Research Article

Thermoreversible Mucoadhesive Gel for Nasal Delivery of Anti Hypertensive Drug

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ABSTRACT

The present study was aimed to develop a mucoadhesive in-situ gel of Carvedilol for improved bioavailability by circumventing the hepatic first pass metabolism and patient compliance. Carvedilol was incorporated into the blends of thermoreversible polymer pluronic F 188(PF 188) and bioadhesive polymer Carbopol 940 in the form of in-situ gel by cold technique to reduce the muco ciliary clearance, and thereby it will increase the contact of formulation with nasal mucosa and hence improving drug absorption. The prepared gels were characterized by Gelation temp, pH, Drug content, Gel strength, permeation studies, Histopathological evaluation, stability study etc. The results revealed that as the concentration of Carbopol increases there was a decrease in the gelation temperature. pH of all the formulations were found to be within the range between 4.5-6.0 and the nasal mucosa can tolerate the above mentioned pH of the formulations. The drug content of all formulations was found to be 97.44 to 99.17%. Tests also revealed that as the level of carbopol increases mucoadhesive strength also increase. Viscosity measurement of the formulations at temperatures 25°C & 37°C, shows that there was increase in viscosity with increase in the temperature and it was found that all formulations were in liquid state at room temperature and were converted into gel at nasal physiological temperature. The optimized formulation showed a drug release of 93.98% in 480 min. The biopolymers used and their compositions in the in-situ gels preparation greatly affected the drug release which allows absorption in the nasal mucosa.

Keywords: Carvedilol, in-situ gel, nasal delivery, Pluronic F188.

INTRODUCTION

Nasal mucosa has also been considered as a potential administration route to achieve faster and higher level of drug absorption because it is permeable to more compounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents.[1] The nasal epithelium is a highly permeable monolayer, the submucosa is highly vascularised with large and fenestrated capillaries facilitating rapid absorption. Moreover, direct systemic absorption avoids hepatic first-pass metabolism.[2]

Carvedilol, a racemic lipophilic aryloxypropanolamine is a Nonselective β-adrenergic blocking agent with selective α1-adrenergic blocking activity. Chemically (CAR) is 1-(9H-Clarbazol-4-yloxy)-3-[2-(2-methoxy phenoxy) ethyl [amino]-2-propanol. It is a lipid soluble compound, practically insoluble in water and poorly absorbed from the gastrointestinal tract. The slow absorption of Carvedilol was attributed to its poor water solubility. It has absolute bioavailability 25% as it undergoes stereo-selective first pass metabolism and will be eliminated from body through urine (16%) and feces (60%). Carvedilol is a weak base and its pKa value is approximately 7.8, which satisfies the criterion for the selection of the drug. The log PC (partition coefficient) value for Carvedilol is about 3.967. The t_{max} of Carvedilol is 1.2 h by peroral route, which is long and variable.[3] The dose of Carvedilol is 25 mg twice a day, however, a lower effective dose is reported to be approximately 3.125 mg and is used to treat moderate hypertension, angina pectoris and congestive heart failure.[4]

In-situ gels are the novel drug delivery systems that favors the ease and convenience of administration and delivery of accurate dosage forms which are the major problems encountered by the normal semi solid dosage forms. An in situ muco adhesive gel appears to be very attractive since they are fluid like prior to administration which makes them easy to administer as a drop allowing accurate dosing. [5, 6] Formulation of in situ gels depends on various physical and chemical stimuli.

Poloxamers or Pluronics are a class of thermo reversible gels that have the capacity to make, break and modify the bonds responsible for holding the network together. Their thermo reversible property make them useful as a carrier for most routes of administration including oral, topical, intranasal, vaginal, rectal, ocular and parenteral routes.[7] Reverse thermal gelation and low toxicity have been the basis of research into the use of Pluronics as a possible drug delivery system in man.[8]

MATERIALS AND METHODS

Materials

Carvedilol was obtained as a gift sample from Anugraha chemicals; Bangalore, Poloxamer (Research fine laboratories), Carbopol 940 (Loba chemie Pvt Ltd), Propylene Glycol, Methyl paraben and Triethanol amine used were of AR grade.

Methods

Determination of λ max of the drug

A stock solution of 100µg/ml of Carvedilol was prepared by dissolving 10mg in 100ml of phosphate buffer pH 6.4. The resulting solution was scanned between 200-400nm using UV 1700 Spectrophotometer.

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of pure drug and physical mixture were obtained using KBr pellet method (applying 6000 kg/cm^2). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Japan).

Pre-formulation studies

The plain and drug loaded PF188 gels were prepared by cold method described by Schmolka.[9] For drug loaded PF188 gels,15mg drug was stirred with sufficient quantity of distilled water while for plain PF188 gels, only sufficient quantity of double distilled water without drug was kept overnight at 4°C in refrigerator. The PF188 was added slowly with continuous stirring. The dispersions were then stored in a refrigerator until clear solution was obtained and finally volume was adjusted. Optimization of plain and drug loaded PF127 gels were done by varying concentration of PF188 and evaluating them for gelation temperature.

Optimized concentration of PF188 was used for further study of effect of mucoadhesive polymer on gelation temperature, mucoadhesive strength and spreadability. The concentration of mucoadhesive polymer was screened in the range of 0.2 to 0.5%.

Preparation of In Situ Gels

Thermo reversible gels were prepared using cold method described by Schmolka.[9] This method involved slow addition of polymer PF188, in cold water containing 1% propylene glycol with continuous agitation. The formed mixtures were stored overnight at 4°C. The PF188 vehicles used throughout this study were composed of 18% wt/v of PF188. The liquid was left at 4˚C until a clear solution was obtained. Bioadhesive anionic polymer C940 P which was allowed to swell overnight was slowly added to the Poloxamer solution with continuous agitation. C940P was added in concentration range of 0.2% wt/v to 0.5% wt/v to PF188 solution. To the above solution add the 15mg drug dissolved in DMF. Finally tri ethanol amine was added to adjust the pH and these prepared gels were used for further evaluation (Table 1).

Characterization of the Prepared Formulations

Surface pH of the Gel

A digital glass electrode pH meter was used for this purpose. pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 minute. The pH meter was first calibrated using solutions of pH 4 and pH 7.

Drug Content

1ml of each formulation was taken in 10ml volumetric flask, diluted with distilled water and volume adjusted to 10ml. 1ml quantity from these solutions was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 285 nm by using UV visible spectrophotometer (Shimadzu UV-1700).[10]

Inflection point

The inflection point is defined as the temperature at which there is a sudden change in the viscosity of the prepared in-situ gels. Three readings were taken for each formulations and the average was calculated.

Measurement of Gelation Temperature (T1) and Gel Melting Temperature (T2)

It was determined by using method described by Miller and Donovan technique. A 2ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5min at each new setting. The sample was then examined for gelatin, which was said to have occurred when the meniscus would no longer moves upon tilting through 900. After attaining the temperature T1, further heating of gel causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 i.e. gel melting temperature. It is a critical temperature when the gel starts flowing upon tilting test tube through 900.[11]

Table 1: Composition of different batches of gel formulations

Ingredients	Formulation code				
	G1	G2	G3	G4	G5
Drug (mg)	15	15	15	15	15
Poloxamer PF188 (%w/v)	18	18	18	18	18
Carbopol 940 (%)	----	0.2	0.3	0.4	0.5
Propylene Glycol (%)	1	1	1	1	1
Methyl Paraben (%)	0.001	0.001	0.001	0.001	0.001
Triethanolamine	q.s	q.s	q.s	q.s	q.s
Water (ml)	10	10	10	10	10

Viscosity Study

Viscosity of the prepared gels was studied using Brookfield viscometer DV II at constant temperatures of 25°C and 37°C.

Gel strength determination

A sample of 50 g of the nasal gel was put in a 100 ml graduated cylinder. A weight of 20 g was placed on the gelled form. The gel strength, which is an indication of the viscosity of the nasal gel at physiological

temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.[12]

Determination of Muco adhesive Strength[13, 14]

The muco adhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. 50mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The muco adhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

Muco adhesive Strength (dynes/cm^2) = mg/A

Where, m = weight required for detachment in gram,

g= Acceleration due to gravity (980cm/s^2),

A = Area of mucosa exposed

***In-vitro* Release Studies**

Release of drug from various gel formulations was studied employing the permeation apparatus designed as described by Fites et al. A glass cylinder with both ends open, 10 cm height, 3.7 cm outer diameter and 3.1 cm inner diameter. Cellophane membrane was tied to one end of donor compartment. Gel was accurately weighed, was taken in donor compartment and the cell was immersed in a beaker containing 30 ml of phosphate buffer (receptor compartment) of pH 6.4 were used for study. The cell was immersed to a depth of 1cm below the surface of phosphate buffer in the receptor compartment, and temperature maintained at 34 ± 1°C throughout the study. Aliquots of 1ml were withdrawn at periodic intervals and each time equal volume was replaced with fresh phosphate buffer. The amount of drug release was estimated using UV spectrophotometer at 285 nm.

***In-vitro* Permeation Study**

For the permeation studies goat nasal mucosa was obtained from local slaughter house and used within 2 hours of slaughtering. The nasal mucosal membrane was separated from the underlying tissues. The surface of the tissue was cleaned with Ringer's solution and the mucosal membrane was allowed to equilibrate for approximately 1 hour in receptor buffer to regain the lost elasticity.

Permeation studies were carried out for the optimized formulation by Modified nasal diffusion cell 15 using Franz diffusion cell apparatus of surface area 3.14 cm^2 and receptor compartment capacity of approximately 15ml. Goat nasal mucosa was used as the model membrane. The receptor compartment was filled with phosphate buffer pH 6.4 solution. The epidermal side of the tissue was exposed to donor compartment and the other side was bathed with receptor solution. Caution was taken to remove all air bubbles between the underside of the nasal epithelium and the receptor solution. The tissue was also checked for the presence of furrows. To mimic the body condition during the experiment, the temperature was maintained as 37 ± 0.5°C with and external constant water circulation and the receiver medium was continuously stirred with a small magnetic bar in order to prevent any boundary layer effects.

At a predetermined time of intervals 1 ml of sample were withdrawn and the cell was refilled with the same fresh receptor solution. Amount of drug permeated was determined spectrophotometrically at 285 nm using UV-visible spectrophotometer.[16]

Histo pathological Evaluation of Mucosa

Histo pathological evaluation of nasal mucosa incubated in Phosphate Buffer Saline (pH 6.4) after collection was compared with nasal mucosa permeated with the G-4 formulation in the Franz diffusion cell. Nasal mucosa was fixed in 10% buffered formalin (pH 7.4) and was cut into section vertically. Each section was hydrated using ethanol, and embedded in paraffin for fixing. Paraffin sections (7 µm) were put on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope, to detect any damage to the tissue during *in-vitro* permeation.

Stability studies

Medicated gels were subjected to stability testing. Gels were placed in a glass beaker covered with aluminum foil and kept in room temperature (30±2°C) and in refrigerator temperature (4±2°C) for 45 days to determine physical and chemical stabilities. Changes in appearance, drug content and gelation temperature of the stored gels were investigated at the end of every week. The data presented were the mean of three determinations.

RESULTS AND DISCUSSION

Carvedilol exhibited λ max at 285 nm. The IR spectrum of pure Carvedilol (Figure 1) showed the peaks 3346.61 cm^{-1} (N-H, str), 2995.55 cm^{-1} (CH, str, Sp2), 2924.18 cm^{-1} (C-H, str, Sp3), and 1106 cm^{-1} (C-O, str). These peaks can be considered as characteristic peaks of Carvedilol and were not affected and prominently observed in IR spectra of Carvedilol along with polymers (Figure II) indicated there was no interaction between Carvedilol and polymers.

The preliminary studies indicated that the minimum concentration of PF-188 that formed gel below 35°C was 18%w/w. It was observed that as the PF-188 concentration increased, the gelation temperature decreased. As the concentration of PF188 increases,

there is micellar formation, followed by micellar aggregation. The gel phase can only occur when the concentration is above the micellar concentration.[17]

Surface pH of the gel

The pH of all the formulations was found to be in the range of 4.5-6.0. Lysozyme was present in the nasal secretions, which was responsible for destroying certain microbes at acidic pH. Under alkaline pH Lysozyme is inactive and nasal tissue is susceptible to microbial infection. Lower pH acts as hypertonic solutions, causing the shrinkage of epithelial cells and also inhibits ciliary activity. It was therefore advisable to keep the pH of formulation in the range of 4.5-6.5.

Drug Conten

The percentage drug content of all the formulations was found to be in the range of 97.44 -99.17%. The percentage drug content of formulations from same batch was found to be uniform.

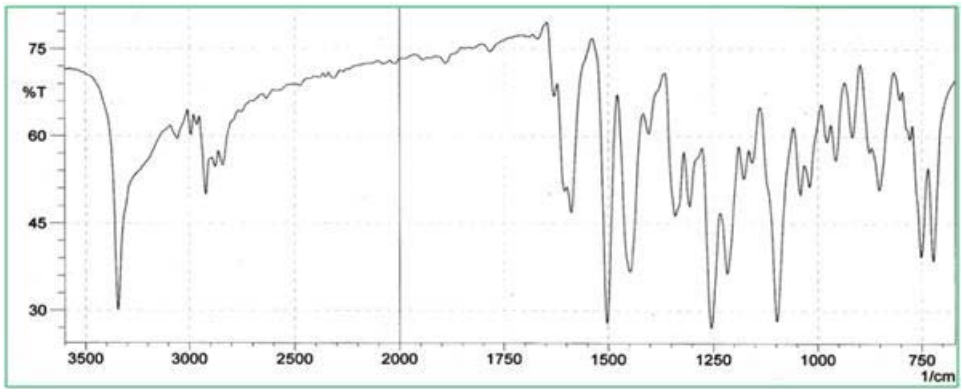


Figure 1: FTIR of Carvedilol

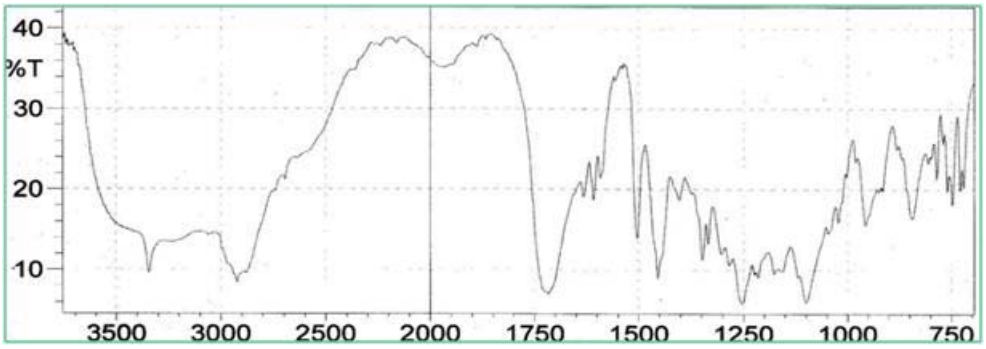


Figure 2: FTIR of Physical mixture

Inflection Point

All the prepared gels showed a sudden change in viscosity at a temperature range of 21-33°C.

Gelation temperature (T1) and gel melting temp (T2)

At gelation temperature, liquid phase makes transition into gel. Due to the addition of carbopol there is a change in T1 of gel formation. Study shows that formulation G1 has gelation temperature of 36O°C (mucoadhesive polymer absent) where as G5 has a T1 of 24O°C which is having high level of Carbopol (0.5%).The results presented in Table. This indicates that the mucoadhesive polymer, carbopol has significant T1 lowering effect. The gelation temperature lowering effect might be caused due to increased viscosity after dissolution of mucoadhesive polymer. The gel melting temperature (T2) was also found to increase with increasing concentration of Carbopol from 0.2%-0.5%W/V.

Viscosity

Viscosity measurement of the formulations at various temperatures (25 & 370°C), shows that there was increase in viscosity with increase in the temperature. Figure III shows viscosity profiles of formulations at 25 & 370°C and the mucoadhesive polymer had a viscosity enhancing effect.

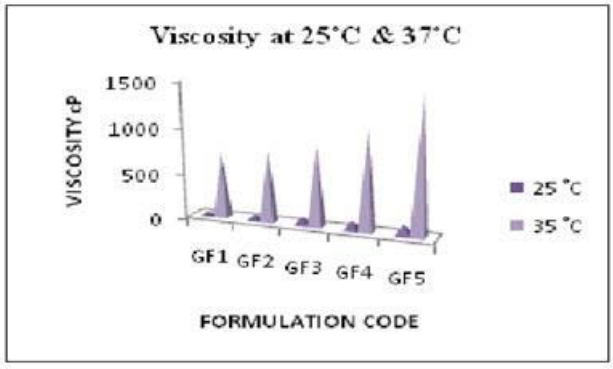


Figure 3: Viscosity of gels at 25°C & 37°C

Gel strength

At high gel strength, it is difficult to insert the gel. On the other hand, at low gel strength the gel leaked from the nose. It has been previously reported that the optimal in situ gelling and muco adhesive liquid gels must have suitable gel strength, in the range of 20 to 60 seconds. In the present study it was found that all formulations showed gel strength between 25-75 sec, the addition of muco adhesive polymers increased the gel strength of Poloxamer mixture in a concentration dependent manner. The large increase in gel strength caused by the addition of CP might be attributed to the strong cross-linking bonding of CP with the cross-linking reticular Poloxamer gel forming more closely packed micelles. It was found that formulations having high muco adhesive force had high gel strength.

Determination of muco adhesive strength

Assessment of the muco adhesive strength in terms of detachment stress showed that the PF188 preparations possessed adhesive properties that increased with the addition of C940P concentration. It shows that as level of carbopol increases, muco adhesive strength also increases. The results are presented in Table 2. This was due to wetting and swelling of carbopol, permits intimate contact with nasal tissue, interpenetration of bioadhesive carbopol chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains. But higher ratio of carbopol responsible for excessive bioadhesive force and the gel can damage the nasal mucosal membrane. Earlier work with carbopol polymers has clearly indicated that it is the availability of carboxyl groups that determines bioadhesion. Carbopol has a very high percentage (58%- 68%) of carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus membrane.

Table 2: Physical evaluation of in-situ gel

Code	Gelation temp(˚C)	Gel melting temp(˚C)	pH	Gel strength (SEC)	mucoadhesive strength (Dynes/cm^2)	Inflection pt (˚C)	Drug content
G1	36.33 ± 1.52	46.6 ± 1.52	4.81 ± 0.37	25.33 ± 1.15	3985.33	33 ± 2	0
G2	32.67 ± 1.53	53.67 ± 1.154	5.13 ± 0.14	31.33 ± 1.52	4285.86	28.3 ± 2.081	97.44 ± 1.183
G3	30 ± 1.73	58.3 ± 2.081	5.28 ± 0.157	47.58 ± 2.08	4886.93	26.67 ± 2.171	97.65 ± 0.84
G4	28.6 ± 2.08	63.32 ± 1.527	5.64 ± 0.091	51.6 ± 1.54	5951.86	25 ± 1	99.177 ± 0.352
G5	24 ± 1	69.6 ± 1.5	5.74 ± 0.12	75.3 ± 1.43	6291.6	21.42 ± 1.154	98.416 ± 0.291

***In-vitro* diffusion study**

Diffusion profiles of formulation series are elaborated in Figure 4. Permeability of G1 was less because the presence of PF188 in the gel retards the drug release rate slightly owing to reduction in dimension of water channels resulting for enhanced micellar structure. More percent of drug diffused in case of G2, G3, G4 & G5 because of 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. This result could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoiling of the polymer chain, resulting in the relaxation of the polymer network. At this stage, drug is rapidly dissolved and released from the gels as a result of very high swelling of the ionized carbopol.

***In-vitro* Permeation Study**

In-vitro permeation was observed for the aqueous drug solution and formulation G4. Formulation G4 exhibited good drug release profile with favorable gelation and rheological properties. Hence, the formulation G4 was chosen as an optimized formulation, to see the permeation of drug through goat nasal mucosa. It was observed that permeation of drug from buffer solution was 64.55% where as the optimized formulation G4 shows release of 87.09% at the end of 480min as shown in Figure 5.

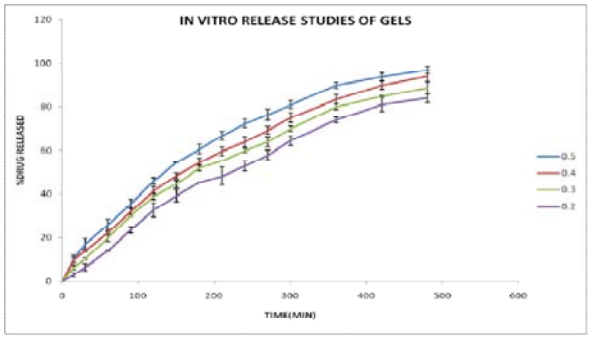


Figure 4: *In-vitro* release studies of gel

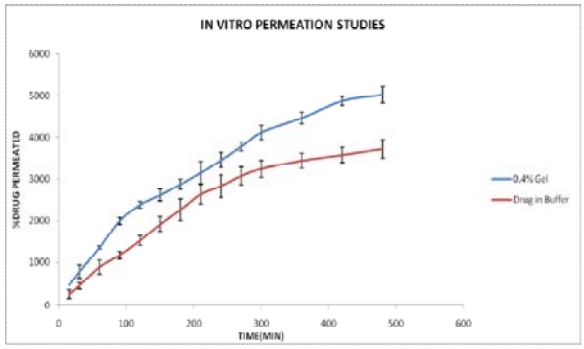


Figure 5: *In-vitro* permeation studies

Addition of anionic polymer, carbopol to the PF188 has dramatically increased permeation coefficient. This result could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational changes in the polymer chain.

Histological Studies

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of nasal mucosa. As shown in Figure 6 and 7, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of G4. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of sub mucosa as compared with PBS-treated mucosa. Thus, gel formulations seem to be safe with respect to nasal administration.

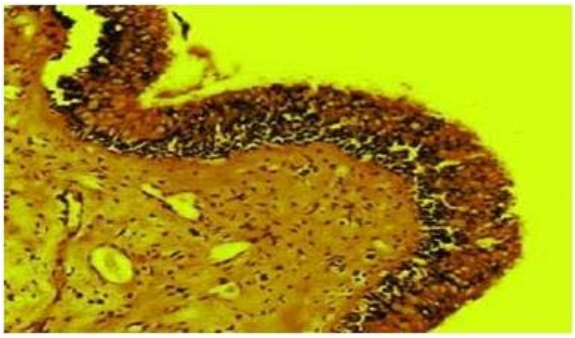


Figure 6: Histopathology of PBS treated mucosa

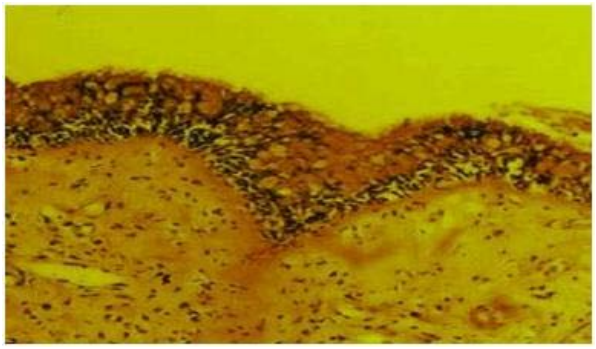
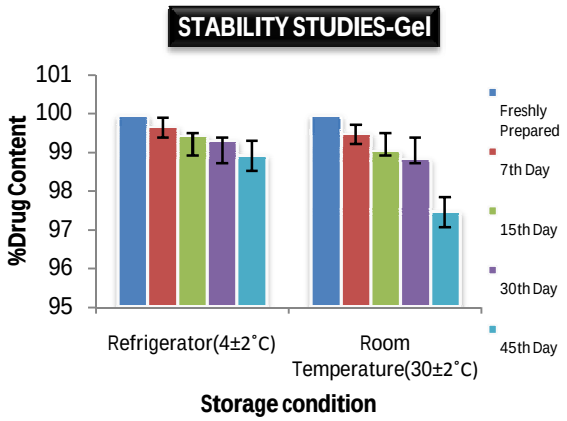


Figure 7: Histopathology of Gel treated mucosa



Stability study

Formulations containing 0.4% of muco adhesive polymer carbopol were subjected to stability studies at room temperature (30±2°C) and at refrigerator temperature (4±2°C) for 45 days. The gels were evaluated for drug content and pH on 7th, 15th, 30th & 40th day. The pH and drug content results of the gel at low temperature indicated that there was no significant change in the Carvedilol gel formulations after 45 days when compared with the initial values. There was slight decrease (0.5 – 1.5°C) in the gelation temperature of the formulation studied. Thus above results (Figure 8) indicated that refrigeration condition (2 to 8°C) was suitable for the storage of nasal in-situ gel formulations.

CONCLUSION

The present study was aimed to develop suitable drug delivery systems for the management and immediate use of Carvedilol used in the treatment of Hypertension and angina pectoris. The purpose of the study was to overcome the inherent drawbacks, associated with conventional drug delivery of Carvedilol and will have an improved bioavailability, fast therapeutic action and patient compliance with an added advantage of circumventing the hepatic first pass metabolism. Among all the formulated gels G4 was selected as the optimized formulation with respect to its evaluation parameters like gelation temperature, pH, drug release & mucoadhesive strength. The pre-formulation studies were carried out; from the study it was clear that it satisfy the entire characteristic for nasal delivery. The study indicates that much work can be done on the nasal formulations of Carvedilol. Furthermore suitable animal models should be studied in order to establish *in vitro-in vivo* correlation.

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