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FORMULATION AND EVALUATION OF ION-ACTIVATED *IN SITU* GEL OF METOPROLOL TARTRATE FOR NASAL ADMINISTRATION

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ABSTRACT

Metoprolol tartrate, a selective beta-blocker is completely absorbed from GIT (95%) but is only 40% bioavailable owing to first-pass metabolism. The present investigation has been carried out with the aim of avoiding first-pass metabolism by formulating the drug for nasal administration, based on the concept of ion-activated *in situ* gelation using gellan gum. *In situ* gels were formulated using different concentrations of gellan gum. To all the formulations 2.5%w/v of metoprolol tartrate was added, pH adjusted to 4.5 by 0.5 M NaOH solution and terminally sterilized by autoclaving at 121^{0}C and 15 psig for 20 min. Formulations were slightly viscous solutions (1.330-3.130Pa.s) at pH 4.5 and viscosity increased markedly (19.350-48.970Pa.s) in phosphate buffer saline (PBS) of pH 7.4 depending upon concentration of gellan gum. Mucoadhesive force from 23.53±0.58 to 35.73±1.02dynes/cm^2 was found. *In vitro* percent drug release after 8hrs from different formulations was from 79.57±0.29-99.05±0.42. Drug release followed Higuchi's diffusion mechanism. On the basis of viscosity, mucoadhesive force and *in vitro* release, the optimized formulation G4 containing 0.4%w/v of gellan gum was subjected for *in vitro* permeation studies and histological studies using porcine nasal mucosa. The permeability coefficient of optimized formulation was found to be 1.39x10^{-5}cm/sec. Histological study shows no change in mucosa as compared to control. All formulations showed good stability with no significant change after exposure to 40^{0}C and 75% relative humidity for 3 months.

Key words: *Gellan gum; Metoprolol tartrate; Nasal delivery; In situ gel; Nasal permeation*.

INTRODUCTION

The interest in and importance of the systemic effect of drugs administered via the nasal route have expanded over recent decades. Nasal administration offers an interesting alternative to the parenteral route for achieving systemic drug effects. Parenteral route may be inconvenient or oral administration may sometimes result in unacceptably low bioavailabilties[1]. The nasal epithelium is a highly permeable monolayer, the submucosa is richly vascularised and hepatic first-pass metabolism is avoided after nasal administration. Other attractive feature includes the rather large surface area(180cm^2 because of the presence of large number of microvilli) of the nasal cavity and the relatively high blood flow, which promotes rapid absorption, porous endothelial membrane and highly vascularised tissue providing an attractive site for rapid and efficient systemic absorption, furthermore, self-medication is easy and convenient[2]. Nasal route is a potential route for administration of peptides like insulin. Inhalation insulin formulation EXUBERA® formulated by Pfizer delivers insulin by oral inhalation and is absorbed as quickly as subcutaneously administered rapid-acting insulin analogs for type 1 and 2 diabetes. It consists of blisters containing human insulin inhalation powder produced by recombinant DNA technology utilizing a non-pathogenic laboratory strain of Escherichia coli (K12)[3].

Currently, nasal administration is used therapeutically for the systemic absorption of drugs in a variety of indications, including sumatriptan for migraine[4], the antidiuretic desmopressin for the treatment of diabetes insipidus and oxytocin for the stimulation of breast milk ejection[5]. The nasal dosage forms include solutions, sprays, microspheres, gels and liposomes. Although solutions are easy to use, they achieve poor bioavailability, due to their short residence time in the nasal muscosa because of the mucociliary clearance. It had been demonstrated that a significant improvement in the bioavailabilty would be achieved if the nasal residence time of the drug could be increased. From the point of view of patient acceptability a liquid dosage form that can sustain drug release and remain in contact with the nasal mucosa for extended period of time is ideal. Drug delivery systems based on the concepts of *in situ* gel formation should provide these properties. Such delivery systems consists of phase transition due to physiological stimulus (pH, temperature, ionic content)[6,7] . The aim of the present work was to develop an ion-activated *in situ* gelling system of metoprolol tartrate, an antihypertensive drug having oral bioavailability of only 40% due to hepatic

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first-pass effect. Gellan gum was investigated as a vehicle for the formulation of nasal drops of metoprolol tartrate, as it undergoes gelation when instilled into the nasal cavity and can provide sustained release of the drug by increasing residence time due to its mucoadhesive property. Ion activated in situ gels have been formulated for various applications. Ion activated in situ gel containing Gelrite® was formulated for indomethacin for ophthalmic administration. The developed formulations provided sustained release of the drug over an 8 hour period and were therapeutically effective in uveitis induced rabbit eye model[8]. Oral sustained delivery formulation of paracetamol using gellan gum was prepared by Watarn Kubo et al[9]. Aqueous solution of gellan gum containing calcium ions in complexed form resulted in the formation of gel depots in rabbit and rat stomachs. In vitro studies showed release of paracetamol from gels over a period of 6 h.

MATERIALS AND METHODS

Metoprolol tartrate was generously gifted by Polydrug Laboratories, Mumbai (India), C.P. Kelco, Mumbai, India provided gellan gum (KELCOGEL®). All other reagents used were of analytical grade.

FORMULATION OF *IN SITU* GEL

Gellan gum solutions were prepared by adding polymers to deionised water and heating to 90^{0}C while stirring. After cooling the solution below 40^{0}C, metoprolol tartrate 2.5% w/v was added to the polymeric solution and stirred until dissolved. Mannitol was added to adjust the isotonicity. Sodium taurocholate, benzalkonium chloride and sodium bisulphite were then added and mixing was continued until a uniform and clear solution was formed. The formulations were filled in amber glass container and were terminally sterilized by autoclaving at 121^{0}C and 15psig for 20min. Composition of the formulations is given in Table 1.

***Table 1**. Composition*, mucoadhesive force and duration of mucoadhesion of in situ gels*

Formulation	Composition (%w/v)			Mucoadhesive force, n=3±S.D (dynes/cm^2)x10^3	Duration of mucoadhesion (hr) n=3± S.D
	Metoprolol Tartrate	Gellan gum	Mannitol		
G1	2.5	0.1	4.0	10 ±2.16	< 1 (gel losses integrity rapidly)
G2	2.5	0.2	4.0	23.53 ±0.58	4.10± 0.05
G3	2.5	0.3	4.0	26.74± 0.32	5.35 ±0.1
G4	2.5	0.4	4.0	30.85 ±0.91	7.5 ±0.12
G5	2.5	0.5	4.0	35.73 ±1.02	>8

**Sodium taurocholate (1% w/v) as penetration enhancer, benzalkonium chloride (0.02% w/v) as preservative & sodium bisulphate (0.25% w/v) as antioxidant were added to all formulations.*

EVALUATION OF FORMULATIONS

Drug content uniformity

400µl of the preparation was transferred to 100ml volumetric flasks with a micropipette and final volume was made up with phosphate buffer saline pH 7.4. Drug concentration was determined spectrophotometrically at 274nm.

Gelation study[8]

Gelation studies were carried out in gelation cells, fabricated locally using teflon. The cells were cylindrical reservoirs holding 3ml of gelation solution. Within the cells at the bottom, a 400µl transparent plastic cup was located to hold the gel sample in place after its formation. The studies were carried out using nasal fluid of composition 7.45mg/ml NaCl, 1.29mg/ml KCl and 0.315mg/ml $CaCl_2$[10].

Determination of the mucoadhesive force[11]

Mucoadhesive force of nasal *in situ* gel was determined according to previously reported method[11] by means of the mucoadhesive force measuring device (Figure 1) and, using porcine nasal mucosa and phosphate buffer saline as the moistening fluid. At the time of testing a section of tissue (E) was secured, keeping the mucosal side out, onto each glass vial(C) using a rubber band and an aluminum cap. One vial with a section of tissue was connected to the balance (A) and other vial was kept in petridish (F). To the exposed tissue on the vial a constant amount of 0.5g polymer gel (D) was applied .The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of both vials. A constant force was placed on the upper vial and applied for two minutes, after which it was removed and weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the two vials were separated. The mucoadhesive force expressed as the detachment stress in dyne /cm^2 was determined from the minimal weights that detached the two vials using the following equation;

Detachment stress = m.g/A

Where, m is the weight added to the balance in grams, g is the acceleration due to gravity taken as 980cm/sec^2, A is the area of the tissue exposed and is equal to ð r^2(r is the radius of the circular hole in the aluminum cap). Mucoadhesive force of different formulations is given in Table 1.

Duration of mucoadhesion

Duration of mucoadhesion of phase transition system was determined by means of the mucoadhesive force measuring device (Figure 1) and according to previously reported methods[11], using porcine nasal mucosa and phosphate buffer saline as the moistening fluid. At the time of testing a section of tissue (E) was secured, keeping the mucosal side out, onto each glass vial(c) using a rubber band and an Aluminium cap. One vial with a section of tissue was connected to the balance (A) and other vial was kept in petridish (F). To the exposed tissue on this vial a constant amount of 0.5 g polymer gel (D) was applied .The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of both vials. A constant force was placed on the upper vial and applied for two minutes, after which it was removed and weight of 10 g was added to the pan on the other side of the modified

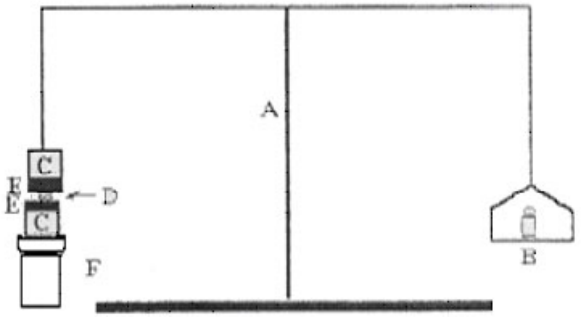


Figure 1: Assembly of the apparatus for mucoadhesion test. (A-Balance, B-Petridish, C-Glass vial, D-Polymer gel, E-Section of tissue, F-Support to vial to adjust height)

balance of the used device until the two vials were separated. Duration of mucoadhesion was noted as the time in hours taken to separate two vials and is depicted in Table 1.

Viscosity measurements

The apparent single viscosity values were measured with Brookfield digital viscometer DV-E with RV spindle system at 10rpm after 2min using spindle No. 7.

Rheological study[8]

Rheological characteristics were studied using Brookfield viscometer. Thixotrophic behavior was studied by keeping the formulations under fixed spindle speed of 10rpm and measuring viscosity at different time till a constant viscosity. After that stress was removed and gain in viscosity was again measured at different time till constant viscosity was obtained. Flow behavior was also studied and area of hysteresis loop was calculated. To study flow behavior, shear rate and stress were calculated for increasing spindle speeds from 10 -100 rpm (10, 20, 30, 50, 60 &100 rpm) and then for decreasing speeds to construct the up and down curves. At each speed spindle was rotated for 5 min. The results are shown in Figures 2 and 3.

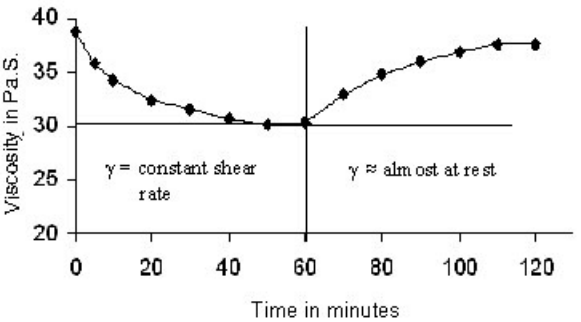


Figure 2: Viscosity-time –curve for G4

***In vitro* release study[12]**

In vitro release study of the formulated *in situ* gel were carried out in two chamber diffusion cell through dialysis membrane-70 (Himedia) with molecular weight cut off 12000-14000kDa. Diffusion cell with receiver

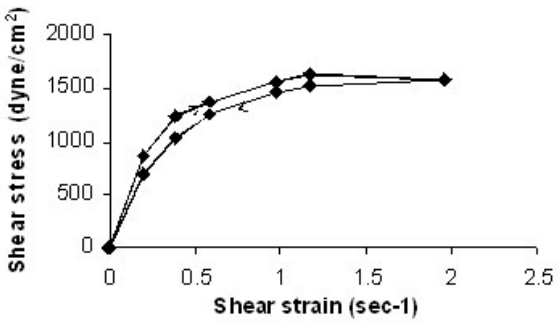


Figure 3. Rheogram of formulation G4

compartment of diameter 1cm and 12ml capacity was used for the study. To prepare artificial membrane, pieces of dialysis membrane were soaked in PBS of pH 7.4 for 24hrs before mounting on the diffusion cell. Diffusion membrane was mounted in a two chamber cells at 30°C. 400µl of formulation was placed in the donor compartment; PBS of pH7.4 was placed in the receptor compartment. The formulation was mounted on the chamber in the sol form. The temperature of the receiver was maintained at 37°C ±1.0 °C during the experiment, an aliquot of 1ml was withdrawn from receiver compartment initially after 15 and 30min and then at 1hr interval and replaced with the same amount of the medium. Aliquots so withdrawn were suitably diluted and analyzed using UV spectrophotometer (UV 2401, Shimadzu) at 274nm for drug. *In vitro* release study was carried out for 8hrs (Figure 4). To know precisely the mechanism of drug release from the gel the release data obtained from *in vitro* release experiments were treated to Peppas exponential equation[13].

$$Mt/M = kt^n$$

Mt/M is the fraction of drug released at time t, n is diffusion exponent indicating release mechanism. The log-log plot of fraction of drug released vs time is depicted in Fig 5.

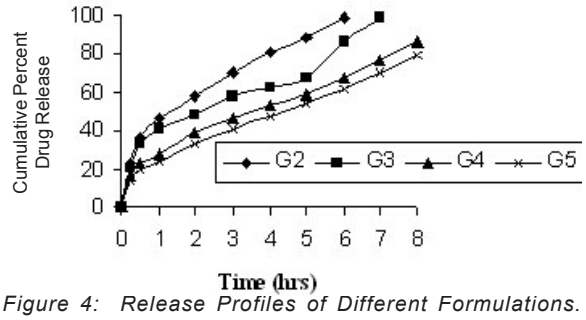


Figure 4: Release Profiles of Different Formulations. (G1 is not shown due to failure to retain gel integrity)

***In vitro* permeation study[12]**

The skin around the nasal region was removed and snout was separated from the porcine and opened up to expose the conchae. The mucosa covering the ventral nasal conchae (cavity mucosa) was carefully removed using forceps and a scalpel. After being rinsed in saline solution and then distilled water, a piece of

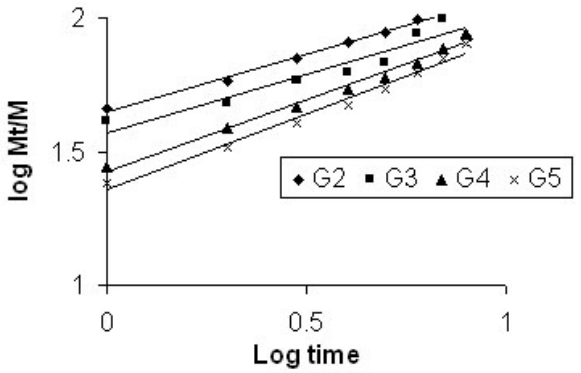


Figure 5: Log-Log Plot of Fraction of Drug Released vs Time (G1 is not shown due to failure to retain gel integrity)

nasal mucosa was mounted as flat sheet in a two chamber diffusion cell at 37 ± 1^{0}C.

On the basis of results of tests done on formulations, G4 was selected for *in-vitro* permeation study of 8 hrs. Four hundred microlitres of formulation containing 10mg of drug was placed on the mucosal surface with effective surface area of 0.785cm^2 in the donor compartment. PBS of pH 7.4 was placed in the receptor compartment. An aliquot of 1ml was withdrawn from receiver compartment initially after 15 and 30min and then at 1hr interval and replaced with same amount of PBS 7.4. Aliquots so withdrawn were suitably diluted and analyzed at 274nm for drug. The permeability coefficient (P)was calculated using following equation

$$P=dQ/dt/Co.A$$

Where, dQ/dt is permeability rate at steady state, Co is the initial concentration in the donor chamber and A is the effective surface area of the mucosa. *In vitro* permeation profile is shown if Figure 6.

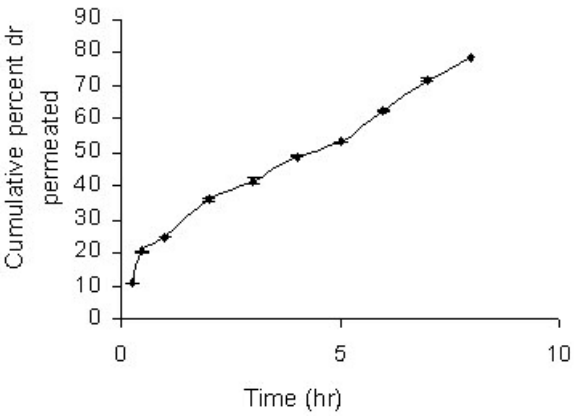
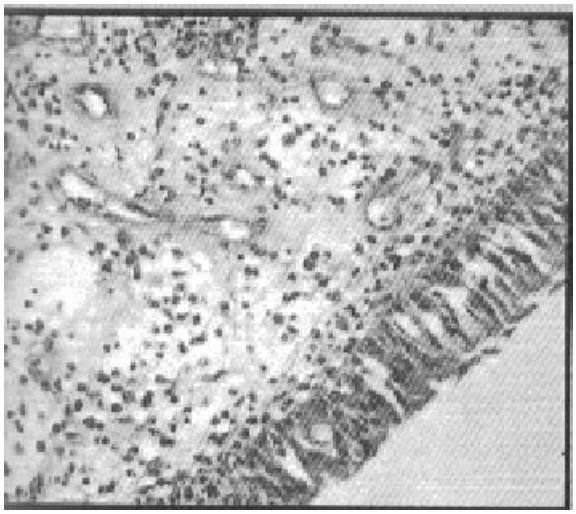


Figure 6: In Vitro Permeation Profile of Optimized Formulation G4 through Porcine Nasal Mucosa

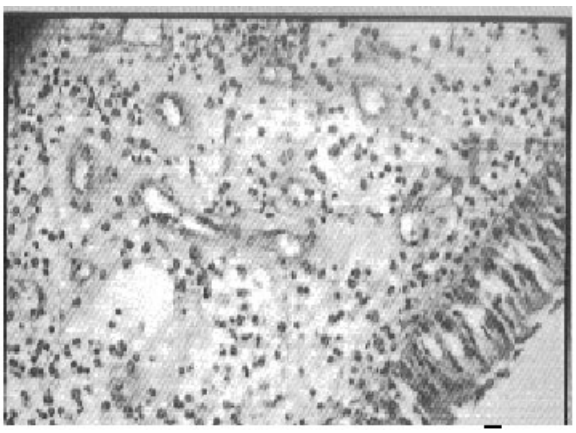
Histological examination of the porcine nasal mucosa[14]

Ventral nasal conchae was immediately removed from the anterior nasal cavity after sacrificing the porcine, and rinsed with saline solution. Eight hours after applying the nasal *in situ* gel, the nasal mucosa was fixed in 10% neutral carbonate buffered formalin for at least 24hrs before routine processing and then cut vertically into sections against the nasal mucosa at the central region in 4mm widths.

Each section was dehydrated using a graded series of ethanol solutions and was then embedded in paraffin wax .Tissues were divided into small pieces (about 3 micrometer in thickness) and stained with hematoxylin and eosin, all sections were examined by an optiphoto light microscope. The state of nasal epithelium on the dosed side of the septum was qualitatively compared with untreated tissue (Figure 7).



(a)



(b)

Figure 7: Microscopic photos of control untreated nasal mucosa (a) and mucosa after applying formulation G4 for 8 hrs (b).

Stability study

Formulation G4 showing optimum gelation, gel strength, mucoadhesive force and high release rate was selected for stability studies. It was stored at 40^{0}C and 75 % relative humidity for a period of 3 months and evaluated at periodic intervals for clarity, drug content, sol to gel transition, viscosity, thixotrophy, flow behavior and *in vitro* drug release.

RESULTS AND DISCUSSION

Concentration of gellan was kept at a maximum of 0.5%w/v. Increasing the concentration beyond 0.5% caused gelation upon cooling to 40^{0}C. All the formulations had clear appearance and the drug content was found between 98.46-100.20%w/w.

The two main prerequisite of a gelling system are viscosity and gelling characteristics (speed and extent of gelation). The formulations should have an optimum viscosity, which will allow its easy instillation into nasal cavity as a liquid (drop or spray), which will then undergo rapid sol to gel transition due to ionic interaction for ion-induced *in situ* gel. Moreover to facilitate sustained release of the drug to the nasal cavity, the *in situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period of time. All the formulation of gellan gum showed instantaneous gelation when contacted with the nasal fluid. However, the nature of gel formed depended upon the polymer concentration. The formulation G1 showed weak gelation, which could be due to the presence of minimal amount of the gellan gum (0.1%).

Marked increased in viscosity of all formulations of gellan gum in presence of artificial nasal fluid due to sol to gel conversion was observed. The viscosity was found between 19.350-48.970 Pa.s. In ion-free aqueous medium at room temperature, the polymer chains form double helices, resulting in a fluid that has a viscosity close to that of water, whereas upon contact with cations present in artificial nasal fluid, a portion of the helices associate and cation-mediated aggregates were formed, acting as cross-links in the gel network leading to the increase in viscosity.

The formulation exhibited pseudoplastic flow as evidenced by shear thinning with the increase in speed of spindle and absence of yield value. The formulations also showed thixotrophic behavior as indicated by time dependent change in viscosity at constant rate of shear and recovery of consistency after removal of stress. Area under the hysterisis loop was found to be 106.32dynes cm $^{-2}$ sec^{-1} for G4. The thixotrophic behaviour and rheogram is shown in the Figures 2 and 3 respectively.

The mucoadhesive force is an important physicochemical parameter for *in situ* gelling nasal formulations since it prevents gels from nasal clearance and increase residence time in the nasal cavity, but if the bioadhesive force is too excessive the gel can damage the nasal mucous membrane. Thus the nasal *in situ* gel must have an optimum bioadhesive force. With the increase in gellan gum concentration (Table 1) mucoadhesive force was found to increase. Mucoadhesive force of G4 containing 0.4% gellan gum was found to be 30.85x10^{3}dynes/cm^2 while duration of mucoadhesion was 7.5 hrs (Table 1).

The initial rates of release were very rapid due to incomplete gel formation, but the release became slow after complete gel formation and remained so. The release profiles exhibited an inflection point, which indicated gel formation on the diffusion membrane in donor compartment of diffusion cell. During gel formation portion of the drug might be loaded into the gel phase, thus drug release became slow, also the initial fast release of metoprolol tartrate from the prepared systems could be explained by the fact that these systems were formulated in aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation could no longer limit the drug release. *In vitro* release study indicated that the release of drug varies according to concentration of polymer .The results further showed that the amount of the drug released in first hour decreased with the increasing polymer concentration and this trend continued for the entire duration of the study.

FormulationG1, prepared with 0.1% gellan gum was found to form weak gel and could not retain its integrity up to 8hours and thus removed from the release study. Formulation G5 with 0.5%gellan gum formed stiff gel having highest viscosity and drug release was only 69.57% in 8hours. As the release was very less and the gel was very stiff therefore, discarded from further studies. Drug release of 86.89% from G4 in 8hours was considered satisfactory (Figure 4).

It was not possible to give the release mechanism in the early stages of the drug release because of the incomplete gel formation, however after complete gel formation; the drug release profiles of each preparation were linear with square root of time and followed Higuchi's equation. Plot of log (Mt/M) versus log (t) (Figure 5) of all the formulations gave the slope values less than or near 0.5 suggesting drug release by Fickian diffusion control.

Formulation G4 showed cumulative percent drug permeation of 78.65 % in 8 hours and permeability coefficient (P) was found to be 1.39x10 $^{-5}$cm/sec. *In vitro* permeation profile of formulation G4 are shown in Figure 6.

It is necessary to examine histological changes in the nasal mucosa caused by formulations, if it is to be considered for practical use. Figure 7a shows the control mucosa, normal respiratory epithelium stained with hematoxylin-eosin and Figure 7b shows mucosa treated with the formulation for 8hrs. No change in mucosal structure was seen when treated with formulation G4 as compared to the control. Thus from the histological study it can be said that gellan gum does not have any destructive effect on the mucosa and can be used safely for nasal administration.

Stability study was carried out on the optimized formulation at 40 ^{o}C ± 75%RH for 3 months. Optimized formulation showed good stability with no change in drug content, gelling capacity, viscosity, thixotrophic behavior and *in vitro* release.

CONCLUSION

From the results it can be concluded that metoprolol tartrate was successfully formulated as an *in situ* gelling system using gellan gum. The formulated system provided sustained *in-vitro* release of the drug over an extended period of 8 hours. Gellan gum was found suitable for nasal administration with no adverse effect on nasal mucosa. The developed formulation can be a viable alternative to conventional nasal drops by virtue of its ability to enhance bioavailability through its longer residence time and ability to sustain drug release after the pharmacokinetic studies.

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