# SECTION IV
# In vivo studies in rat model- Pharmacokinetic study,Pharmacodynamic study And Haemodynamic study

## Chapter 7
## Pharmacokinetic study of formulations in rat

## 7.1 INTRODUCTION

Development of an efficient antimigraine therapy is required whereby drawbacks associated with oral administration; subcutaneous administration and intranasal administration of solution form of sumatriptan are overcome. The major factor limiting the bioavailability of nasally administered polar drugs is poor ability to cross mucosal membranes and mucociliary clearance mechanism in the nasal cavity that rapidly removes non bioadhesive solutions from the absorption site. To overcome these problems and to facilitate nasal absorption of polar molecules two main approaches have been used, the modification of permeability of the nasal mucosal membrane by employment of absorption enhancers and reduction of mucociliary clearance by use of bioadhesive systems or by increasing the viscosity of the formulation. A crucial improvement in the treatment of migraine would be a treatment for acute attacks with increased neural action as the receptors to sumatriptan are located intracranially. In addition, rapid onset of action and enhanced absorption to the cranially active target sites would be required for effective therapy. Nasal drug delivery systems that can enhance the residence time of sumatriptan succinate in the nasal cavity and enhance the permeability across the olfactory epithelium to cranially located target sites would be highly beneficial as it would not only result in quicker onset of action but also result in reduced prevalence of recurrent headache at 2 hours, moreover targeted action to intracranially located sites will result in dose reduction, further reducing cardiac side effects. Hardly any research has been reported on exploring possibility of increasing brain and CSF concentration for effective treatment of migraine Some reports are available on clinical studies pertaining to sumatriptan nasal spray for treatment of migraine, while no reports are available on the study of absorption of sumatriptan succinate across the nasal cavity to brain and cerebro spinal fluid.

In this study, we attempted to determine pharmacokinetic profile of all the optimized formulations (chitosan glutamate microspheres, carbopol 934P microspheres, pluronic F127 thermorevrsible gel, pluronic F127 thermorevrsible gel with chitosan glutamate and pluronic F127 thermorevrsible gel with carbopol 934P) in rat model after intranasal administration. Drug levels were estimated in blood, brain and cerebro spinal fluid and compared with the levels obtained after intranasal administration of sumatriptan succinate solution and

subcutaneous administration of sumatriptan succinate solution to investigate transport of drug across the nasal membrane into the CNS using rat model. The chapter also describes the influence of route of administration and formulation on the pharmacokinetics and distribution in brain and CSF.

## 7.2 EXPERIMENTAL

### 7.2.1 Pharmacokinetic studies

All experiments described in present report were approved by the Institutional Animal Ethics Committee (IAEC) of M S University, Baroda and are in accordance with guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Male wistar rats (200-250gms) were anesthetized with urethane (i.p., 1.2 g/kg) and kept under anesthesia throughout the whole experiment. Three rats for each formulation per time point were used in the study An incision was made along the neck, the trachea was severed and the upper part was tied off with a suture, the lower part was cannulated with a PE tubing to aid air breathing. The anesthetized animals were placed on a warming pad to maintain normal body temperature. After surgery, optimized sumatriptan succinate microspheres (chitosan glutamate (CGM) or carbopol microspheres (CM)) were insufflated into the nasal cavity of rat with a tube attached to a syringe device as described by (Lim et al, 2002) Thermoreversible gel formulations (Pluronic F127-chitosan glutamate gel (PLCG) and Pluronic F127-carbopol934P gel (PLC)) were instilled into the nasal cavity of group of rat via micro pipette tip. Another group obtained an intranasal (i.n) aqueous solution of sumatriptan succinate with micro pipette tip. To the other group, equivalent dose was administered subcutaneously (s.c) in the solution form. The rats received 10mg/kg of body weight of sumatriptan. The subcutaneous injections were used for relative bioavailability calculations The intranasal solution was used to ascertain the influence of powder formulation on nasal absorption and bioavailability. Cases with incomplete administration, estimated by the weight of the tip, rendered the experiment void and were repeated. Blood, CSF and brain samples were collected at 0, 5, 15, 30, 45, 60, 120, 240,480 and 720 min. CSF

samples were withdrawn by cisternal puncture (Dahlin et al, 2000). Terminal blood samples were collected from the descending aorta in tubes containing EDTA and centrifuged for 10 min at 8000 rpm for plasma separation. After the completion of the blood collection, the skull was opened and the brain removed. The tissue samples were weighed and homogenized in PBS (pH=7.4). All the samples were stored at -20°C until analysis. Processing and estimation of sumatriptan in blood, CSF and brain samples was performed by high performance liquid chromatography as described in chapter 3 using Dionex HPLC with a UV-visible detector (UVD170U).

### 7.2.2 Data analysis

Results obtained from the HPLC analyses were plotted as drug concentration versus time curves in plasma, brain and CSF. The pharmacokinetic parameters were determined by analyzing the data by wagner nelson method.

The relative bioavailability was calculated by dividing the mean plasma AUC after nasal administration by the mean value after s.c. administration. The apparent CSF and brain availability was defined as the ratio of $AUC_{CSF,in}/AUC_{CSF,s.c}$ and $AUC_{brain,in}/AUC_{brain,s.c}$. According to Hunt et al. (Hunt et al, 1996), the degree of sumatriptan succinate targeting to CSF and brain after intranasal administration can be evaluated by the drug targeting index (DTI), which can be described as the ratio of the value of AUCCSF or AUCbrain /AUCplasma following intranasal administration to that following subcutaneous route. The higher the DTI is, the further degree of sumatriptan succinate targeting to CSF and brain can be expected after intranasal administration Brain or CSF drug-direct-transport percentage (DTP (%)), which represents the percentage of drug directly transported to the brain or CSF via olfactory pathway.

DTP (%) has been calculated using following equations

$$DTP\ \% = \{(Bi.n. - Bx) / Bi.n\} * 100 \quad \ldots\ldots$$

Where, $Bx = (Bs.c. / Ps\ c) * (Pi.n.)$ ……

Bx = Brain or CSF AUC fraction (i.n.) contributed by systemic circulation through the BBB.

Bs.c. = $AUC_{0\rightarrow720}$ (brain) or (CSF) following subcutaneous administration

Ps.c. = $AUC_{0\rightarrow720}$ (blood) following subcutaneous administration.

Bi.n. = $AUC_{0\rightarrow720}$ (brain) or (CSF) following intranasal administration.

Pi.n. = $AUC_{0\rightarrow720}$ (blood) following intranasal administration

Literature citation reveals that the drug uptake into the brain from the nasal mucosa occurs via two different pathways. One is systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The other is the olfactory pathway by which part quantity of drug can travel from the olfactory region in the nasal cavity directly into CSF and/or brain tissue (Illum et al, 2000).

### 7.2.3 Statistical analysis

All the data sets are reported as mean ± S D of three experiments. Statistical comparision of the data was done by ANOVA followed by Dunnet's multiple comparision test at a significance level of P<0 05.

## 7.3 RESULTS AND DISCUSSION

Sumatriptan succinate formulation's {chitosan glutamate microspheres(CGM), carbopol 934P microspheres(CM), pluronic F127 gel(PLB), pluronic F127-chitosan glutamate gel(PLCG) and pluronic-F127-carbopol 934P gel(PLC) }optimized as described in previous chapters were administered to rats. Figure 7 1, 7.2 and 7.3 represents drug concentration profiles as a function of time for all the intranasal formulations, intranasal solution of sumatriptan succinate and subcutaneous solution of sumatriptan succinate in plasma, brain and CSF respectively It was found that the sumatriptan levels in CSF and brain after intranasal administration of drug solution and formulations were higher than those obtained after s.c route despite the statistically significant ($P<0.05$)lower sumatriptan succinate relative bioavailability in plasma after intranasal administration of the solution form as well as all the formulations

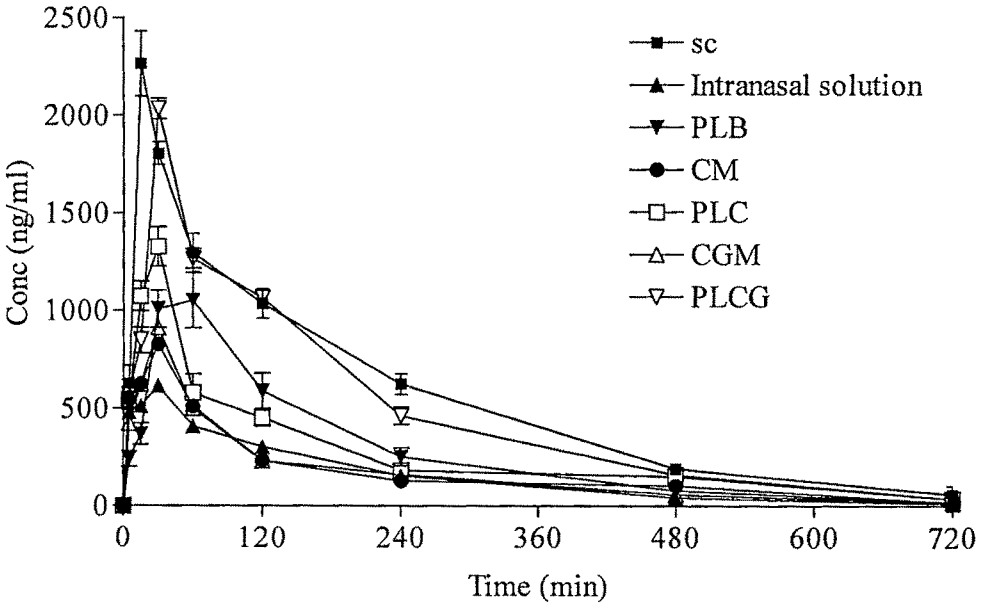


**Figure 7.1 Concentration time profile of sumatriptan in plasma, administered in solution form by s.c and intranasal route and various formulations administered intranasally. Data are expressed as mean ± S.D (n=3).**

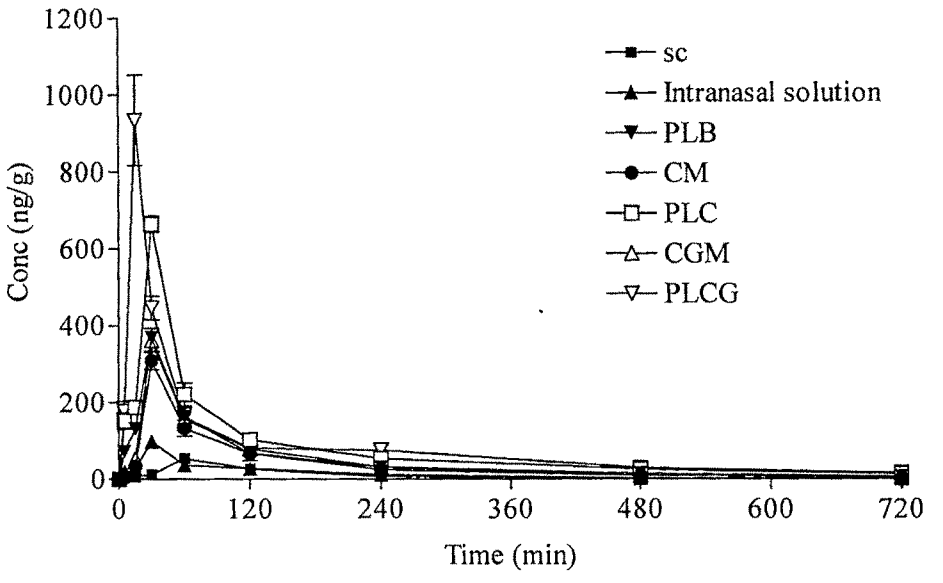


**Figure 7.2 Concentration time profile of sumatriptan in brain, administered in solution form by s.c and intranasal route and various formulations administered intranasally. Data are expressed as mean ± S.D (n=3).**

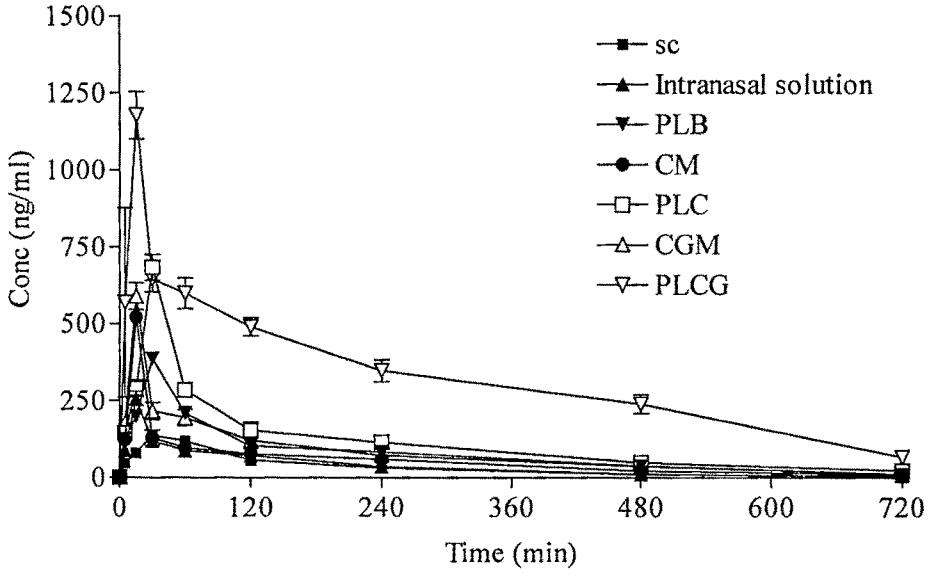


**Figure 7.3 Concentration time profile of sumatriptan in CSF, administered in solution form by s.c and intranasal route and various formulations administered intranasally. Data are expressed as mean ± S.D (n=3).**

**Table 7.1 Pharmacokinetic parameters for sumatriptan succinate administered in solution form by subcutaneous route, intranasal route and various intranasally administered formulations.**

| Formulation and route of administration | Organ/ tissue | Tmax (h) | Cmax(μg/ml) mean ± S.D. | $AUC_{0\rightarrow12}$ (h.μg/ml or g) mean ± S.D. | $AUC_{0\rightarrow8}$(h.μg/ml or g) mean ± S.D. | (AUCformulation/ AUC s.c)*100 mean ± S.D. | $T_{1/2}$(h) mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Subcutaneous route | Plasma | 0 25 | 2 26 ± 0 17 | 6 51 ± 0.16 | 6 73 ± 0 31 | 100 00 ± 0 00 | 2 33 ± 0 39 |
| | CSF | 0 5 | 0 14 ± 0 01 | 0.39 ± 0.01 | 0 40 ± 0.02 | 100 00 ± 0 00 | 2 39 ± 0 18 |
| | Brain | 1 | 0 05 ± 0.00 | 0.12 ± 0 02 | 0 12 ± 0 02 | 100 00 ± 0 00 | 2 01 ± 0 21 |
| Intranasal solution | Plasma | 0 5 | 0 62 ± 0 02* | 1 84 ± 0 1* | 1 87 ± 0 01* | 28 20 ± 1.25* | 2 10 ± 0 13 |
| | CSF | 0 25 | 0 26 ± 0 02* | 0 44 ± 0 04 | 0 46 ± 0 03 | 113 19 ± 7 35 | 2.36 ± 0 29 |
| | Brain | 0 5 | 0.10 ± 0 00 | 0 16 ± 0.02 | 0 16 ± 0.02 | 136.68 ± 19 54 | 1.85 ± 0 11 |
| CGM(i n) | Plasma | 0.5 | 0 91 ± 0.03*,a | 2.01 ± 0 14* | 2 08 ± 0.21* | 30 86 ± 2 36* | 2.30 ± 0 45 |
| | CSF | 0.25 | 0 59 ± 0.04*,a | 0.94 ± 0 14*,a | 0 98 ± 0 16*,a | 240 27 ± 30 66*,a | 2.52 ± 0 27 |
| | Brain | 0 5 | 0.36 ± 0 03*,a | 0 53 ± 0 08*,a | 0 54 ± 0 08*,a | 449 70 ± 37 79*,a | 2 02 ± 0 12 |
| CM(i.n) | Plasma | 0 5 | 0.83 ± 0 03* | 2.09 ± 0.17* | 2 14 ± 0 16* | 31 98 ± 1 85* | 2 38 ± 0 05 |
| | CSF | 0 25 | 0.52 ± 0 02*,a | 0 64 ± 0.02 | 0.68 ± 0.02 | 164 40 ± 11 08 | 3.17 ± 0 11 |
| | Brain | 0.5 | 0 31 ± 0 02*,a | 0 44 ± 0 01*,a | 0.46 ± 0.10*,a | 376 80 ± 26 11*,a | 1 99 ± 0 15 |
| PLB(i.n) | Plasma | 1 | 1.05 ± 0 14*,a | 3 28 ± 0 43*,a | 3 33 ± 0 41*,a | 50.41 ± 7 68*,a | 2 17 ± 0.22 |
| | CSF | 0 5 | 0 38 ± 0 01*,a | 0 93 ± 0.06*,a | 0 97 ± 0 09*,a | 237.76 ± 8 18*,a | 2 65 ± 0.67 |
| | Brain | 0 5 | 0 37 ± 0 01*,a | 0.56 ± 0 05*,a | 0 59 ± 0 06*,a | 486 05 ± 74 81*,a | 2 46 ± 0.36 |
| PLCG(i n) | Plasma | 0 5 | 2 03 ± 0 05*,a | 5 66 ± 0 32a | 5.79 ± 0.30*,a | 86 98 ± 5 07*a | 2 24 ± 0.15 |
| | CSF | 0 25 | 1 18 ± 0 08*,a | 3 86 ± 0 34*,a | 4 19 ± 0 45*,a | 990 83 ± 59 96*,a | 3 42 ± 0.33 |
| | Brain | 0 25 | 0 93 ± 0.12*,a | 1 00 ± 0 07*,a | 1 08 ± 0 08*,a | 869 60 ± 177.83*,a | 3 47 ± 0.27 |
| PLC(i n) | Plasma | 0 5 | 1 33 ± 0 10*,a | 3 14 ± 0 46*,a | 3 27 ± 0 5*,a | 48 06 ± 6 19*,a | 2 58 ± 0.15 |
| | CSF | 0 5 | 0.68 ± 0 04*,a | 1 35 ± 0 12*,a | 1.44 ± 0 12*,a | 347.39 ± 19 74*,a | 2 88 ± 0 38 |
| | Brain | 0 5 | 0.66 ± 0 02*,a | 0 91 ± 0 03*,a | 0.97 ± 0.04*,a | 793 98 ± 135 69*,a | 2 95 ± 0.23 |

* Significant difference as compared to s.c. route ($P<0.05$),[a] significant difference as compared to intranasal solution ($P<0.05$)

Analysis of the results given in Table 7 1 showed that relative bioavailability of sumatriptan in plasma was about 28% after intranasal administration (solution form) compared to subcutaneous route. However, relative bioavailability of drug in plasma was increased for all the formulations as compared to intranasal solution ranging from about 31.98% to 86.98% As all the formulations possessed mucoadhesive potential as well as permeation enhancing effect (except pluronic F-127 gel) their retention in the nasal cavity coupled with increased permeability would have increased bioavailability of drug from the formulations in plasma compared to solution form. Mucoadhesive microspheres exhibited comparatively lower relative bioavailability as compared to thermoreversible gel formulations; this could be probably attributed to different patterns of insufflation expected due to different aerodynamics of liquid and solid particles (Shand et al., 1970; Provasi et al., 1994). This will affect the deposition pattern and the site of liquid vs. powder formulation within the nasal cavity. Variation in the deposition site may have affected both the rate and extent of absorption and clearance by the beating cilia. Moreover thermoreversible gel formulations could have formed uniform layer of gel on the nasal mucosa, reducing the mucociliary clearance and enhancing the residence time in the nasal cavity due to higher viscosity coupled with mucoadhesive potential and permeation enhancing effect.

Although, apparent availability of sumatriptan in CSF and brain after intranasal administration of solution form was higher compared to that achieved after s.c route, it was not statistically significant($P<0.05$). However for all the formulations apparent availability in brain was significantly higher compared to that obtained with s c route and intranasal solution, apparent availability in brain was in the order of PLCG<PLC<PLB<CGM<CM. As explained previously thermoreversible gels with chitosan glutamate exhibited the maximum apparent availability in the brain, reason for this could be as explained previously. Apparent availability in CSF was significantly enhanced for all the formulations (except CM, where the enhancement was there but was not statistically significant) compared to s c route as well as intranasal solution.

Peak plasma concentrations (Cmax) were significantly lower whereas peak CSF (Cmax) were significantly higher for all the intranasally administered formulations including intranasal solution as compared to s.c injection. Whereas peak brain concentrations were significantly higher for all the intranasally administered formulations as compared to s.c route, however peak brain concentration for intranasal solution was not significantly

higher compared to s.c route. All the nasally administered formulations demonstrated significantly higher peak CSF and brain concentrations compared to intranasal solution. Also, peak CSF and brain concentrations for intranasal formulations were achieved much earlier than that of s c injection. These findings suggested that the existence of an alternative transport pathway to the CSF and brain other than the penetration across the BBB from the systemic circulation One suggested anatomical pathway is that, where compounds are transported by the olfactory sensory neurons. Neuronal transport is generally believed to be a slow process. Another plausible explanation is that foreign substances can diffuse into the nasal submucosa and subsequently travel into the olfactory perineuronal channels, transport of substances into the CNS via the epithelial pathway could be more rapid than axonal transport. It is likely that sumatriptan succinate that appeared rapidly in CSF and brain after nasal administration have been transported through this pathway. The statistical significant difference of apparent CSF and brain availability and Cmax values between the intranasally administered formulation and solution form is probably due to loss by drainage from the deposition site within the nasal cavity for the solution form resulting in short duration available for direct transport of drug across olfactory epithelium to CSF and brain regions, an effect that was absent for the microspheres as well as thermoreversible gels. In order to increase the total absorption of drug through the nasal mucosa in brain and CSF and thereby the apparent availability we have explored the possibility of obtaining slow nasal clearance times for the delivery systems in the form of mucoadhesive microspheres and mucoadhesive thermoreversible gel Apart from reduced mucociliary clearance (due to mucoadhesion / increased viscosity of the formulations) allowing formulations to be in contact with olfactory epithelium for longer time periods, formulations also exhibited in-vitro permeation enhancing effect (except PLB) as shown in previous chapter, which additionally contributes in increasing drug absorption of hydrophilic drug sumatriptan succinate across olfactory epithelium. All of the above factors culminating together increased availability of drug across olfactory epithelium to CSF and brain tissues

**Table 7.2 Drug targeting Index and direct nose-to-brain transport* following intranasal administration of solution form and various intranasal formulations**

| Formulation and route of administration | Organ/ tissue | Drug Targeting Index mean ± S.D. | Drug direct transport percentage mean ± S.D. |
|---|---|---|---|
| Intranasal solution | CSF | 4.01 ± 0 08 | 75 07 ± 0.50 |
| | Brain | 4.87 ± 0.89 | 78.96 ± 4 27 |
| CGM(i n) | CSF | 7 79 ± 0 80 | 87.06 ± 1.41 |
| | Brain | 14.59 ± 1.00 | 93.12 ± 0 48 |
| CM(i.n) | CSF | 5.17 ± 0 66 | 80.44 ± 2.36 |
| | Brain | 11.81 ± 1.10 | 91.48 ± 0.82 |
| PLB(i.n) | CSF | 4.80 ± 0.81 | 78.72 ± 3.89 |
| | Brain | 9.66 ± 0.64 | 89.61 ± 0.69 |
| PLCG(i.n) | CSF | 11.40 ± 0.67 | 91.21 ± 0.52 |
| | Brain | 10 02 ± 2.10 | 89.74 ± 2.00 |
| PLC(i.n) | CSF | 7.27 ± 0.54 | 86.20 ± 1.01 |
| | Brain | 16.96 ± 3.10 | 93.74 ± 1.85 |

* Parameters are derived using mean ± S D.

DTI (drug targeting index) – brain and CSF values for all the intranasally administered formulations including intranasal solution were much greater than 1(s.c route). Table 7.2 shows DTI and % DTP values for various intranasally administered formulations. It is evident from the results given in Table 7.2 that DTI values for all the intranasally administered formulations were higher compared to that of solution form. This suggests that sumatriptan succinate has a measurable degree of targeting to CSF and brain attributed to direct nose to brain transport (olfactory pathway), which is increased when sumatriptan succinate is incorporated in mucoadhesive microspheres or thermoreversible gel (with or with out mucoadhesive polymer). The degree of targeting for CSF was in order of PLCG>CGM>PLC>CM>PLB>IN(solution), it is evident that formulations containing chitosan glutamate had higher degree of targeting followed by formulation containing carbopol 934P and thereafter formulation without mucoadhesive polymer and least for intranasal solution form. However in case of DTI for brain no such established trend was observed but one thing common between DTI values for CSF and brain was that DTI for brain was lowest for intranasal solution followed by slightly higher DTI for pluronic gel (without mucoadhesive and permeation enhancing material), whereas for rest of the formulations DTI was higher compared to above two without any particular trend with respect to polymer. This suggests importance of mucoadhesive polymers associated with permeation enhancing effect in targeting of sumatriptan succinate to intracranially located sites

DTP (%) drug direct transport percentage to CSF and brain was higher for all the intranasally administered formulations including intranasal solution (table 7.2). It could be seen that DTP (%)-CSF and brain was higher for all the formulations compared to solution form. It is also evident that among the formulations, PLB (without any mucoadhesive material) showed lowest DTP (%) for CSF and brain This suggests that formulations containing mucoadhesive materials associated with permeation enhancing effect have substantial direct nose to brain transport. This may be due to tight junction opening characteristic of both chitosan glutamate and carbopol, resulting in enhanced paracellular transport of sumatriptan succinate across olfactory mucosa.

The direct pathways for the transfer of low BBB permeability drugs from the nasal cavity via the olfactory mucosa into the CNS have been supported in various researches (Sakane et al 1991) reported that cephalexin was preferentially to enter CSF after nasal administration as compared to i.v. and intraduodenal administration in rats. The levels in

CSF were 166-fold higher, 15 min after nasal administration than those of the other two routes

This is also the case in another study with methotrexate, in one of the study the AUCCSF ratio of i n dosing was more than 13 tımes as high as ı v injection. Illum illustrated that the direct pathway from nose–brain may only be sıgnificant for compounds that have low BBB transport properties (Illum, 2000) Here we can conclude that, for the poor BBB penetration drugs like sumatriptan succınate, ıt is promisıng to obtain high CSF and brain drug concentrations through nasal drug delivery.

It could also been seen from the data in Table 7.1 , that $t_{1/2}$ for all the intranasally admınistered formulations were higher for plasma, CSF and brain compared to intranasal solution, this further confirms longer residence time of the formulations at the absorption sıtes in the nasal cavıty. Increased $t_{1/2}$ for the formulations, particularly in CSF and brain can be explored as basis for potential nasal drug delivery system whereby repeated dosing sometimes required with intranasal administration of solution form can be ruled out.

## 7.4 CONCLUSION

Sumatriptan succinate, poorly permeable through BBB, when administered nasally has a characteristic of brain targetıng; it may be helpful for both increasing the CSF and brain therapeutic levels and reducing the systemic side effects. After intranasal administration, sumatriptan succinate was able to penetrate into the brain and CSF directly from the nasal cavity, with the olfactory epithelium being path of direct transport. Maximum concentration ın brain and CSF after intranasal administration was higher than s c route and was also achieved earlier, thus onset of action for drug required for immediate treatment of migraine attack would be very early. Nasal administration of sumatriptan succinate in the form of mucoadhesive microspheres (chitosan glutamate and carbopol 934P) and thermoreversible gel (pluronic F127 gel, pluronic F127 gel with chitosan glutamate and pluronic F127 gel with carbopol934P), increased relative availabılity of drug in CSF and brain, peak concentrations in CSF and brain, drug targeting index and drug nose to brain direct transport percentage compared to intranasal solutıon It also slightly increased half life of drug in brain and CSF compartment further exploring the possibility of prolonged delivery of drug to the target sites and thereby possibly ruling out the necessity of repeated doses required during the attack. Thus nasally administered sumatriptan succinate in mucoadhesive formulations which increases nasal residence tıme due to mucoadhesıve potential/ıncreased viscosity as well as permeation enhancing effect

is promising to become an effective non-invasive route for treatment of migraine, although it has not been clinically proven.

## 7.5 REFERENCES

Dahlin M, Bjork E (2000). Nasal absorption of (s)-UH-301 and its transport into the cerebrospinal fluid of rats Int J Pharm , 195, 197-205.

Hunt CA, MacGregor RD, Siegel RA (1986). Engineering targeted in vivo drug delivery: I The physiological and physicochemical principles governing opportunities and limitations. Pharm Res., 3, 333–344.

Illum L (2000). Transport of drugs from the nasal cavity to central nervous system. Eur J Pharm Sci , 11(1), 1-18.

Lim ST, Forbes B, Berry DJ , Martin GP , Brown MB (2002). In vivo evaluation of novel hyaluronan/chitosan microparticulate delivery systems for the nasal delivery of gentamicin in rabbits Int J Pharm., 231, 73–82.

Provasi D, De Ascentiis A, Minutello A, Colombo P, Catellani DL (1994). Nasal powders of progesterone: manufacturing and bioavailability. Eur J Pharm Biopharm , 40, 223–227.

Sakane T, Akizuki M, Yamashita S (1991). Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. J Pharm Pharmacol., 43, 449–451.

Shand DG, Nuckolls EM, Oates JA (1970) Plasma propranolol levels in adults, with observations in four children. Clin Pharmacol Ther., 11, 112–120.