percent. Prepared ethosomes were loaded into the thermoreversible gel and evaluated for the parameters to confirm the stability in the nasal physiology. Where the results revealed the mucoadhesive strength and pH as $4400 \pm 45$ to $5500 \pm 78.10$ dynes/cm$^2$ and $6.0 \pm 0.3$ to $6.2 \pm 0.1$ respectively. In-vitro drug release from optimized ethosomal gel formulation (G4) was found to be $75 \pm 2$ percent and ex-vivo drug permeation was $67 \pm 1$ percent with permeability coefficient of $4.02 \pm 0.06 \times 10^{-5} \text{ cm/s}$ after 8hrs. Histopathological study of the nasal mucosa inveterate the non-toxic nature of ethosomal gels. In conclusion it suggests that formulated EH loaded ethosomal thermoreversible gel could serve as the better alternative for brain targeting via intranasal route which in turn could subsequently improve its bioavailability.

In the final segment of the research was planned to investigate formulation of zolmitriptan loaded ethosomes and preparation of its thermoreversible intranasal gel for treatment in migraine. Ethosomes were formulated by using three level factorial design and evaluated for its drug entrapment efficiency, vesicle size and zeta potential. It was observed that independent variables (concentration of soya lecithin and ethanol) affected dependent variables (vesicle size and entrapment efficiency) where optimized ethosomal formulation (E6) showed desirable vesicle size (171.67 nm) and best entrapment efficiency (66%) when compared to other formulations. Optimized ethosomes were then finally formulated into in-situ thermoreversible intranasal gel using poloxamer 407 as thermoreversible polymer and carbopol 934, HPMC K100 as mucoadhesive agent. Gels were evaluated for gel strength, gelation temperature, mucoadhesive strength, in-vitro drug release and ex-vivo drug permeation. Permeability coefficient of thermoreversible gel formulations G3 and G6 was found to be 5.92 and 5.9 $\mu g/cm^2$. It was observed that intranasal gel formulation G3 and G6 showed optimized results in terms of gelation temperature and release kinetics. In conclusion, zolmitriptan loaded ethosomal intranasal thermoreversible could be a potential dosage form for prolonged treatment of recurrent migraine.
Overall the triptans were envisaged for designing the preparation of in-situ gels involving the nanotechnology which facilitate the release of the drug in sustained manner also helps avoiding the first pass metabolism, decreasing the dosing frequency, improving the bioavailability and enhancing the therapeutic efficacy.