Chapter 6
Thermoreversible gels

6.1. INTRODUCTION

Viscous solutions are also reported to increase residence time in the nasal cavity. However, application of the viscous solutions to the nasal cavity is unlikely. Therefore, application of in-situ gelling solutions of low molecular weight triblock copolymers of poly(ethylene oxide) and poly(propylene oxide) (PEO/PPO/PEO) (Pluronics) exhibiting thermoreversible properties have been proposed to render convenient instillation of the nasal formulations at the temperature generally below the body temperature (Illum, 1999). These polymers make it possible to obtain low viscosity solutions at room temperature, which can be applied for instance by nasal spray, which increases the viscosity at body temperature and ensures a better and longer contact with the nasal mucous membrane. Pluronic F-127(Poloxamer 407), a non-toxic (PEO/PPO/PEO) triblock copolymer with a weight-average molecular weight of 12,600, contains 70% hydrophilic ethylene oxide units and 30% hydrophobic propylene oxide units. It forms a gel on warming to body temperature by undergoing a sol–gel transition. As a result of this reverse thermal gelation and extremely low toxicity, the administered solution containing drug turns into a gel and renders slow release characteristics to the drug delivery system in the pharmaceutical fields (Cho et al, 2003). By modulating the gelation temperature of different pluronic solutions, liquid bases for nasal use can be formulated which form a gel in the nasal cavity at body temperature with suitable gel strength resulting in enhancement of the residence time in the nasal cavity (Zhou et al, 1996). They have been widely used for parenteral administration (Gariepy et al, 2004). Besides injectables, other administration routes such as rectal, vaginal, transdermal and ophthalmic have also been evaluated (Gariepy et al, 2004). Pluronics are also reported to exhibit mucoadhesive potential. Furthermore, in order to fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers such as polycarbophil, hydroxypropyl cellulose, polyvinylpyrolidone have been added to the in situ-gelling liquids (Chu et al , 1991). However, there are very few reports on evaluating pluronics for intranasal drug delivery (Bromberg et al. 2001), in this report by Bromberg et al effect of addition of polycarbophil to pluronic on nasal clearance rate was studied. Recently, the use of thermo-reversible pluronic F-127 gels of vitamin B_{12} for nasal delivery has been reported by Pisal et al (Pisal et al, 2004).

Thermoreversible gels of pluronic F127 could be one of the potential carriers for intranasal drug delivery, due to their enhanced viscosity and moderate mucoadhesive strength in the nasal cavity, which would enhance residence time and thereby nasal

absorption of drug by reducing mucociliary clearance rate. The addition of bioadhesive polymers can lengthen the residence time and enhance bioavailability of drugs delivered to the nasal cavity. The nasal bioadhesive gels might be used to provide an enhanced bioavailability compared with oral delivery (D'souza et al, 2005). A very good example for such a system is EnerB (Nature's Bounty Inc, NY), a vitamin B-12 supplement available in gel form. These systems could be effectively used in treatment of migraine by intranasal route as it would increase residence time in the nasal cavity associated with increased drug absorption to intracranially located target sites for sumatriptan succinate. This local transfer of sumatriptan to target sites will not only result in rapid onset of action but increased residence time of the formulation in the nasal cavity will result in highly effective antimigraine therapy. Effect of addition of mucoadhesive polymer on rheological behavior, mucoadhesive strength and in vitro permeation across nasal mucosal membrane has never been studied. In the past no attempt has been ever done to study effect of pluronics or its combination with mucoadhesive polymer on permeation of drug across the mucosal membrane. Moreover, combination of non-ionic surfactant pluronic as thermoreversible polymer and cationic polymer chitosan glutamate as mucoadhesive polymer and absorption enhancing material has never been explored before as potential drug delivery system with number of advantages. Therefore this chapter deals with development of effective intranasal delivery systems of sumatriptan succinate using thermoreversible polymer pluronic and mixed gels prepared by addition of mucoadhesive polymer carbopol 934P or chitosan glutamate to pluronic The formulations were evaluated for its gelation temperature, rheological characteristics, mucoadhesive strength, in vitro permeation across sheep nasal mucosa and in vitro toxicological effects of the vehicles on sheep nasal mucosal membrane

6.2. PREPARATION METHODS

6.2.1. Preparation of Pluronic F-127 gels

Different concentration of pluronic gels were prepared by cold process (Schmolka et al, 1972) Briefly, weighed amount of pluronic F-127(PF127) and sumatriptan succinate (7%w/v) was slowly added to cold distilled water at about 4°C with mild agitation. This liquid was left at 4°C until a clear solution was obtained. Pluronic vehicles with varying concentration 12%, 14%, 16%, 17%, 18%, 19% and 20 %w/v were screened. Benzalkonium chloride (0.01%w/v) was added to the formulations. pH of the formulations was measured

6.2.2. Preparation of mixed gels of Pluronic F-127 and carbopol 934P

Thermoreversible gels containing pluronic F-127 and carbopol 934P (C934P) were prepared using cold method (Schmolka et al, 1972) . PF127(18%w/v) and sumatriptan succinate(7%w/v) were solubilized in cold distilled water containing 1% propylene glycol at 4°C. The pluronic F-127 vehicles used throughout this study were composed of 18% w/v of PF127. The concentration of pluronic F-127 was selected so as to obtain thermoreversible gel at minimum possible concentration. The mixture was left at 4°C until a clear solution was obtained. Bioadhesive anionic polymer C934P was slowly added to the solution with continuous agitation. C934P was added in concentration range of 0.1% w/v to 1% w/v to pluronic F-127 solution. Benzalkonium chloride (0.01%w/v) was added to the formulations. pH of the formulations was measured.

6.2.3. Preparation of mixed gels of Pluronic F-127 and chitosan glutamate

Thermoreversible gels containing pluronic F-127 and chitosan glutamate were prepared using cold method (Schmolka et al, 1972). Pluronic F-127 and sumatriptan succinate (7%w/v) were solubilized in distilled water at 4°C. The pluronic F-127 vehicles used throughout this study were composed of 18% w/v The mixture was left at 4°C until a clear solution was obtained Bioadhesive cationic polymer chitosan glutamate was slowly added to the solution with continuous agitation. Chitosan glutamate was added in concentration range of 0.1% w/v to 1% w/v to poloxamer solution. Benzalkonium chloride (0.01%w/v) was added to the formulations. pH of the formulations was measured.

6.3. CHARACTERISATION OF FORMULATIONS

6.3.1. Measurement of gelation temperature by visual inspection

Gelation temperature defined as the temperature, at which the liquid phase undergoes a transition to gel, was determined as described previously (Choi et al, 1998). In brief, a 10-mL transparent vial containing a magnetic bar and each formulation was placed in a water bath. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

6.3.2. Gelling Temperature Determination by rheological method

Rheological studies were performed with a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LVDV III) fitted with CP-52 spindle. The cone/plate geometry was used. The cone had a 1.2 cm radius and an angle of 3°. Pluronic F-127 formulations were evaluated rheological measurement of gelation temperature by brookfield rheometer at 1.66 s^{-1} and 10 s^{-1} shear rate. Whereas, for mixed gels of Pluronic F-127 carbopol 934P and Pluronic F-127 chitosan glutamate the shear stress was controlled to maintain a shear rate of 10 s^{-1} shear rate. This value was chosen to allow precise determination of the gelling temperature. The temperature was increased in steps of 1°C per minute, from 20°C to 40°C to locate the sol/gel transition point. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (mPa s) as a function of the temperature (°C). Each preparation was tested thrice to control the repeatability of the measurement.

6.3.3. Evaluation of the mucoadhesive strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method described by Jones et al (Jones et al, 2000). Briefly, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse Tissues were immediately used after separation. At the time of testing a section of nasal tissue was secured, keeping the mucosal side out, to upper probe using a cyanoacrylate adhesive. Upper probe was attached to precalibrated force displacement transducer SS12LA, (BIOPAC systems, Inc , CA, USA) connected to the Biopac MP-30 data acquisition system (BIOPAC Systems, Inc. , CA, USA). The surface area of each exposed mucosal membrane was 0 785cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe The probes were equilibrated and maintained at 34°C Probe with nasal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 2 min to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation (Chng et al, 1985)

$$Detachment\ stress(dyne/cm^2) = \frac{mg}{A}$$

Where, m: the weight added to the balance in gram, g: acceleration due to gravity taken as 980 cm/sec^2, A: area of tissue exposed. Measurements were repeated thrice for each of the gel preparations, but before each measurement fresh smooth gel surface was created.

6.3.3a.Effect of initial contact time on mucoadhesive strength

Effect of varying contact time (1min, 2min, 3min, 5min and 10 min) was investigated for some of the gel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact times (1min, 2min, 3min, 5min and 10 min) and the bioadhesive force was determined as discussed above. Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time required for adequate adhesion.

6.3.4. In vitro Permeation Studies

Performed as described in section 5.2.6

6.3.5. Histopathological evaluation of mucosa

Histopathological evaluation of tissue incubated in PBS (pH 6.4) after collection was compared with tissue incubated in the diffusion chamber with optimized gel formulations. Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (H & E). Sections were examined under a light microscope, to detect any damage to the tissue during *in vitro* permeation by a pathologist blinded to the study.

6.3.6. Statistical Analysis

Data were expressed as mean with standard deviation (SD). Statistical analysis of data was performed using ANOVA followed by Dunnett's Multiple Comparison test. A *p*-value of less than 0.001 was considered significant.

6.3.7. Stability Studies

The optimized gel formulation samples were stored in well sealed glass vials for a period of 90 days at Room temperature (37°C) and at 4°C. After storage, samples were collected

and evaluated for physical appearance and gelation temperature (rheological method) was evaluated Sumatriptan succinate content of samples was determined by method described in Chapter 3. Prior to analysis of gel samples, a weighed amount was reconstituted with water.

6.4. RESULTS AND DISCUSSION

6.4.1. PLURONIC F-127 GELS

Thermoreversible polymer-based liquid formulations that provide *in situ* gelling property in nasal cavity were designed to delay clearance of the formulations from the nasal cavity and enhance retention and thereby increase absorption of drug from the nasal cavity. Usually, the gelation temperatures have been considered to be suitable if in the range of 25–34 °C. If the gelation temperature of a thermoreversible formulation is lower than 25 °C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering If the gelation temperature is higher than 34 °C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. As the temperature of the nasal cavity is 34°C (Keck et al, 2000), our study is aimed at preparing the liquid formulations of PF127 that may gel below 34°C and determining the lowest possible concentration of pluronic which gels below 34°C with suitable rheological and mucoadhesive potential. pH of all the samples was between 5-6.5, which is suitable for nasal administration.

a. Viscosity and gelling temperature determination

PF127 formulation of sumatriptan succinate studied existed as a free flowing viscous liquid at the storage temperature (4°C), formed a semisolid gel at experimental temperatures (i.e., 34°C), and returned to the liquid state upon cooling below gelation temperature. For preparation of in situ gel that gels upon instillation into the nasal cavity at temperature of 34°C, the sol-gel transition temperature has to be lower than 34°C. The gelation of pluronic F-127 vehicles is known to result from the change in miceller number with temperature. With increasing temperature, number of micelles formed are increased which is consequence of the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed (Kabanov et al, 2002). Recently, Cabana et al (Cabana et al,

1997) suggested a mechanism of gelation based on micelles packing and entanglements. Also, conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon (Rassing et al, 1983). At 20°C, all PF127 solutions were in liquid state with viscosity ranging from 21 to 73 mPa s (at 10 s^{-1} shear rate) for 12%w/v to 20% w/v pluronic F127. Rheological behavior of different concentration of pluronic F-127 solutions measured at two different shear stress conditions (1.66s^{-1} and 10s^{-1}) is given in Figure 6.1. All solutions exhibited Newtonian behavior at 20°C, all the formulations were too soft and remained as a liquid and no gel formation was observed.

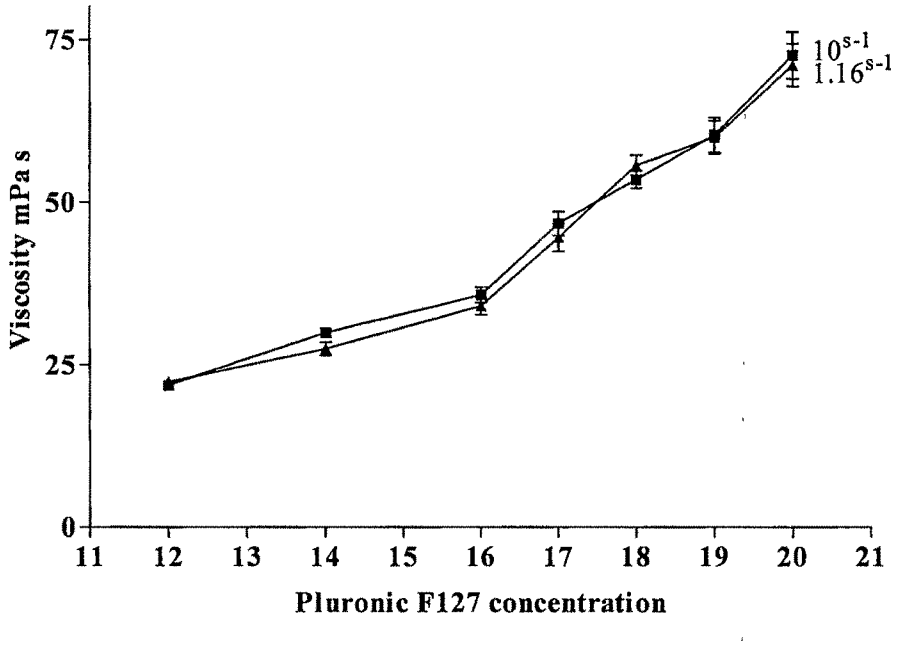

Figure 6.1 Viscosity measured at two different shear rates for different concentration of pluronic F-127 at 20°C. Values are expressed as mean ± SD (n=3).

However at 34°C, the behavior of PF127 solutions changed, depending on the polymer concentration. The 12% to 17% w/v solution remained fluid and showed a constant viscosity between 32 mPa s and 73 mPa s. Pluronic solutions ranging between 12% to 17% w/v exhibited Newtonian flow at 34 °C also. When Newtonian viscosity values were investigated, no significant difference was observed between temperatures for 12% to

17% w/v pluronic F-127 demonstrating that the temperature does not dramatically affect the viscosity of the non-concentrated solutions of pluronic. Results are shown in Figure 6.2.

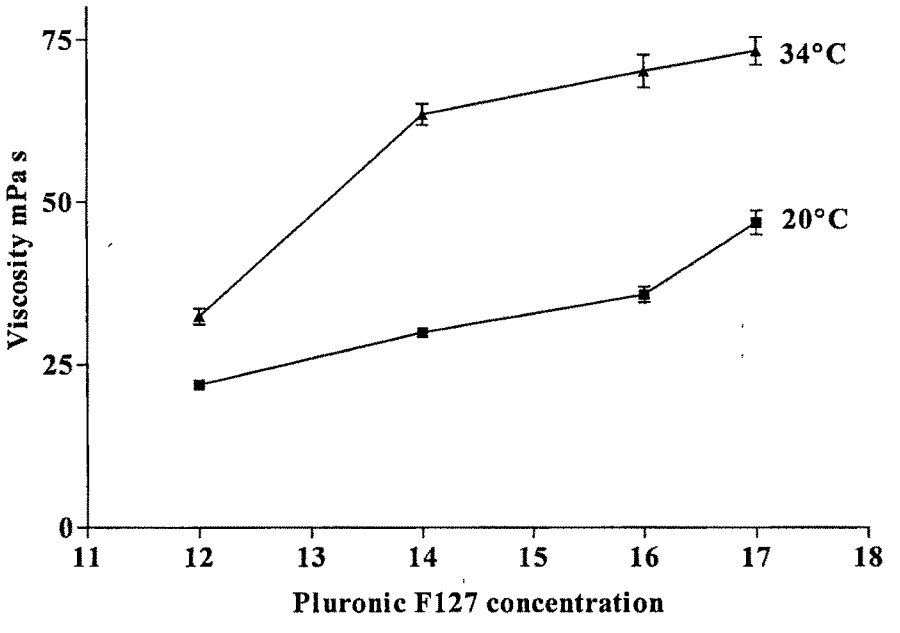

Figure 6.2 Viscosity values for different concentration of pluronic F-127 at constant shear rate ($10s^{-1}$) measured at 20°C and 34°C. Values are expressed as mean ± SD (n=3).

Beyond 18% w/v concentration, the preparations gelled and showed a shear-thinning (pseudoplastic) behavior. The reason for this is that the preparation containing pluronic 18%w/v or greater has a sol-gel transition temperature below 34°C. They formed semisolid gels at 34 °C, increased concentration of pluronic resulted in large resistance to flow, primarily due to entanglement of molecule chains. Gelation temperature of gels prepared with 18%w/v, 19%w/v and 20%w/v was much below 34°C. The curves of viscosity (mPa s) of each gel above 18%w/v PF127, measured at a shear rate of 10 s-1, as a function of the temperature (°C) were composed of three phases. In the first part, the viscosity was nearly constant; in the second part, it increased dramatically and reached its maximum; in the third part, it remained constant at the plateau. Figure 6.3 represents the viscosity (mPa s) of each gel, measured at a shear rate of 10 s^{-1}, as a function of the temperature (°C).The gelling temperature, determined graphically as the inflection point

of the second part of the curve, decreased as the polymer concentration increased i e. 29°C, 27°C and 24.5°C respectively for 18%, 19% and 20%w/v PF 127.

The results of gelation temperature obtained by visual inspection and rheological study did not vary more than ± 1°C; results are shown in Table 6.1. Results of the rheological study for the formulation containing 18%w/v pluronic exhibited pseudo plastic (i.e., shear thinning) flow behavior at its gelation temperature (exhibiting maximum viscosity of 1810 at $10s^{-1}$. and 5888 mPa s at 1.66 s^{-1}), same is true for formulation containing 19%w/v and 20%w/v pluronic F-127. Objective of our work was to assess whether pluronic F127 was compatible with components of the formulation and to determine the range of concentrations that could provide solutions that were liquid at room temperature and gelled at the physiological temperature in the nasal cavity. The threshold concentration was 18%, and the viscosity of the gels increased with the PF127 concentration At higher concentrations, a polymolecular micelles forms and several micelles come together and minimize their interaction with water, whereas at lower concentration monomolecular micelle is formed At lower temperature, water molecules around the polymer chain are ordered and the hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxypropylene) units of pluronic molecules dominate polymer chains approach closer and squeezed ordered water molecules Thus the viscosity of the gel increases and high viscosity or rigid gel is formed. These data are in agreement with a recent study on 14% to 25% w/w pluronic F127 solutions in ultrapure water, which showed that below 18%, solutions did not gel, and 18%, 20%, and 22% preparations, newtonian at 20°C, were pseudoplastic at 35°C, with viscosity that increased with the polymer concentration (Edsman et al, 1998) Our determination of the sol-gel transition temperature demonstrated that, the higher the concentration, the lower the transition temperature and the higher the final viscosity.

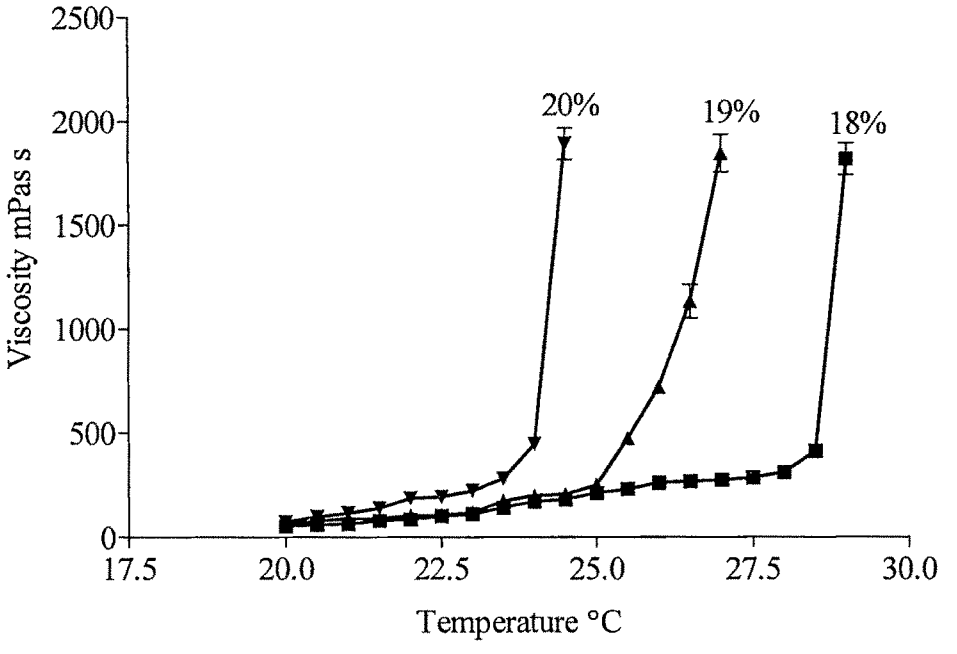

Figure 6.3 Effect of temperature on the viscosity of various pluronic gels with varying concentration of pluronic F127 measured at 10 s^{-1} shear rate. Values are expressed as mean ± SD (n=3).

Table 6.1 Gelling temperature determined rheologically and by visual inspection for various formulations containing different concentration of pluronic F127

Composition	Sol-gel transition temp. (°C)	
	Rheology	Visual Inspection
18%PF127(without drug)	28.7	28.0 ± 0.9
18% PF127(with drug)	29	28 2 ± 1.0
19% PF127(with drug)	27	26.3 ± 0.6
20% PF127(with drug)	24.5	23.8 ± 0.3

The sol–gel transition temperature can be changed with addition of the drug. The graphs of sol–gel transition temperature for the concentration of pluronic solution of 18% with sumatriptan succinate or absence of the drug are shown in Figure 6.4. The viscosity, is low for the solutions and increases with the temperature as a result of gel formation

process. The presence of the sumatriptan succinate in the gel did not significantly change the transition temperature (Table 6.1). The addition of sumatriptan succinate did not affect the process of gelation and sol–gel transition temperature. Sumatriptan succinate is a hydrophilic drug and it is primarily located outside the pluronic micelles of the gels. It can interfere with the gelification process and with the physical-chemical characteristics of the polymer solution. In the present study, the sol–gel transition temperature was not affected by the addition of the drug (Table 6.1). The gel viscosity was slightly affected by the addition of the drug and 18%w/v pluronic F127 gel containing 7% sumatriptan succinate is less viscous than the gel in the absence of the drug. However, decrease in viscosity was not significant and hence, addition of drug did not affected gelation temperature or viscosity of the gel.

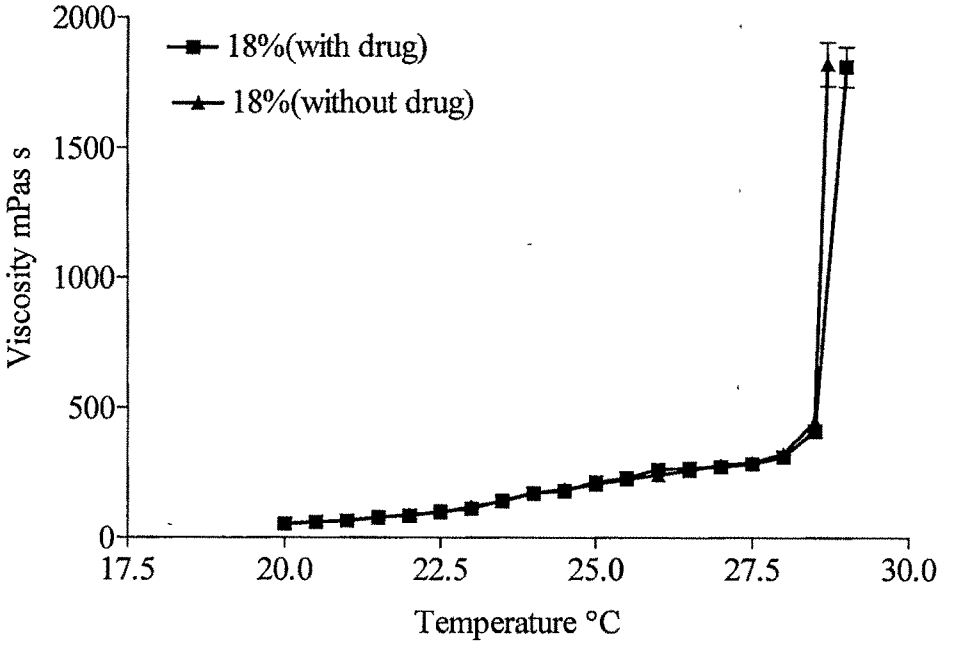

Figure 6.4 Effect of addition of sumatriptan succinate on the viscosity of 18%w/v pluronic gels with as a function of temperature measured at 10 s^{-1} shear rate. Values are expressed as mean ± SD (n=3).

b. Mucoadhesive strength

The model used for mucoadhesive strength measurement was validated by studying effect of initial contact time of the tissue with formulation. 2 minutes was found to be optimum time to achieve maximum detachment stress. At lower contact time formulations did not have sufficient time to interact with mucosal membrane whereas increase in contact time more than 2 min did not affected mucoadhesive strength further. The assessment of the mucoadhesive strength at 34°C showed that the PF-127 preparations possessed adhesive properties that increased with the polymer concentration. The mucoadhesive strength expressed as detachment stress was very low with 12% to17% w/v PF-127 and significantly increased from the 18% concentration. Further increase in the polymer concentration up to 20%w/v did not significantly affected mucoadhesive strength compared to 18%w/v.Mucoadhesive strength data is shown in Figure 6.5. The poor adhesion observed with the lower PF-127 concentration could be explained by the fluidity of the preparation at 34°C, whereas the significantly higher adhesive properties of the gels containing 18% to 20% PF-127 compared to 12%w/v PF-127 could be due to their higher viscosity Indeed, it has been demonstrated that concentrated pluronic F127 solutions, characterized by high viscosities, presented better adhesive properties to ileal mucosa (Juhasz et al, 1991) or an increased ocular contact time (Edsman et al, 1998) than diluted preparations. The three highest concentration of PF-127 could therefore provide adhesive sumatriptan succinate gels likely to prolong the residence time at the absorption site in the nasal cavity. However, there was no significant difference($P<0.001$) in the mucoadhesive strength of 18%w/v, 19%w/v and 20%w/v PF127 formulations and hence 18%w/v being lowest possible pluronic F-127 concentration exhibiting thermoreversible property with good mucoadhesive potential was selected further for in vitro permeation study.

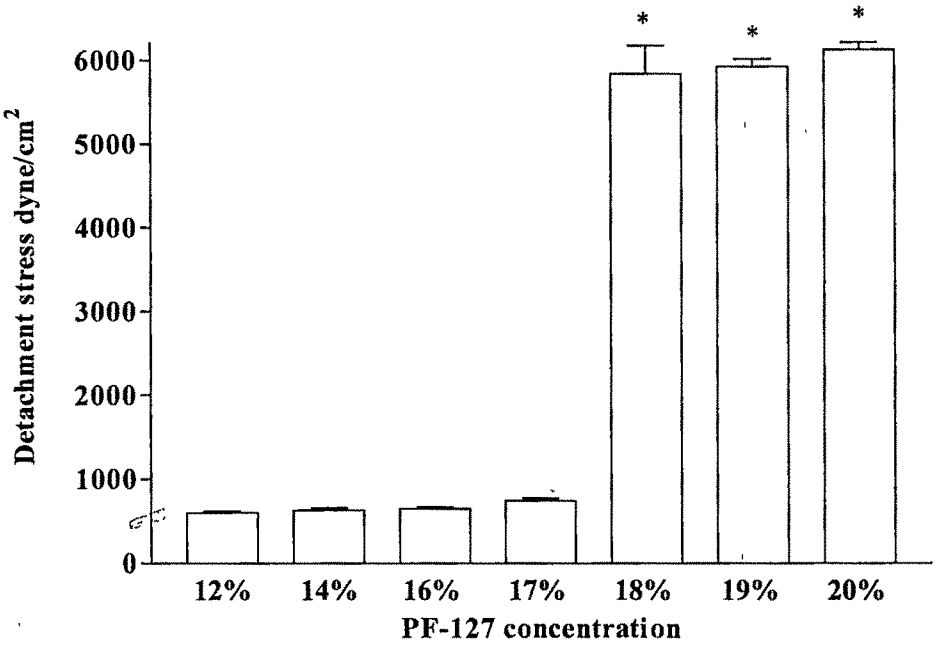

Figure 6.5 Influence of pluronic F127 concentration on the detachment stress measured *in vitro*, mean ± SD (n=3);*P <0.001 versus 12% pluronic F-127 concentration.

c. In vitro permeation study

Cumulative amount of drug permeated as function of time is given in figure 6.6. It is evident that cumulative amount of drug permeated with time was reduced for PLB (18%w/v PF127) compared to pure drug solution. Peff (effective permeability) of sumatriptan succinate was found to be 5.6 $x10^{-5}$ (± 0.37 x 10^{-5}) cm/s for pure drug solution and 4.5 $x10^{-5}$ (± 0.28 x 10^{-5}) cm/s for PF 127 18%w/v thermoreversible gel (PLB). Effective permeability of sumatriptan reported in literature was found to be 1.4 x 10^{-5} (cm s^{-1}) (Wadell et al, 2003), in this study effective permeability of 5.58 x 10^{-5}(cm s^{-1}) was obtained. This difference in the reported and obtained value may be due to different mucosal membrane used (reported method used bovine nasal mucosa), also the diffusion cell as well as diffusion medium were different in the two methods. Moreover reported method determined effective permeability using sumatriptan base whereas in this study succinate salt of sumatriptan has been used. Formulation was to be selected based on the maximum effective permeability obtained. It is evident that effective permeability

coefficient for sumatriptan succinate is significantly lower for formulation PLB ($P<0.001$) as compared to that of pure drug solution. Since pluronic gels are viscous, isotropic liquid crystals consisting of micelles, it was hypothesized that the drug is released by diffusion through the extramicellar water channels of the gel matrix and a higher concentration of Pluronic causes smaller-sized water channels, a lower micellar growth rate, or greater tortuosity(You Fang et al, 2002). Presence of pluronic in the gel retards the drug release rate slightly due to reduction in dimension of water channels resulting for enhanced micellar structure. Similar observations have been reported by Moore et al (Moore et al, 2000) and Bhardwaj and Blanchard (Bhardwaj and Blanchard, 1996). Concerning, local tissue damage staining of sheep nasal mucosa with trypan blue after permeation studies with various formulations did not show any dead cells.

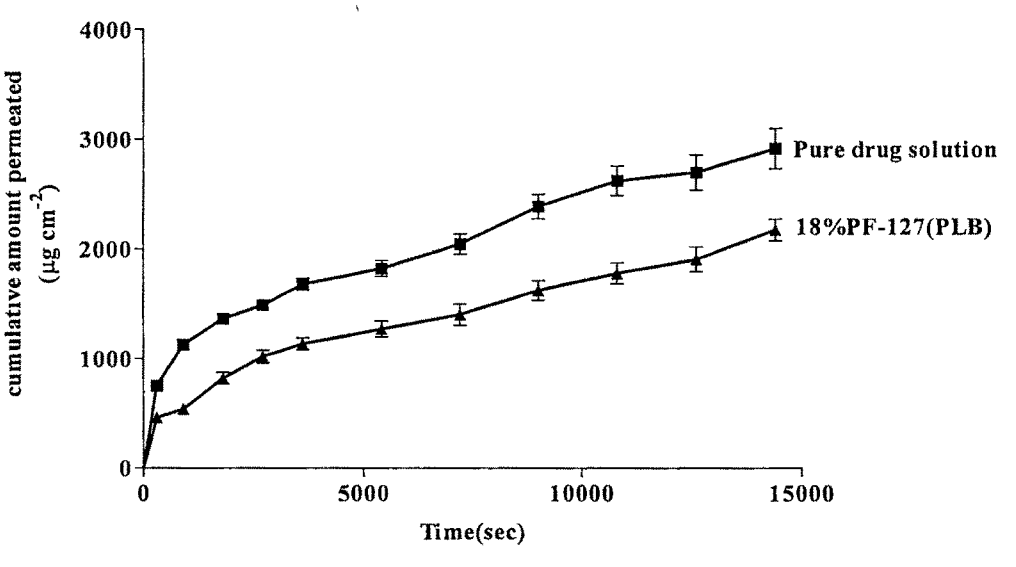

Figure 6.6 Cumulative amount of sumatriptan permeated across sheep nasal mucosal membrane from pure drug solution and from 18%w/v pluronic F-127 gel formulation at 34°C in franz diffusion, data are expressed as mean ± S.D (n=3).

d. Histological Evaluation of Mucosa after in vitro permeation study

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa As seen in figure 6.7 neither cell necrosis nor was removal of the epithelium from the nasal mucosa observed after permeation of PLB. The epithelium layer was intact and there were no alterations in basal membrane

and superficial part of submucosa as compared to PBS treated mucosa. Thus gel formulations seem to be safe with respect to nasal administration.

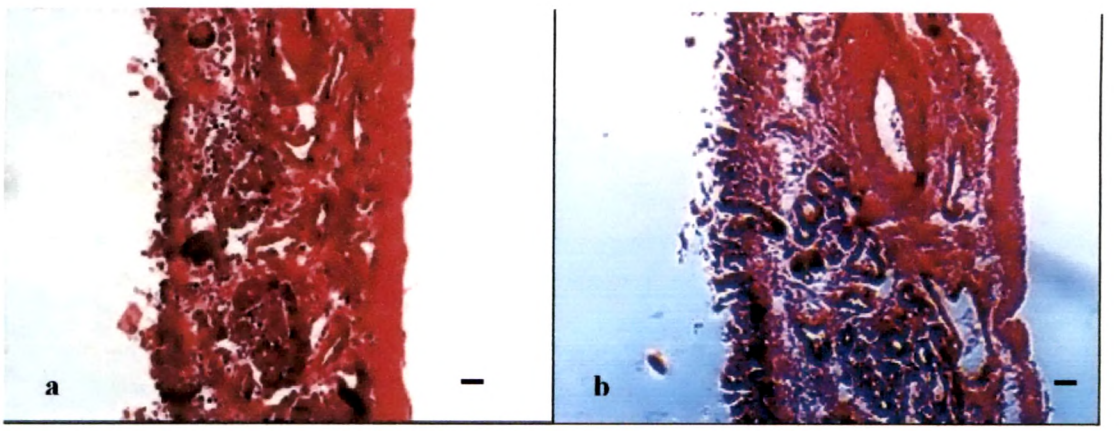

Figure 6.7 Histopathological evaluations of sections of sheep nasal mucosal membrane (a) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber, (b) Mucosal layer after incubation in diffusion chamber with gel formulation (PLB). (H x E), (line = 20μm).

e. Stability study

PF127 gel (18%w/v) showed good physical stability, as there was no discoloration, precipitation, or any other physical changes after storage. Sumatriptan succinate showed good chemical stability in the gel formulation. The drug content of the gel was 99.53(±1.02) and 98.79(±0.87) determined after storage at 4°C and room temperature respectively. The gelation temperature was 29.1°C and 29.3°C after storage at 4°C and room temperature respectively. The gel stability results are similar to published studies, which show that the stability of urease, interleukin-2 (Wang et al, 1993) and recombinant human growth hormone (Katakam et al, 1995) was enhanced when formulated in PF127.

6.4.1.1. CONCLUSION

Pluronic F127 thermoreversible gel formulation for nasal administration was prepared by incorporating antimigraine drug sumatriptan succinate. Formulations containing pluronic F127 (18%w/v or greater) exhibited thermoreversible property well below physiological temperature in the nasal cavity and they also exhibited mucoadhesive properties.

Formulations containing pluronic(18% and above) were newtonian at 20°C and pseudoplastic at its gelation temperature, with viscosity that increased with the polymer concentration Gelation temperature reduced with increase in pluronic F-127 concentration. However, permeation enhancing effect was not observed with pluronic F-127 formulations. Although Pluronic F127 gel formulation does not have permeation enhancing effect, it appears to be promising nasal drug delivery system for antimigraine drug that would enhance nasal residence time attributed to increased viscosity and mucoadhesive characteristics. In conclusion, this study demonstrated that the use of *in situ* gelling vehicles could effectively and safely improve the nasal residence time and thereby absorption of sumatriptan succinate to the target sites.

6.4.2. MIXED GELS OF PLURONIC F-127 AND CARBOPOL 934P

In order to fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers have been added to the in situ-gelling vehicles of pluronic F-127(Chu et al, 1991) Enhancement of the absorption of drugs loaded into Pluronic-PAA micro-gels through the epithelial cell monolayer of the upper small intestine has been reported by one of the research group (Bromberg et al, 2003). However combination of thermoreversible polymer pluronic and mucoadhesive polymer carbopol has never been screened as effective intranasal delivery system. This part of the chapter deals with development of system containing effective amount of sumatriptan as succinate salt , thermoreversible polymer pluronic F127and mucoadhesive polymer (carbopol 934P) which has the property of increasing the residence time in the nasal cavity and increasing absorption of sumatriptan across nasal membrane with enhanced delivery to intracranially located target sites Effect of concentration of carbopol 934P on viscosity, gelling temperature, mucoadhesive potential and in vitro permeation was also studied. pH of all the samples was between 5-6.5, which is suitable for nasal administration

a. Viscosity and gelling temperature determination

It is evident from the data shown in Table 6 2 that the gelation temperature obtained using two different methods (visual inspection and rheological method) did not vary more than ± 1.5°C. Figure 6.8 shows the viscosity vs. temperature curves of all the formulations. The gelation temperatures of pluronic F-127 vehicles as determined by rheological method were lowered from 29 °C to 28.5°C by the presence of 0.1 % mucoadhesive polymer C934P (Table 6 2). It is to be noted that addition of increasing concentrations of C934P from 0.1 % to 0 5 % further lowered the gelation temperature from 28.5°C to 23.9°C. The gelation temperature-lowering effect of mucoadhesive polymers might be partly due to the increased viscosity after dissolution of mucoadhesive polymers When Carbopol is exposed to water, the polymer begins to uncoil, generating an increase in viscosity and gel formation, uncoiling and expansion of the molecule results in polymer swelling and elastic gel formation The formulations containing concentration of C934P higher than 0 5 % were found to have very high viscosity, and so were difficult to administer into the nostril. Regardless of the concentration of mucoadhesive polymers, all the formulations gelled at the temperature ranging from 23.9°C to 29°C. These

temperatures seem to be proper for *in situ* gelling of the various vehicles at the nasal cavity, minimizing the loss of administered drug due to clearance from the site of application.

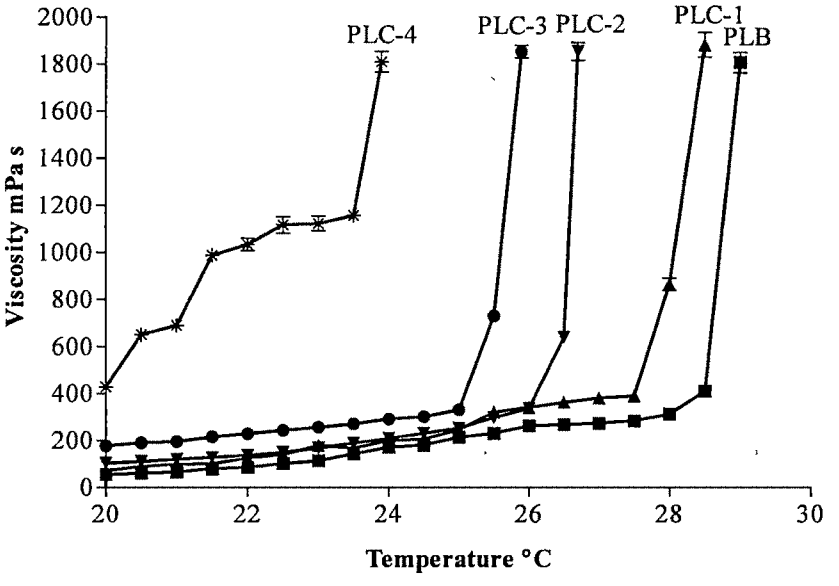

Figure 6.8 Effect of temperature on the viscosity of various pluronic gels with varying concentration of C934P (0.0% to 0.5%) measured at 10 s^{-1} shear rate. Values are expressed as mean ± SD (n=3).

Table 6.2 Gelling temperature determined rheologically and by visual inspection for various formulation

Sample	Composition	Sol-gel transition temperature (°C)	
		Rheology	Visual Inspection, Mean ±S.D (n=3)
PLB	18% PF127	29	28.2 ± 1.04
PLC-1	18% PF127, 0.1%C934-P	28.5	27.3 ± 0.58
PLC-2	18% PF127,0.2% C934-P	26 7	25.8 ± 0.29
PLC-3	18% PF127,0.3% C934-P	25.9	24.8 ± 0.76
PLC-4	18% PF127,0.5% C934-P	23.9	23.0 ± 1.00

b.Mucoadhesive strength

Assessment of the mucoadhesive strength in terms of detachment stress showed that the PF127 preparations possessed adhesive properties that increased with the addition of C934P concentration (Figure 6.9). Mucoadhesive strength for formulations PLC-2, PLC-3, PLC-4 with 0.2 %,0.3 % and 0.5 % C934P concentration respectively increased significantly ($P<0.001$) with respect to PLB whereas increase in mucoadhesive strength of PLC-1(0 1% C934P) was not significant. Difference in the mucoadhesive strength for PLC-3 and PLC-4 was not statistically significant. Earlier work with carbopol polymers has clearly indicated that it is the availability of carboxyl groups that determines bioadhesion, (Efentakis et al , 2000) carbopol has very high percentage (58% - 68%) of carboxylic groups that gradually undergoes hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane resulting in formation of strengthened network between polymer and mucus membrane. Thus carbopol having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. In addition, carbopol may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of delivery system may lead to the prolonged retention and increased absorption across mucosal tissues (Kunisawa et al, 2000).

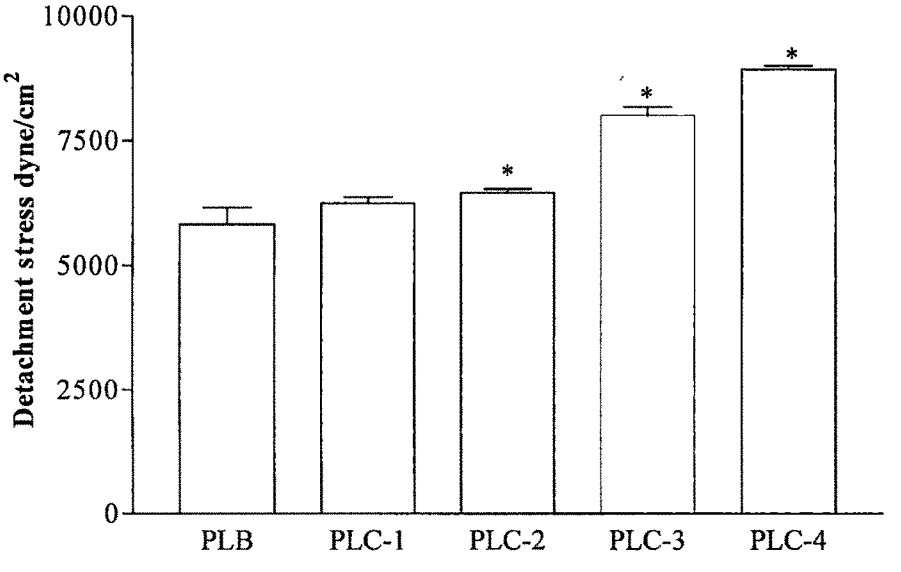

Figure 6.9 Influence of C934P concentration on the detachment stress measured *in vitro*, mean ± SD (n=3);*$P < .001$ versus PLB (18% pluronic F-127 concentration).

c. In vitro permeation study

Our previous experiments on the effect of calcium chelator, ethylene-glycol-bis-[β-aminoethylether]-N,N,N',N'-tetraacetic acid (EGTA) on permeability of sumatriptan across sheep nasal mucosal membrane exhibited increased permeability of sumatriptan in pure drug solution on exposure of membrane to EGTA, indicated by upward shift of time-permeation profile which was significant over the whole time period of 240 min. This study confirms importance of chelating calcium for enhancing permeability of sumatriptan. Anionic polymers such as polycarbophil or carbopol are reported to demonstrate permeation enhancing properties. These polymers were shown to express a high Ca^{2+} binding ability. Therefore, it was important to address the question to what extent increase in *in-vitro* permeation across the nasal membrane could be attained by thermoreversible gels of pluronic F-127 and carbopol. Effective permeability coefficient (Peff) determined for sumatriptan in each of the gel formulations are given in Table 6.3 and cumulative amount of sumatriptan succinate permeated as a function of time across sheep nasal membrane for various mixed gel formulations of pluronic F127 and carbopol 934P is given in figure 6.10. It is evident from the results that effective permeability coefficient for sumatriptan was significantly lower for formulation PLB and PLC-1 ($P<0.001$) as compared to that of pure drug solution. Since pluronic F-127 gels are viscous, isotropic liquid crystals consisting of micelles, it was hypothesized that the drug is released by diffusion through the extramicellar water channels of the gel matrix. Peff was not significantly different from the pure drug solution in case of PLC-2 ($P<0.001$), while Peff was significantly higher for formulation PLC-3 and PLC-4. Addition of anionic polymer (above 0.3%) to the pluronic F-127 have dramatically increased permeation coefficient. Presence of carbopol results in very rapid dissolution and release of highly soluble drug due to rapid swelling and dissolution of carbopol at pH 6 4. However presence of pluronic F-127 in the gel retards the drug release rate slightly due to reduction in dimension of water channels resulting for enhanced miceller structure. As seen from the results, addition of 0.1% carbopol to pluronic F-127 had no permeation enhancing effect, due to insufficient concentration of carbopol (0 1%) to enhance drug dissolution from the gel by circumventing pluronic F-127 gel network. Although addition of 0.2% carbopol had permeation enhancing effect as compared to PLB, it was not significantly greater than that obtained with pure drug solution. Addition of 0.3% and 0.5% carbopol enhanced permeation of drug from gel. This could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational

changes in polymer chain. Electrostatic repulsion of ionized carboxyl group results in de-coiling of polymer chain resulting in the relaxation of the polymer network (Chen et al, 1997). At this stage drug is rapidly dissolved and released from the gels due to very high swelling (or fast dissolution) of the ionized carbopol (Chen et al, 1997). Increase in permeation of the drug from the formulation can be further explained on the basis that increase in carbopol concentration will not only result in increased Ca^{2+} binding sites but also increase in inter-accessibility of Ca^{2+} binding sites due to relaxation of polymer network. . Similar results were also obtained in previous studies where the depletion of Ca^{2+}ions from the extra cellular cell medium has been shown to increase the permeation of sodium-fluorescein, bacitracin, a vasopressin analogue and insulin (Lueben et al, 19994 and Lueben et al 1997). Indications also exist that Pluronic-Poly Acrylic Acid can also bind Ca^{2+} in biological milieu (Bromberg et al, 2001). Concerning, local tissue damage, staining of sheep nasal mucosa with trypan blue after permeation studies with various formulations did not show any dead cells.

Considering rheological behavior, gelation temperature, mucoadhesive strength and effective permeability formulation containing 0.3%w/v and 0.5%w/v carbopol 934P were found to be the best. However, addition of 0.5% carbopol 934P (PLC-4) had no statistically significantly ($P<0.001$) enhancement of mucoadhesive potential or effective permeability compared to formulation containing 0.3% carbopol 934P (PLC-3). Hence, PLC-3 was selected as optimized formulation exhibiting ideal characteristics with respect to gelation, mucoadhesion and permeation enhancement and therefore selected for further studies.

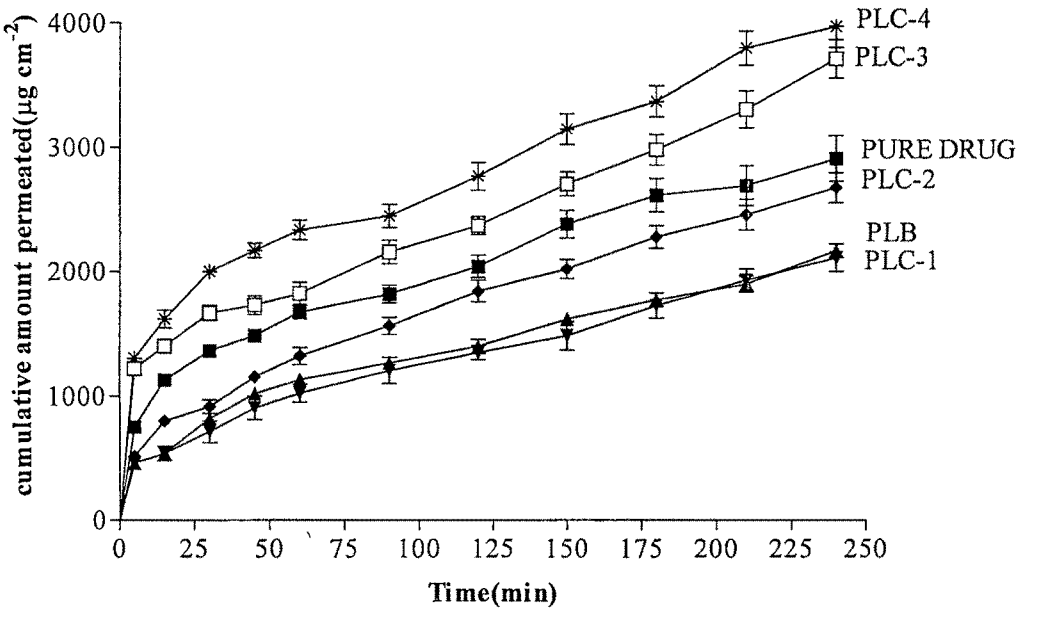

Figure 6.10 Cumulative amount of sumatriptan permeated across sheep nasal mucosal membrane from pure drug solution and from various pluronic F-127 gel formulations at 34°C in franz diffusion, data are expressed as mean ± S.D (n=3).

Table 6.3 Effective permeability coefficient determined for various formulations across sheep nasal mucosal membrane

Sample	Peff (x 10^{-5}) (cm s^{-1})	
Pure drug	5 58	± 0 365
PLB	4.46	± 0.275*
PLC-1	4 51	± 0 174*
PLC-2	5.81	± 0.276
PLC-3	6.61	± 0 170*
PLC-4	7 00	± 0 364*

* P<0.001 vs Pure drug

d. Histological Evaluation of Mucosa after in vitro permeation study

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As seen in figure 6.11 neither cell necrosis nor was removal of the epithelium from the nasal mucosa observed after permeation of PLC-3. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared to PBS treated mucosa. Thus gel formulations seem to be safe with respect to nasal administration.

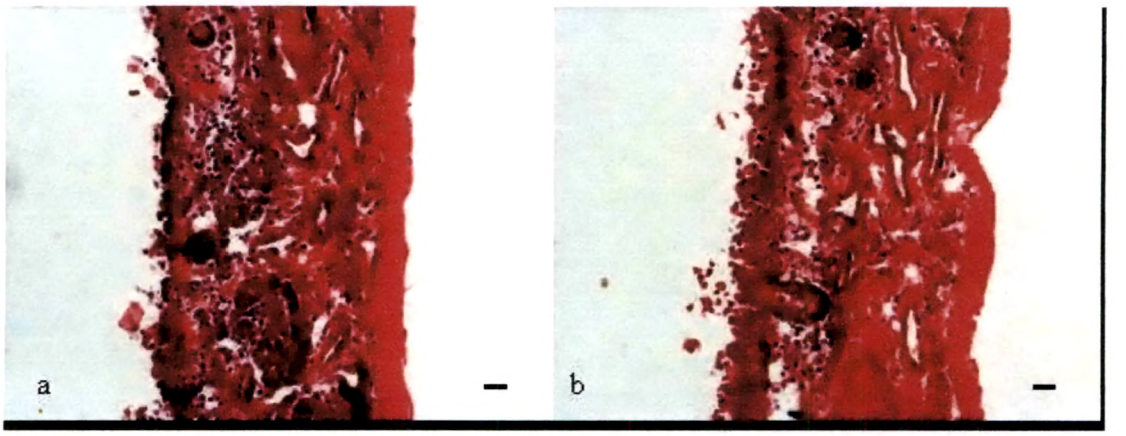

Figure 6.11 Histopathological evaluations of sections of sheep nasal mucosal membrane (a) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber, (b) Mucosal layer after incubation in diffusion chamber with gel formulation (PLC-3). (H x E), (line = 20μm).

e. Stability study

PLC-3 showed good physical stability, as there was no discoloration, precipitation, or any other physical changes after storage. Sumatriptan succinate showed good chemical stability in the gel formulation. The drug content of the gel was 98.22(±0.92) and 98.11(±0.96) determined after storage at 4°C and room temperature respectively. The gelation temperature was 26.2°C and 26.4°C after storage at 4°C and room temperature respectively.

6.4.2.1. CONCLUSION

Pluronic F127 thermoreversible and mucoadhesive gel formulation for nasal administration was prepared by incorporating mucoadhesive polymer carbopol 934P and antimigraine drug sumatriptan succinate. Formulations exhibited thermoreversible property well below physiological temperature and they also exhibited mucoadhesive properties. Addition of anionic mucoadhesive polymer C934P to pluronic F127 resulted in reduction of gelling temperature along with increased mucoadhesive potential. Incorporation of carbopol (above 0.3%) resulted in significant increase in effective permeability coefficient of drug. Histopathological evaluation after in vitro permeation studies did not exhibited any toxicity on the nasal tissue. Thus, pluronic F127 gel formulation with 0.3% carbopol is a promising nasal drug delivery system for antimigraine drug sumatriptan succinate that would enhance nasal residence time attributed to increased viscosity and mucoadhesive characteristics; furthermore it also exhibited permeation enhancing effect. In conclusion, this study demonstrated the potential of *in situ* gelling mucoadhesive vehicles of pluronic F-127 for improvement of the nasal residence time and absorption of sumatriptan succinate with improved efficacy and safety.

6.4.3. MIXED GELS OF PLURONIC F-127 AND CHITOSAN GLUTAMATE

In order to fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers have been added to the in situ-gelling vehicles of pluronic F-127(Chu et al, 1991). Combination of polyoxyethylene-polyoxypropylene block copolymers (pluronic F-127) as thermoreversible polymer and cationic polymer chitosan glutamate as mucoadhesive polymer and absorption enhancing material has never been explored before as potential drug delivery system with number of advantages. The research work is aimed at development of system containing effective amount of sumatriptan as succinate salt, thermoreversible polymer pluronic F127and mucoadhesive polymer (chitosan glutamate) which has the property of increasing the residence time in the nasal cavity and increasing absorption of sumatriptan across nasal membrane with enhanced delivery to intracranially located target sites. Effect of concentration of chitosan glutamate on viscosity, gelling temperature, mucoadhesive potential and in vitro permeation was also studied pH of all the samples was between 5-6.5, which is suitable for nasal administration.

a. Viscosity and gelling temperature determination

As evident from the results in Table 6 4 the gelation temperatures of pluronic vehicles as determined by rheological method were lowered from 29 °C to 27.9°C by the addition of increasing concentrations of mucoadhesive polymer chitosan glutamate i.e. 0% to 1%w/v. Figure 6.12 shows the viscosity of various pluronic gels with varying concentration of chitosan glutamate (0.0% to 1%) measured at 10 s^{-1} shear rate as a function of temperature Gelation temperature determined by rheological method and visual method did not varied more than ±1.5°C The decrease in gelation temperature with increase in chitosan glutamate concentration may be due to enhanced viscosity of the formulation. This temperature range seems to be proper for *in situ* gelling of the various vehicles at the nasal cavity, minimizing the loss of administered drug due to clearance from the site of application.

Table 6.4 Gelling temperature determined rheologically and by visual inspection for various formulation and compositions of the formulations

Sample	Composition(%w/v)	Sol-gel transition temperature (°C)	
		Rheology	Visual Inspection, Mean ±S.D (n=3)
PLB	18% PF127	29	28 2 ± 1 04
PLCG-1	18% PF127, 0.1%Chitosan glutamate	28 9	28 3 ± 0 58
PLCG-2	18% PF127,0 2% Chitosan glutamate	28.4	28 1 ± 0 29
PLCG-3	18% PF127,0 3% Chitosan glutamate	28 3	27.8 ± 0 76
PLCG-4	18% PF127,0.5% Chitosan glutamate	28	27.0 ± 1 00
PLCG-5	18% PF127,1% Chitosan glutamate	27 9	26.7 ± 0 76

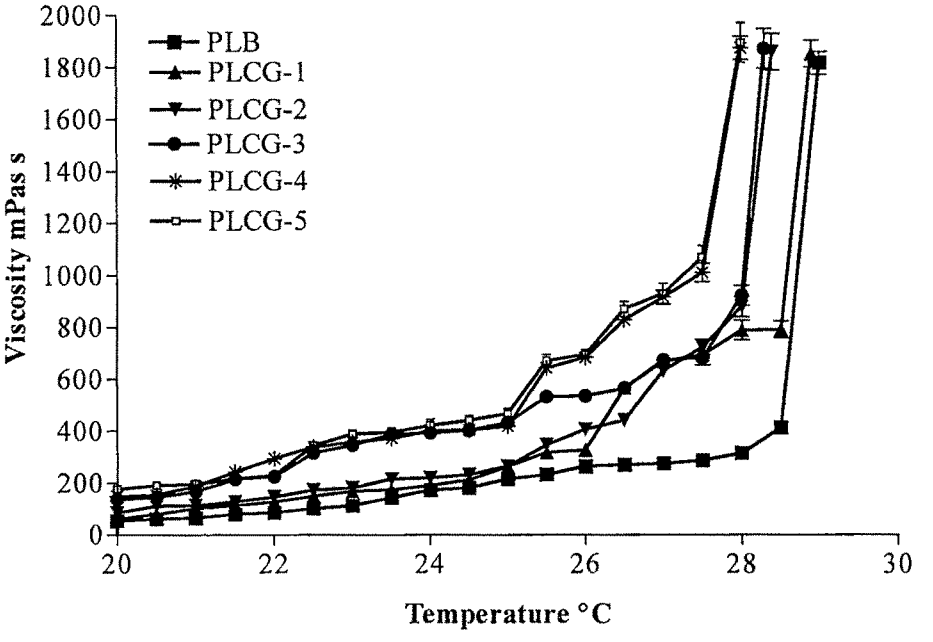

Figure 6.12 Effect of temperature on the viscosity of various pluronic gels with varying concentration of chitosan glutamate (0.0% to 1%) measured at 10 s^{-1} shear rate. Values are expressed as mean ± SD (n=3).

b. Mucoadhesive strength

Assessment of the mucoadhesive strength in terms of detachment stress showed that all the preparations possessed adhesive properties that increased with the chitosan glutamate concentration as seen in figure 6.13 Increase in chitosan glutamate concentration (0.2% and above) resulted in statistically significant ($P<0.001$) increase in detachment stress compared to pluronic vehicle alone (PLB) Concentration below 0.2%w/v was not sufficient enough to cause significant increase in mucoadhesion. This enhanced mucoadhesive strength resulted from interaction between increased number of positively charged amino group of chitosan and negatively charged sialic acid residues of mucus glycoprotein with increase in chitosan glutamate concentration Moreover, increase in polymer concentration will result in increased amount of polymer on the surface and hence more number of amino groups responsible for binding with sialic acid residues is available and oriented towards mucosal membrane resulting in increased mucoadhesive strength

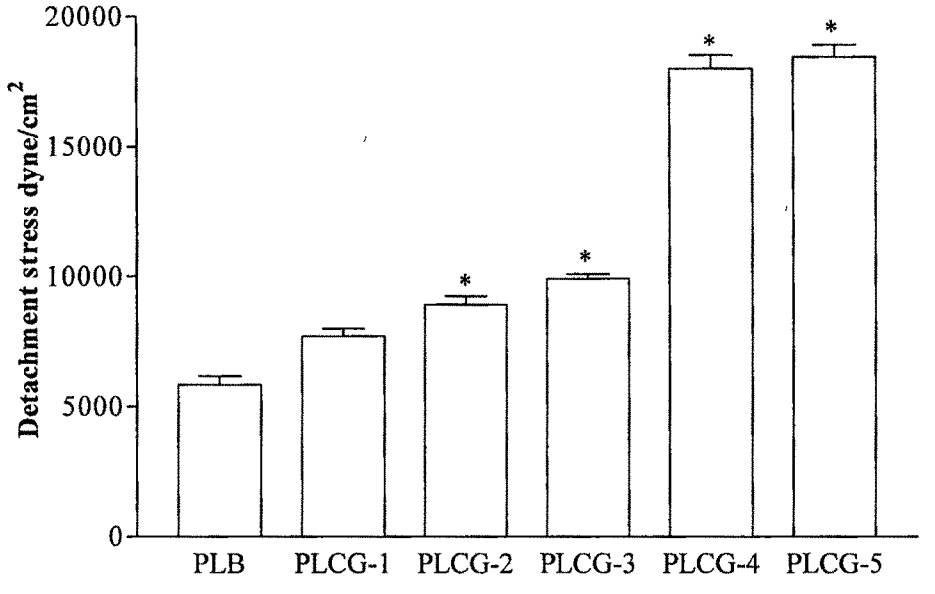

Figure 6.13 Influence of chitosan glutamate concentration on the detachment stress measured *in vitro*, mean ± SD (n=3);*P < 0.001 versus PLB (18%w/v pluronic concentration).

c. In vitro permeation study

Results from in vitro permeation study revealed that addition of increasing concentration of chitosan glutamate to the pluronic increased effective permeation coefficient linearly with cationic polymer concentration. Figure 6.14 shows cumulative amount of sumatriptan succinate permeated across sheep nasal mucosal membrane in presence of different concentration of chitosan glutamate. Chitosan is a reported polymeric permeation enhancer due to its tight junction opening activity. The mechanism underlying this permeation enhancing effect seems to be based on the positive charges of the polymer, which interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins (Schipper et al, 1997). It is evident from the results in table 6.5 that effective permeability coefficient for sumatriptan was significantly lower for formulation containing pluronic without chitosan glutamate (PLB) ($P<0.001$) as compared to that of pure drug solution. Since pluronic gels are viscous, isotropic liquid crystals consisting of micelles, it was hypothesized that the drug is released by diffusion through the extramicellar water channels of the gel matrix resulting in retardation of drug

diffusion across the system due to reduction in dimension of water channels. Addition of chitosan glutamate resulted in increased effective permeability of drug. With increasing concentration of chitosan glutamate effective permeability of drug increased, this could be due to hydrophilic nature of chitosan glutamate which allows water molecules to penetrate inside the gel network. The hydration force between the chains is responsible for swelling and this facilitates release of sumatriptan succinate. However, for formulation PLCG-1(0.1% w/v chitosan glutamate) increase in effective permeability of drug compared to pure drug solution was not significant, further increase in chitosan glutamate concentration (above 0.1%) resulted in statistically significant increase in effective permeability of the drug ($P<0.001$).This could be due to the fact that 0.1%w/v concentration of chitosan glutamate is not sufficient for circumventing the barrier to drug release caused by reduction in aqueous path way, by increasing hydration force between the chains and thereby drug release. Concentration above 0.1% was sufficient enough to circumvent the barrier to drug release and thereby resulted in significant enhancement of effective permeability Additionally, with increase in chitosan glutamate concentration number of positive ions interacting with membrane will increase and is possibly responsible for altering tight junction integrity, thereby enhancing permeation. Also the vicinity of these reactive groups will increase at the site of interaction due to increased hydration resulting in disentanglement of chains and chain relaxation thereby enhancing permeation of drug. Concerning, local tissue damage staining of sheep nasal mucosa with trypan blue after permeation studies with various formulations did not show any dead cells.

Considering rheological behavior, gelation temperature, mucoadhesive strength and effective permeability formulation containing 0.5%w/v and 1%w/v chitosan glutamate were found to be the best. However, addition of 1% chitosan glutamate (PLCG-5) had no statistically significantly ($P<0.001$) enhancement of mucoadhesive potential or effective permeability compared to formulation containing 0 5% chitosan glutamate (PLCG-4). Hence, PLCG-4 was selected as optimized formulation exhibiting ideal characteristics with respect to gelation, mucoadhesion and permeation enhancement and therefore selected for further studies.

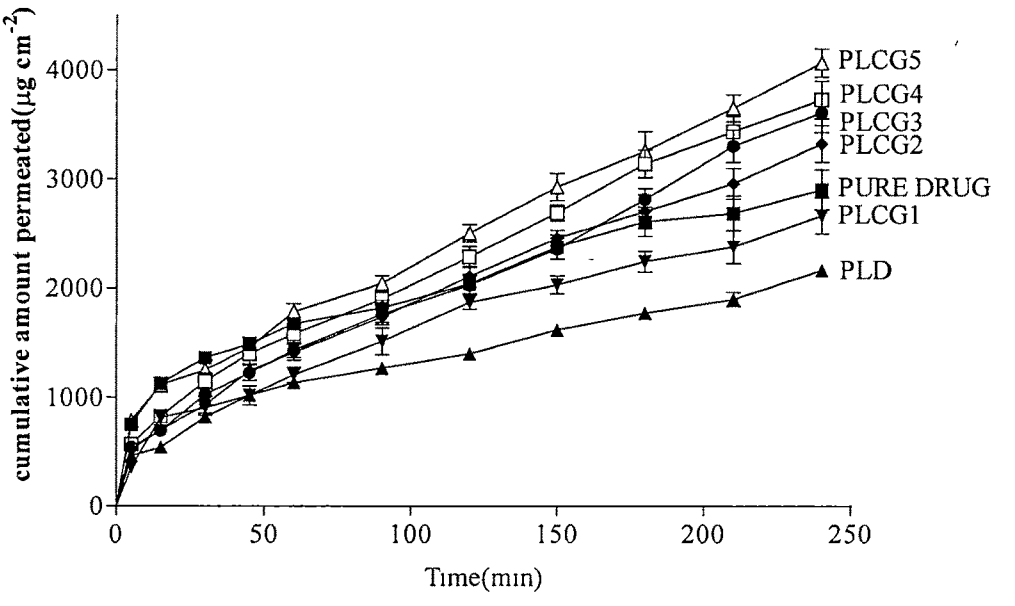

Figure 6.14 Cumulative amount of sumatriptan permeated across sheep nasal mucosal membrane from pure drug solution and from various pluronic gel formulations at 34°C in franz diffusion, data are expressed as mean ± S.D (n=3).

Table 6.5 Effective permeability coefficient determined for various formulations across sheep nasal mucosal membrane

Sample	Peff (x 10^{-5}) (cm s^{-1})
Pure drug	5 58 ± 0 365
PLB	4 46 ± 0 275*
PLCG-1	6 01 ± 0 353
PLCG-2	7 77 ± 0 260*
PLCG-3	8 46 ± 0 212*
PLCG-4	8 78 ± 0 257*
PLCG-5	9 01 ± 0 172*

P<0.001 vs pure drug solution

d. Histological Evaluation of Mucosa after in vitro permeation study

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As seen in figure 6.15 neither cell necrosis nor was the removal of the epithelium from the nasal mucosa observed after permeation of PLCG-4. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared to PBS treated mucosa. Thus gel formulations seem to be safe with respect to the safety of nasal mucosa following nasal administration of the formulation.

e. Stability study

PLCG-4 showed good physical stability, as there was no discoloration, precipitation, or any other physical changes after storage. Sumatriptan succinate showed good chemical stability in the gel formulation. The drug content of the gel was 98.76(±0.56)% and 98.43(±0.77)% determined after storage at 4°C and room temperature respectively. The gelation temperature was 28.1°C and 28.3°C after storage at 4°C and room temperature respectively.

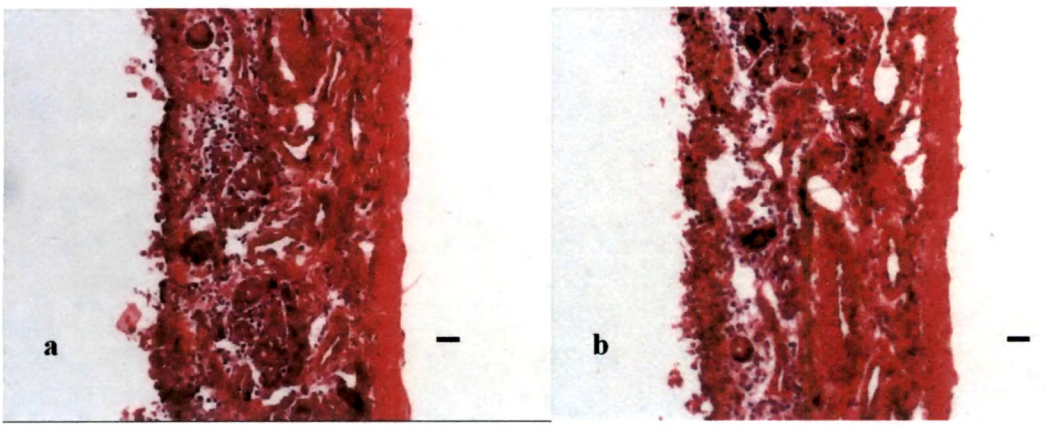

Figure 6.15 Histopathological evaluations of sections of sheep nasal mucosal membrane (a) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber, (b) Mucosal layer after incubation in diffusion chamber with gel formulation (PLCG-4). (H x E), (line = 20μm).

6.4.3.1 CONCLUSION

Mixed gels prepared by incorporating thermoreversible polymer pluronic F127 and mucoadhesive polymer chitosan glutamate, exhibited gelation temperature below 34°C, which reduced with increase in concentration of chitosan glutamate. All formulation possessed mucoadhesive property as well as permeation enhancing effect across sheep nasal mucosal membrane, which significantly increased with increase in chitosan glutamate concentration upto 0.5%w/v. Further increase in concentration had no significant effect on above mentioned parameters. Histopathological evaluation did not show any local damage to nasal tissue. Thus, mixed gels possessed good mucoadhesive properties which allowed it to be in contact with nasal epithelium for longer time periods, also permeation enhancing effect of the formulation increased permeation of hydrophilic drug sumatriptan succinate across epithelium, all this culminating in increased availability of drug to the target sites. Thus nasally administered sumatriptan succinate in mucoadhesive thermoreversible gel formulation, by increasing nasal residence time (due to mucoadhesive potential/increased viscosity) as well as permeation enhancing effects is a promising delivery system for an effective, non-invasive route for treatment of migraine.

6.5 REFERENCES

Bhardwaj R, Blanchard J (1996). Controlled release delivery system for the α-MSH analog melanotan-I using poloxamer 407. J. Pharm Sci., 85, 915-919.

Bromberg L (2001) Interactions among proteins and hydrophobically modified polyelectrolytes. J. Pharm Pharmacol , 53, 541–547

Bromberg L, Alakhov A (2003). Effects of polyether-modified poly (acrylic acid) microgels on doxorubicin transport in human intestinal epithelial Caco-2 cell layers. J. Control. Release, 88, 11–22.

Bromberg LE (2001) Enhanced nasal retention of hydrophobically modified polyelectrolyte J. Pharm. Pharmacol , 53, 109–114

Cabana A, AitKadı A, Juhasz J (1997) Study of the gelation process of polyethylene oxide a-polypropylene oxide b-polyethylne oxide a copolymer (Poloxamer 407) aqueous solutions J. Colloid. Interface. Sci , 190, 307–312.

Chen G, Hoffman AS, Kabra B, Randeri K (1997). Temperature-induced gelation pluronic-g-poly(acrylic acid) graft copolymers for prolonged drug delivery to the eye. In· Harris JM, Zalips S, eds. Poly (ethylene glycol)· Chemistry and Biological Applications, USA: Oxford University Press, 441-451.

Chng HS, Park H, Kelly P, Robinson JR (1985). Bioadhesive polymers as platforms for oral controlled drug delivery II. Synthesis and evaluation of some swelling water-insoluble bioadhesive polymers J Pharm. Sci , 74, 339-405.

Cho KY, Chung TW, Kim BC, Kim MK, Lee JH, Wee WR, Cho CS (2003). Release of ciprofloxacin from poloxamer-graft-hyaluronic acid hydrogels in vitro. Int. J. Pharm., 260, 83–91

Choi HG, Oh YK, Kim CK (1998). In situ gelling and mucoadhesive liquid suppository containing acetaminophen: Enhanced bioavailability. Int J. Pharm , 165, 23–32.

Chu JS, Amidon GL, Weiner ND, Goldberg AH (1991). Mixture experimental design in the development of a mucoadhesive gel formulation Pharm. Res., 8, 1401–1407.

D'Souza R, Mutalik S, Vidyasagar S, Udupa N (2005). Nasal Insulin Gel as an Alternate to Parenteral Insulin: Formulation,Preclinical, and Clinical Studies AAPS PharmSciTech., 6, E184-E189

Edsman K, Carlfors J, Petersson R (1998). Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur. J. Pharm. Sci., 6, 105–112

Efentakis M, Koutlis A, Vlachou M (2000) Development and evaluation of oral multiple-unit and single-unit hydrophilic controlled-release systems. AAPS PharmSciTech , 1, E34

Fang JY, Leu YL, Wang YY, Tsai YH (2002). In vitro topical application and in vivo pharmacodynamic evaluation of nonivamide hydrogels using wistar rat as an animal model. Eur J. Pharm. Sci., 15, 417–423.

Gariepy ER, Leroux JC (2004). In situ-forming hydrogels—review of temperature-sensitive systems. Eur. J. Pharm. Biopharm., 58, 409–426.

Illum L (1999) Bioadhesive formulations for nasal peptide delivery In: Mathiowitz E, Chickering DE, Lehr CM. (eds) Bioadhesive Drug Delivery Systems. MarcelDekker, New York, 507-562.

Jones DS, Woolfson AD, Brown AF, Coulter WA, McClelland C, Irwin CR (2000). Design, characterization and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. J. Control Release., 67, 357–368

Juhasz J, Pimienta C, Lenaerts V (1991). Adhesion of poloxamer 407 formulations on dog ileal segments in vitro Eur J Pharm. Biopharm., 37, 262–265.

Kabanov AV, Batrakova EV, Alakhov VU (2002) Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release , 82, 189–212.

Katakam M, Bell LN, Banga AK (1995). Effect of surfactants on the physical stability of recombinant human growth hormone. J Pharm Sci.;84,713-716.

Keck T, Leiacker R, Riechelmann H, Reittinger G (2000). Temperature profile in the nasal cavity. Laryngoscope, 110, 651–654.

Kunisawa J, Okudaira A, Tsutusmi Y, Takahashi I, Nakanishi T, Kiyono H, Mayumi T (2000) Characterization of mucoadhesive microspheres for the induction of mucosal and systemic immune responses Vaccine, 19, 589–594.

Lueben HL, Lehr CM, Rentel CO, Noach ABJ, De Boer AG, Verhoef JC, Junginger HE (1994) Bioadhesive polymers for the peroral delivery of peptide drugs. J. Control. Release., 29, 329–338.

Lueben HL, Rentel CO, Kotze AF, Lehr CM, De Boer AG, Verhoef JC, Junginger HE (1997). Mucoadhesive polymers in peroral peptide drug delivery. IV. Polycarbophil and chitosan are potent enhancers of peptide transport across intestinal mucosa in vitro. J. Control Release., 45, 15–23.

Moore T, Croy S, Mallapragada S, Pandit N (2000). Experimental investigation and mathematical modeling of pluronic F127 gel dissolution: drug release in stirred system. J. Control. Release., 67, 91-202.

Pisal SS, Paradkar AR, Mahadik KR, Kadam SS (2004). Pluronic gels for nasal delivery of Vitamin B12. Part I: Preformulation study. Int. J. Pharm , 270, 37–45.

Rassing J, Attwood D (1983). Ultrasonic velocity and light scattering studies on polyoxyethlene-polyoxypropylene copolymer PF127 in aqueous solution. Int. J. Pharm , 13, 47-55.

Schipper NGM, Olsson S, Hoogstraate JA, DeBoer AG, Varum KM, Artursson P (1997). Chitosans as absorption enhancers for poorly absorbable drugs. 2: Mechanism of absorption enhancement. Pharm. Res., 14, 923–929.

Schmolka IR (1972). Artificial skin. Preparation and properties of pluronic F-127 gels for the treatment of burns. J Biomed. Mater. Res., 6, 571-582.

Wadell C, Bjork E, Camber O (2003). Permeability of porcine nasal mucosa correlated with human nasal absorption. Eur. J. Pharm. Sci , 18, 47–53.

Wang P, Johnston TP(1993). Enhanced stability of two model proteins in an agitated solution environment using poloxamer 407. J Parenter Sci Technol.;47,183-189.

Zhou M, Donovan MD (1996). Intranasal mucociliary clearance of putative bioadhesive polymer gels. Int. J. Pharm., 135, 115-125