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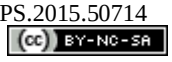
ISSN 2231-3354 

# Nasal Mucoadhesive In-Situ Gel of Granisetron Hydrochloride using Natural Polymers

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**ABSTRACT**

The prolonged residence of drug formulation in the nasal cavity is of utmost importance for intranasal delivery of drug. Present investigation was aimed to develop a mucoadhesive *in situ* gel of Granisetron hydrochloride (GH) with reduced nasal mucocilliary clearance in order to improve the bioavailability of the antiemetic drug, granisetron hydrochloride. The *in situ* gelation upon contact with nasal mucosa was conferred via the use of the thermogelling Pluronic flake 127 (PF 127). Moringa gum (MG), carboxymethyl tamarind gum (CMTG) and sodium alginate (SA) was used to modulate mucoadhesion whereas drug release of optimized formulation was modified by 0.3% polyethylene glycol 6000 (PEG 6000). Results revealed that as the concentration of mucoadhesive polymer increased the mucoadhesive strength increased and gelation temperature decreased significantly. Preformulation studies showed that addition of GH in 18% PF 127 gels modulated gelation temperature significantly while mucoadhesive polymers alters mucoadhesion. Formulation F6, F11 and F15 showed more than 80% of drug diffusion at 240 min. Gelation temperature and mucoadhesive strength of all three formulations were found in the range of 30-31 °C and 963.66±9.60 to 991.33±10.26 dyne/cm$^2$ respectively. Formulation F11 showed optimum results and further histopathological evaluation reveled formulation is safe for use. Addition of PEG 6000 increased drug diffusion in formulation F11 with flux 0.034 mg.cm$^2$/min. This study concluded the potential use of CMTG as mucoadhesive *in situ* nasal gel in terms of ease of administration, accuracy of dosing, prolonged nasal residence and improved nasal bioavailability.

## INTRODUCTION

Nasal drug delivery system acquired a great deal of attention as a convenient and reliable method for the systemic administration of drugs in the recent years. Nasal administration of drug offers various advantages like rapid onset of action by fast absorption, higher bioavailability allowing lower doses, avoidance of liver or gastrointestinal metabolism, avoidance of the gastrointestinal irritation, and enhanced patient compliance by self-medication (Costantino *et al.,* 2007; Cho *et al.,* 2010). Granisetron hydrochloride (GH) is antiemetic drug, effective both intravenously and orally, acts by antagonizing 5HT3 receptor in the chemoreceptor trigger zone and probably in upper gastrointestinal tract. GH is used in management of nausea and vomiting induced by cytotoxic chemotherapy, radiotherapy and for the prevention of post operating nausea and vomiting. GH has 60% oral bioavailability due to hepatic first pass metabolism by 7-hydroxilation (Dollary, 1999; Upward *et al.,* 1990). The conventional oral dosage forms are not suitable for some patients who are suffering from vomiting or, in case of unconscious patients or, for some reason, cannot absorb orally administered drugs efficiently. One modern way to cope with this difficulty is to develop new formulations for alternative routes such as nasal route of administration (Charlton *et al.,* 2007; Ugwoke *et al.,* 2005). The dose of GH is low (1 or 2 mg) and rapid onset of action is required, intranasal formulation of GH would be beneficial alternative to oral and intravenous administration. However, mucociliary clearance is known to be significant limiting factor for nasal drug delivery, which severely limits the residence time for drug to be absorbed.

Therefore, there is a need to design mucoadhesive preparation of GH which will increase contact time between the dosage form and mucosal layers of nasal cavities, thereby enhancing drug absorption. This can be achieved by formulating

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nasal formulation with increased nasal residence, permeation and ultimately bioavailability (Duan *et al.,* 2010). The poor bioavailability and therapeutic response exhibited by the conventional nasal solution due to short residence time is the basic problem of nasal route.

This problem can be overcome by the use of in situ gelling systems, which upon instillation as liquid droplets, undergo sol to gel transition into nasal cavity, which may lead to increased residence time of drug and aids drug absorption with rapid onset of action (Hussein, 1998; Ugwoke *et al.,* 2000). Natural polymers primarily remain attractive because they are easily available, cost effective, biodegradable and biocompatible (Hua *et al.,* 2010; Satturwar *et al.,* 2003).

Tamarind seed polysaccharide (TSP) is a water soluble polysaccharide which is commonly used as thickening, stabilizing and gelling agent in the food industry (Goyal P *et al.,* 2007). Major component of tamarind seed is identified as non-ionic, neutral, branched polysaccharide which consists of cellulose like backbone consisting of xylose, galactoxylose and glucose substituents in the ratio of 2.25:1.0:2.8 (Saettone *et al.,* 1997). TSP is chemically modified by various groups like acetyl, carboxymethyl and hydroxyalkyl, to overcome its drawbacks such as unpleasant odor, dull color and fast degradability.

Carboxymethyl group disrupts the organization of structure thereby exposing the polysaccharide network of hydration resulting in higher viscosity and lower biodegradability thereby enhancing its shelf life (Goyal *et al.,* 2007; Rao *et al.,* 1955; Prabhanjan *et al.,* 1989; Pal *et al.,* 2008). Moringa gum obtained from stem of the plant *Moringa oleifera* Lam, is a polyuronide consisting of arabinose, galactose, and glucoronic acid in the proportion of 10:7:2 mole; rhamnose is present in traces (Panda *et al.,* 2006). Now days, pharmaceutical researchers are getting attracted towards polysaccharides from tamarind and Moringa for development of drug delivery (Singnal *et al.,* 2012; Kaur *et al.,* 2010; Kaur *et al.,* 2012).

In order to formulate thermosensitive *in situ* gel for nasal administration, thermoreversible polymer should have gelation temperature in the nasal physiological temperature range (29 °C to 34 °C). PF 127 has excellent thermosensitive gelling property (Edsman K et at., 1995), low toxicity and irritation, excellent water solubility, good drug release characteristics, and compatibility with other chemicals (Zhang *et al.,* 2002). It is an ABA triblock copolymer consisting of the hydrophilic polyethylene oxide (PEO) and the hydrophobic polypropylene oxide. The temperature-induced gelation of PF127 has been explained on the basis that the polymer exists as a mobile viscous liquid at reduced temperatures but forms a rigid semisolid gel network with an increase in temperature (Johnston *et al.,* 1992).

The objective of present study was to develop a mucoadhesive thermoreversible nasal gel of GH using natural mucoadhesive polymers with a modulated phase transition temperature. In order to enhance nasal residence time and absorption of drug across nasal mucosal membrane.

## MATERIALS AND METHODS

Granisetron HCl was kindly gifted by Medi-orals Pvt. Ltd., Satara, India. Pluronic Flake 127 (PF 127) was gifted by BASF Pvt. Ltd., Mumbai, India. Sodium alginate (SA) and methyl paraben (MP), were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Moringa gum (MG) and carboxymethyl tamarind gum (CMTG) (DS 0.2) extracted, isolated and prepared in the laboratory (Goyal P *et al.,* 2007; Panda D *et al.,* 2006; Rao *et al.,* 1946). All the chemicals used were of analytical grade.

### Preparation and optimization of thermoreversible PF127 gels

The plain and drug loaded PF127 gels were prepared by cold method described by Schmolka *et al.,* (1972). For drug loaded PF127 gels, 10% of drug was stirred with sufficient quantity of distilled water while for plain PF127 gels, only sufficient quantity of distilled water without drug was kept overnight at 4°C in refrigerator. The PF127 was added slowly with continuous stirring. The dispersions were then stored in a refrigerator until clear solution was obtained and finally volume was adjusted. Plain PF 127 and drug loaded PF 127 gels were optimized by varying concentration of PF127 and their evaluation for gelation temperature. Optimized concentration of PF127 was used to study of effect of mucoadhesive polymers on gelation temperature and mucoadhesive strength. Different concentrations of MG, CMTG and SA were screened in the range of 0.1 to 0.5% as a mucoadhesive polymer (Dias *et al.,* 2010).

### Preparation of mucoadhesive thermoreversible nasal gels

GH, mucoadhesive polymer and MP were dissolved in distilled water by agitation at room temperature. After cooling the solution to 4°C, PF127 was added slowly with stirring. The resulting dispersion was then kept overnight at 4°C until clear transparent solution was formed. Then volume was made by using cold distilled water. Composition of various thermoreversible mucoadhesive nasal gel formulations is given in table 1. Evaluation of final formulations was done with respect to clarity, pH, gelation temperature, mucoadhesive strength, gel strength, viscosity, drug content, diffusion through sheep nasal mucosa, FTIR study and histopathological evaluation of mucosa. Optimized formulation was used to study the effect of addition of PEG 6000 on gelation temperature, mucoadhesion and drug diffusion.

### Evaluation of formulations

#### *Clarity*

All formulations were observed visually under black and white background and clarity of formulations was graded as follows: turbid: +, clear ++, very clear (glassy): +++.

#### *pH of Formulation*

pH meter (Equiptronics, Model EQ-610) was used to determine pH of the each formulation at room temperature. A standard solution of pH 4.5 and pH 7.0 was used to calibrate pH meter.

***Gelation Temperature***

Gelation temperature was measured by visual observation method and also by using Anton Paar modular compact rheometer MCR52.

**Visual observation method**

Two ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly at a constant rate of 1°C for 2 min from room temperature to the temperature at which gel formed. The sample was then examined for gelation, which is said to be occurred when the meniscus will no longer moves upon tilting the test tube through an angle of 90° (Choi *et al.,* 1998).

**Anton paar modular compact rheometer MCR52**

The gelation temperature was determined by using Anton Paar Rheometer (model: Gmbh, 3ITT) and probe (PP25-SN 17002) using 1 ml aliquot of the sample. Measurements were performed in oscillation mode using temperature sweep mode. Temperature was increased at constant rate from 10 to 60°C. Storage modulus and loss modulus were plotted against temperature automatically using Rheoplus software to determine gelation temperature.

***Determination of Mucoadhesive strength***

Mucoadhesion testing was carried out using a texture analyzer (CT3, Brookfield, USA) with 50 N load cell equipped with mucoadhesive holder. Sheep nasal mucosa was utilized as the model membrane for mucoadhesive strength determination of various formulations. The tissue (about 20 X 20 mm) was equilibrated for 15 min at 37.0 ± 0.5 °C before placing onto the holder stage of mucoadhesive holder. The probe was lowered at a rate of 0.5 mm/s until there will be contact with the membrane. A contact force of 1N was maintained for 60 s, and the probe was subsequently withdrawn at a rate of 0.5 mm/s to a distance of 15 mm. By using the texture analyzer, the maximum force required to separate the probe from the tissue (i.e. maximum detachment force in grams; $F_{max}$) could be detected directly from Texture Pro CT V1.3 Build 14 software. Following formula is used to calculate mucoadhesive strength (Yong *et al.,* 2001):

$$\text{Mucoadhesive strength (dyne/cm}^2) = F_{max} \times g / A$$

Where, $F_{max}$ = maximum detachment force in grams, g = Acceleration due to gravity [980cm/s$^2$], A = Area of tissue exposed.

***Drug content***

One ml of formulation was taken in 100 ml volumetric flask. 50ml of distilled water was added to it with gentle shaking and final volume was adjusted to 100ml. one ml quantity from this solution was transferred into the 10ml volumetric flask and final volume was made upto 10ml by using distilled water. Finally, the absorbance of prepared solution was measured at 302 nm by using UV visible spectrophotometer, to determine the drug content.

***Viscosity***

The viscosity of in situ gelling formulations was determined at 25°C with Anton Paar Rheometer (model: Gmbh, 3ITT) and probe (PP25-SN 17002) using one ml aliquot of the sample. Measurements on each value were performed in triplicate at a fixed shear rate of 50 (1/sec) using Rheoplus software.

***Gel strength***

A sample of 50g of the nasal gel was put in a 100 ml graduated cylinder and gelled in a thermostatically controlled water bath at 37°C. A weight of 35 g was placed onto the gel. The gel strength was determined by the time in seconds required by the weight to penetrate 5 cm deep into the gel (Choi *et al.,* 1998).

***Ex vivo permeation studies***

Fresh nasal mucosa obtained from the local slaughterhouse was carefully removed from the nasal cavity of sheep. The mucosa was stored in normal saline solution. Blood and bony cartilage from the mucosal membrane was removed and used for further study.

Modified diffusion cell was used to study in vitro drug diffusion profile. 100 ml of simulated nasal electrolyte solution (SNES) pH 5.5 at 34°C was added to the acceptor chamber (Dias *et al.,* 2010). The temperature within the chamber was maintained at 34°C by circulating hot water. After preincubation time of 20 min, formulation equivalent to 2 mg of GH was placed in donor compartment. At predetermined intervals, the samples were withdrawn and replaced with same volume to maintain sink condition. Samples were filtered and amount of drug permeated was determined at 302 nm by using UV- visible Spectrophotometer. In vitro drug permeation was carried out in triplicate for 4h.

***Analysis of drug release data***

To understand the release mechanism of various nasal *in situ* gelling formulation of GH, an attempt has been made to fit data of the rate of release using the semi-empirical model of Korsmeyer and more precisely the equation proposed by Peppas (Fawaz F *et al.,* 2004):

$$M_t/M_\infty = k\, t^n \text{ or the logarithmic form of this equation:}$$

$$\log(M_t/M_\infty) = \log(k) + n \log(t)$$

Where, $(M_t/M_\infty)$ is the fraction of released drug at time (t), (k) a characteristic constant of dosage form and (n) the fraction of release exponent, indicated drug release mechanism. The parameter n and k were calculated from the plot of log $(M_t/M_\infty)$ versus log (t).

When n is 0.45-0.57, the drug is released from polymer with Fickian diffusion mechanism. If n is 0.57-0.84, the drug is released from polymer with a non Fickian (anomalous) release.

Permeability coefficient (P) was calculated from slope of graph of percentage of drug transported v/s time and equations are shown as follows;

$$P = \text{slope} * Vd / S$$

Where, Vd = Volume of donor solution, S = Surface area of tissue.

$$\text{Flux (J)} = P * CD$$

Where, CD = concentration of donor solution

***FTIR Study***

The Fourier-transform infrared spectra of GH and formulations of GH with different excipients were obtained by using FTIR spectroscopy (IR Affinity I, Shimadzu, Japan).

***Histopathological evaluation of mucosa***

Mucosa for histopathological evaluation was incubated in simulated nasal electrolyte solution (SNES) pH 5.5 and gel formulation. Exposed tissues were fixed in 10% buffered formalin (pH 7.2), processed and embedded in paraffin. Paraffin sections (7 μm) were cut 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 by a pathologist blinded to the study (Majithiya *et al.*, 2006).

***Statistical treatment***

Values are expressed as mean ± SD. Statistical analysis of data was performed using paired t-test or one-way ANOVA followed by Dunnett's multiple comparison test. A $p$ value < 0.05 was considered significant.

**RESULTS**

***Optimization of concentration of PF127***

Results of optimized concentration of PF 127 are given in table 2. The concentration of PF 127 was screened from 16 to 20% to get thermoreversible gel on the basis of gelation temperature. It was found that the gelation temperature of plain PF127 gels decreased with increasing concentration of PF127 from 37.87±0.47 to 19.85±0.74 °C. When 10% GH was added to PF 127 gels, it was observed that gelation temperature of formulations increased significantly ($p$<0.05) for each concentration of gelling agent. The physiological range of the nasal mucosal temperature lies between 32-34 °C. So, thermoreversible nasal gels were prepared with the phase transition temperature in the range of 29-32 °C. From results it was found that only 18% w/v of PF127 gel with drug showed ability to form gel in the range of 29 to 32 °C. So 18% w/v concentration of PF127 was used for further studies.

***Optimization of Mucoadhesive Polymer with 18% w/v of PF127***

The concentration of mucoadhesive polymers, MG, CMTG and SA were optimized for gelation temperature and mucoadhesive strength. The results of optimization of mucoadhesive polymer are given in table 3. Formulation batches from F1 to F16 with different mucoadhesive polymers in different concentration were evaluated for mucoadhesive strength and gelation temperature.

As concentrations of mucoadhesive polymer increased, there was significant ($p$<0.05) decrease in gelation temperature and increase in mucoadhesive strength of formulations. From the results (see table 3) it was found that 0.5% MG (F6), 0.5% CMTG (F11) and 0.4% SA (F15) showed optimum results in terms of mucoadhesive strength and gelation temperature. Therefore these concentrations of mucoadhesive polymers were selected for further study.

***Clarity and pH***

Results of clarity and pH of formulation batches are given in table 4. All the prepared sets of formulations were found to be clear. pH of all the formulations was found to be in the range of 5.2-5.5 which is considered within nasal physiological pH range.

***Gelation Temperature***

The gelation temperature of *in situ* gelling formulations F6, F11 and F15 was determined by using Anton Paar Rheometer (model: Gmbh, 3ITT) and probe (PP25-SN 17002) using 1 ml of the sample. The point at which storage modulus crosses over the loss modulus is considered as a gelation point because it is indicative of phase transition from solution to gel. Gelation temperature of formulations F6, F11 and F15 is as shown in table 4 and figure 1.

Each graph obtained from Rheoplus software has two lines of two different parameters. One is of storage modulus **G´´** and other is of loss modulus **G´**. At particular temperature, when the line of loss modulus has higher value than storage modulus; the system is said to be in solution state. As the temperature goes on increasing, values of both parameters change. The temperature at which storage modulus attains higher value than loss modulus is called as a phase transition temperature because at this temperature solution system gets converted in to gel system. So the cross over point of two lines is the gelation temperature.

From the results of gelation study it was seen that all three formulation batches showed gel formation at nasal physiological temperature i.e. 29 to 34 °C.

***Mucoadhesive strength***

As concentrations of mucoadhesive polymer increased, there was significant (p<0.05) decrease in gelation temperature and increase in mucoadhesive strength of formulations. From the results, (table 3) it was found that 0.5% moringa gum (F6), 0.5% CMTSP (F11) and 0.4% sodium alginate (F15) showed mucoadhesive strength in the range of 900 to 1200 dyne/cm$^2$.

***Gel strength***

All formulations exhibited gel strength in the range of 45-48 sec (table 4). The results indicated that all formulations showed suitable gel strength.

**Table 1:** Composition of various thermoreversible mucoadhesive nasal gel formulations.

| Code | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 | F16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GH** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **PF127** | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| **MG** | - | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | - | - | - | - | - | - | - | - | - | - |
| **CMTG** | - | - | - | - | - | - | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | - | - | - | - | - |
| **SA** | - | - | - | - | - | - | - | - | - | - | - | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 |
| **MP** | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |
| **PW** | q.s. | q.s | q.s. | q.s. | q.s. | q.s. | q.s | q.s. | q.s. | q.s. | q.s. | q.s | q.s. | q.s. | q.s. | q.s. |

GH-granisetron hydrochloride, PF127- pluronic flake 127, CMTSP-carboxymethy tamarind seed polysaccharide, MG-moringa gum, SA-sodium alginate, MP-methyl paraben; PW- purified water, *All concentrations in percentage.

**Table 2:** Optimization of PF127 Solutions.

| Composition (w/v) | Gelation Temperature (˚C) Mean ± SD |
|---|---|
| PF127 16 % | 37.87±0.47 |
| PF127 16 % + GH 10 % | 40.54±0.51* |
| PF127 17 % | 34.91±0.36 |
| PF127 17 % + GH 10 % | 37.64±0.40* |
| PF127 18 % | 29.33±0.33 |
| PF127 18% + GH 10 % | 32.62±0.39* |
| PF127 19 % | 24.53±0.41 |
| PF127 19 % + GH 10 % | 27.83±0.51* |
| PF127 20 % | 19.85±0.74 |
| PF127 20 % + GH 10 % | 23.57 ±0.69* |

PF127- pluronic flake 127; GH-granisetron hydrochloride Values are expressed as mean±SD; n=6, * $p<0.05$ considered statistically significant

**Table 3:** Optimization of Mucoadhesive Polymer with 18% w/v of PF127.

| Formulation Code | Gelation temperature (˚C) | Mucoadhesive strength (dynes/cm$^2$) |
|---|---|---|
| F1 | 32.62±0.39 | 429.66±13.50 |
| F2 | 32.7±0.11* | 480.00±10.00* |
| F3 | 32.06±0.2* | 560.66±10.06* |
| F4 | 31.50±0.21* | 632.33±11.53* |
| F5 | 31.06±0.11* | 787.33±7.50* |
| F6 | 30.74±0.28* | 963.66±9.60* |
| F7 | 32.3±0.26 | 449.00±5.56 |
| F8 | 31.56±0.11* | 592.33±2.51* |
| F9 | 31.13±0.23* | 633.00±12.12* |
| F10 | 30.63±0.15* | 801.00±8.54* |
| F11 | 30.36±0.1* | 986.66±9.07* |
| F12 | 32.51±0.18 | 532.00±17.08* |
| F13 | 32.10±0.13* | 673.33±15.27* |
| F14 | 31.06±0.11* | 823.66±25.16* |
| F15 | 30.77±0.28* | 991.33±10.26* |
| F16 | 30.23±0.25* | 1261.66±7.63* |

Values are expressed as mean±SD; n=6 for gelation temperature; n=3 for mucoadhesive strength; * $p<0.05$ considered statistically significant (control F1).

**Table 4:** Evaluation of gels.

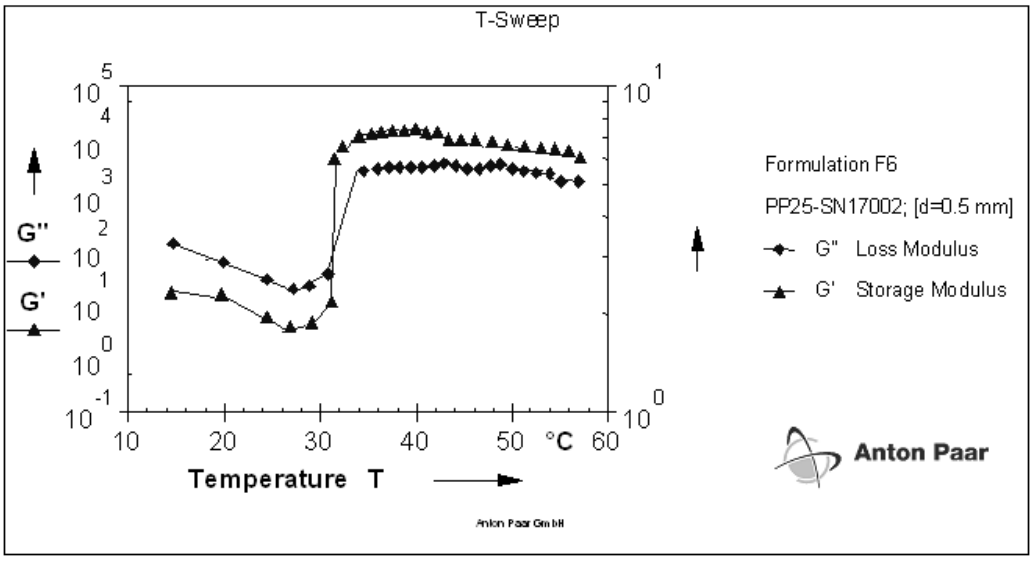
| Formulation Code | Clarity | pH | Gelation temperature (˚C) | Mucoadhesive strength (dynes/cm$^2$) | Gel strength (s) | Drug content (%) |
|---|---|---|---|---|---|---|
| F6 | ++ | 5.4 | 30 | 963.66±9.60 | 45 | 97.26±0.072 |
| F11 | ++ | 5.2 | 31$^0$C | 986.66±9.07 | 47 | 98.55±0.070 |
| F15 | ++ | 5.5 | 31$^0$C | 991.33±10.26 | 48 | 98.78±0.043 |

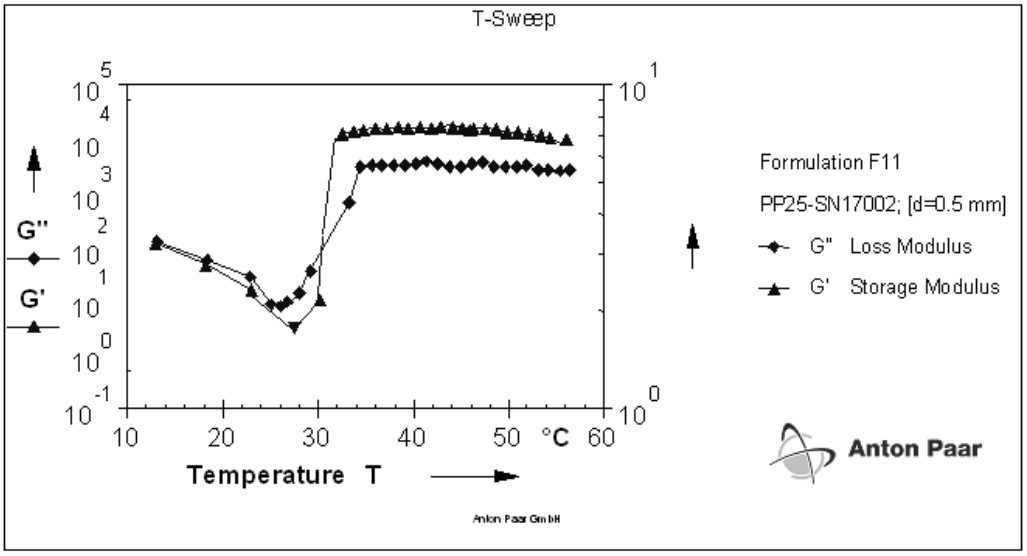
Values are expressed as mean±SD; n=3.

**Table 5:** Result of permeation study.

| Formulation Code | Time (min) | % Drug release | Permeation coefficient (mg.cm/min) | Flux (mg/cm$^2$/min) | n value | $R^2$ |
|---|---|---|---|---|---|---|
| F6 | 240 | 88.80±0.052 | 0.011 | 0.021 | 0.61 | 0.986 |
| F11 | 240 | 92.44±0.073 | 0.013 | 0.026 | 0.52 | 0.986 |
| F15 | 240 | 82.33±0.120 | 0.009 | 0.019 | 0.55 | 0.973 |

Values are expressed as mean±SD; n=3.



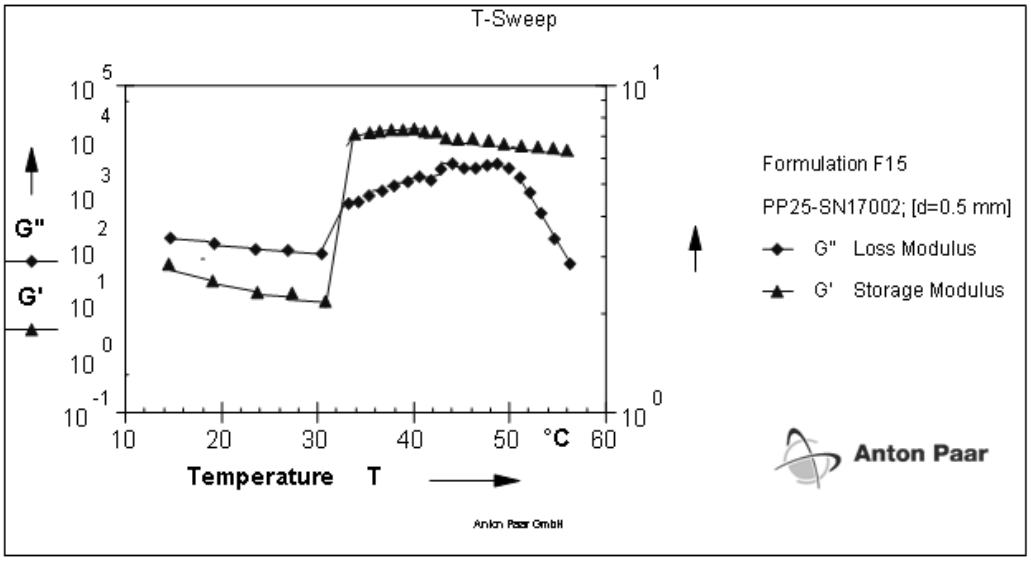




**Fig. 1:** Graph of gelation temperature of formulation F6, F11, F15.

***Drug content***

All the formulations were found to have drug content in between 97.26 and 98.78% w/w (table 4).

***Viscosity studies***

The plot of viscosity versus temperature for *in situ* gelling formulations F6, F11 and F15 is shown in figure 2. There was no considerable change in viscosity up to the point of gelation temperature. Sharp rise in viscosity was observed at the point of $T_{sol\text{-}gel}$ transition. From the plot it was clear that viscosity of each formulation decreased initially with increase in temperature. At about 27 °C, all formulations showed lowest viscosity and were in solution form. Sharp rise in viscosity was observed at gelation temperature and maintained a constant value up to 45 °C. The viscosity studies indicated that all formulations were in liquid state at room temperature and were converted into gel at nasal physiological temperature.

***Ex vivo permeation studies***

The permeation study of F6, F11 and F15 was carried out by using Modified diffusion cell apparatus in which sheep's nasal mucosa was used as a diffusion membrane and SNES pH 5.5 was used as a diffusion medium. Permeation data of GH mucoadhesive nasal *in situ* gels is given in table 5 and figure 3. From the drug release profiles, it was observed that initially there was fast release but after a specified period the release rate was slowed down. The fast release may due to incomplete gel formation and further release rate was retarded due to complete gel formation. Formulation F11 showed 92.44 % drug release in 240 min with flux 0.026 mg/cm$^2$/min.

***FTIR Study***

IR spectra of pure drug GH showed characteristic absorbance peak at 3233.07, 2943.8, 2448.19, 1645.95 and 1542.77 cm$^{-1}$, indicating N-H stretching, C-H stretching, protonated tertiary amine, C=O stretching and bending of N–H. The IR spectra of thermoreversible gel containing MO showed presence of all the characteristics peaks of GH. Peak of GH corresponding to bending of N–H (1542.77cm$^{-1}$) was disappeared in thermo-revesible gel containing CMTG and SA. Peak of GH corresponds to C-H stretching was disappeared in CMTG containing thermo-reversible gel (figure 4).

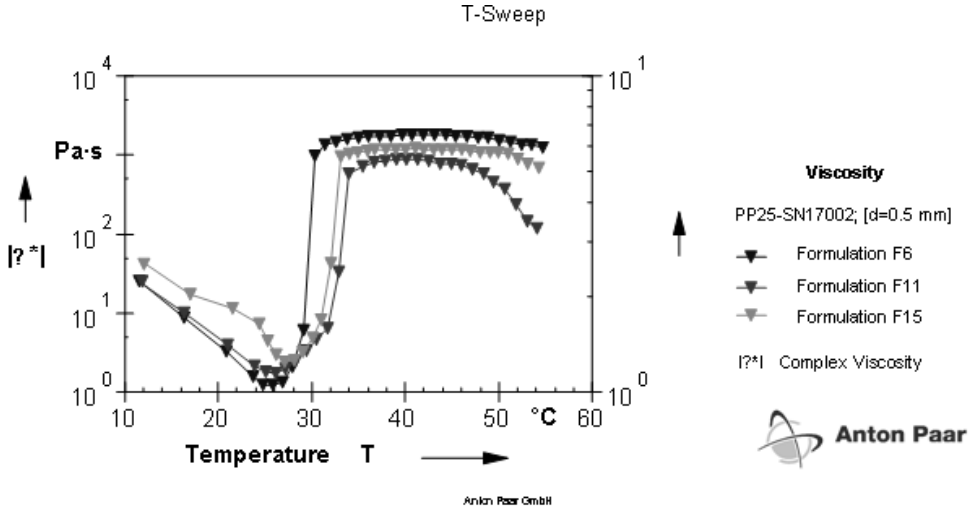



**Fig. 2:** Plot of viscosity vs temperature.

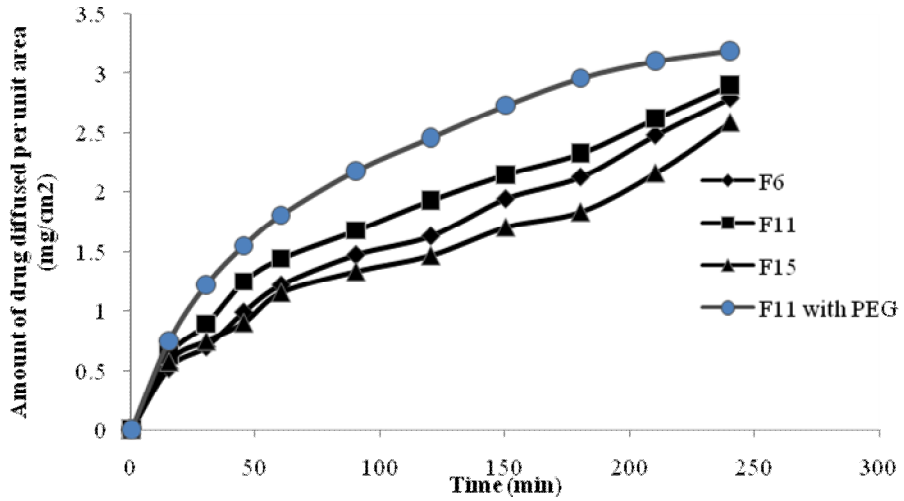



**Fig. 3:** Drug diffusion profiles of optimized formulations.

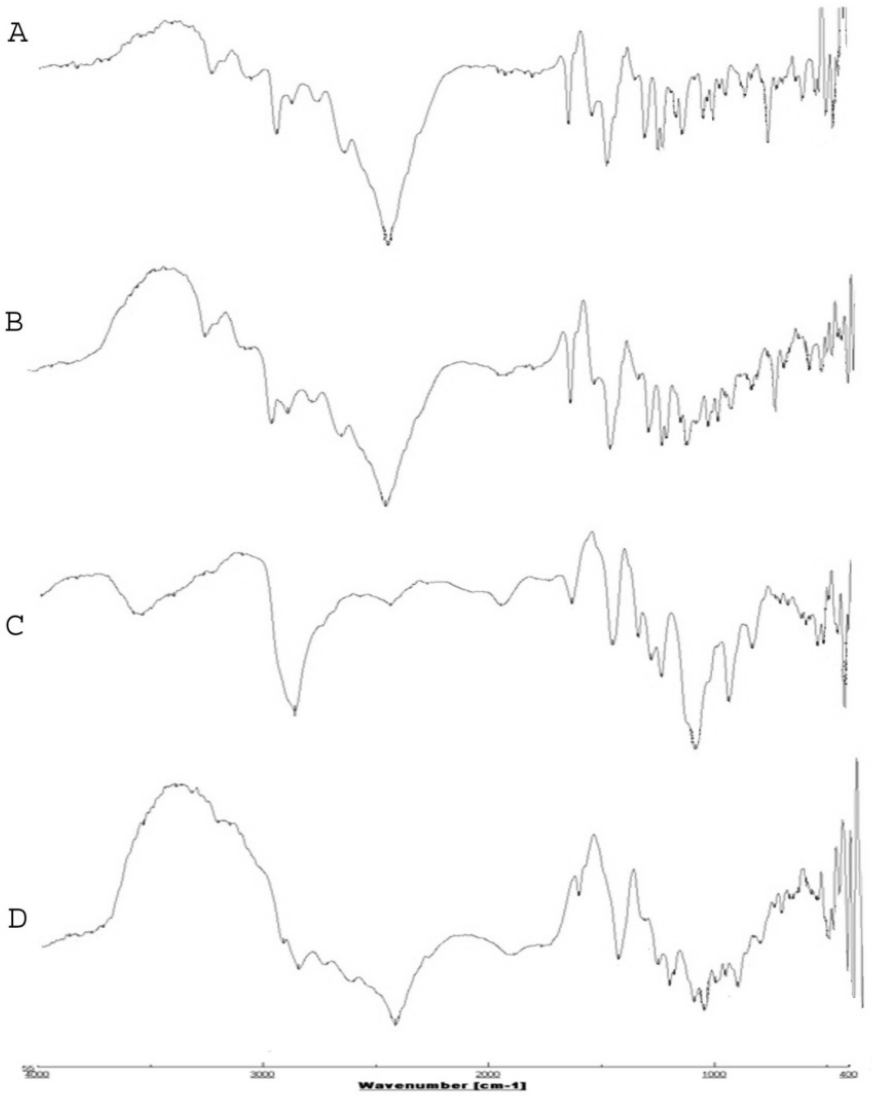



**Fig. 4:** FTIR spectrum of drug and formulations. Pure GH (A), Formulation containing MG (B), Formulation containing CMTG (C), Formulation containing sodium alginate (D).

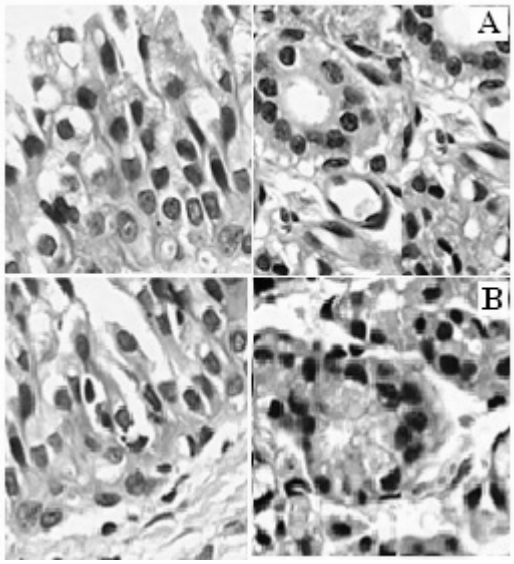



**Fig. 5:** Structure of nasal mucosa before (A) and after (B) exposure to formulation F 11.

***Histopathological evaluation of mucosa***

The compiled result of optimized formulation related to histopathological evaluation of mucosa is shown in figure 5. Histopathological study revealed that prepared formulation was safe for nasal administration as it does not cause necrosis to glandular cells as well as damage to epithelial cells and considered to be safe for nasal administration.

***Effect of PEG 6000 on optimized formulation***

Addition of 0.7 % of PEG 6000 in formulation F11 increased gelation temperature significantly ($p<0.05$) to 31.57± 0.11 with flux 0.034 mg/cm$^2$/min. Plot of amount of drug permeated per unit area verses time is given in figure 3.

## DISCUSSION

In present investigation nasal mucoadhesive thermoreversible gels of GH was prepared with PF 127. Prepared gels were liquid at room temperature and gelled at nasal mucosal temperature (32-34 °C).

The decrease in the gelation temperature with increase in PF127 concentration may be due to the higher number and volume occupied by micelles at low temperature. As the concentration of PF127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern and gelling occurs rapidly at low temperature. Incorporation of GH into *in situ* nasal gels increases the gelation temperature significantly ($p< 0.05$). This may be due to water soluble nature of GH which may cause modification of the process of micellar association of PF127 gels thereby increasing their gelation temperature. The increase in gelation temperature observed upon addition of PEG 6000 in F11 could be attributed to its interference with the process of micellar association of PF 127 chain (Dias *et al.*, 2010).

When concentration of mucoadhesive polymers in formulations were increased gelation temperature lowered significantly ($p<0.05$). This may be due to their ability to bind to PEO chains present in the PF127 molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding (Abd Elhady *et al.*, 2003).

Mucoadhesive strength increases significantly ($p<0.05$) with increasing concentration of mucoadhesive polymer. The mechanism of mucoadhesion may be related to hydrogen bonding between gel formulation and mucosal membrane. Mucoadhesive strength depends on mechanism of mucoadhesion and strength of bonding of polymer with membrane which varies polymer to polymer, therefore difference in mucoadhesive strength of different polymers was observed (Dumortier *et al.*, 2006).

Lysozyme is formed in the nasal secretions, which is responsible for destroying certain microbes at acidic pH. Under alkaline pH, lysozyme is inactive and nasal tissue is susceptible to microbial infection. It is therefore advisable to keep the pH of formulation in the range of 4.5 to 6.5. The gel strength values between 45-48 sec are considered sufficient, as gel strength of less than 25 sec may not preserve its integrity and may erode rapidly while gels with strength greater than 50 sec are too stiff and may cause discomfort.

Comparatively all three formulations F6, F11 and F15 showed similar drug diffusion profile. F11, F11 with PEG 6000 and F15 showed Fickian release kinetics, revealed by (n) values between 0.45-0.57, which may indicate that the drug was released by diffusion mechanism (Table 5). On the other hand F6 showed non Fickian release kinetics (n value between 0.57-0.84) indicating drug followed coupled erosion diffusion mechanism.

The release profiles exhibited an inflection point, which indicated gel formation on the diffusion membrane in donor compartment of diffusion cell. During gel formation, formulation was converted into the gel phase and due to which drug release was decreased. All formulations (F6, F11, F15) showed almost similar release profile because the concentration of PF 127 was constant in all three formulations ($p>0.05$).

CMTG containing formulation (F 11) showed higher diffusion than other formulations. The mucoadhesive polymers contributed in the retardation of drug diffusion. The retardation of drug diffusion with the mucoadhesive polymers may be due to increase in overall gel viscosity. Also, the molecular interactions between drug and mucoadhesive polymers appeared to be involved in the release retarding effect of the mucoadhesive polymers. GH being a weakly basic drug exhibits pKa value of 9.4, and shows low pH in ionic form. Hence, slow release of drug from gels can be also attributed to the formation of ion-pair of "drug and anionic polymer". Further, SNES used to simulate in vivo conditions, contained polyelectrolytes which may also affect drug release from gels. This may be associated to the diffusion of $Cl^-$ and $Na^+$ from the receptor compartment to the gel (donor compartment). On one hand $Cl^-$ may affect the diffusion of drug by acting as a counter ion and on the other hand, the ionic exchange between $Na^+$ and drug may also affect drug diffusion from the polyanion. Addition of PEG 6000 showed a significant release enhancing effect ($p<0.05$) due its higher water solubility and viscosity lowering effect (Dias *et al.*, 2010). FTIR studies revealed that there were no physicochemical interaction between GH and other excipients. The disappearance of peak due to N-H bending may be due to formation of hydrogen bonds between '-CO-NH-' group of GH and '-COOH' group of alginic acid and CMTG. Also, disappearance of peak due to C-H stretching in case of thermoreversible gel containing CMTG may be due to masking of the spectral features of the respective groups by the prominent broad peak at 2872 cm$^{-1}$.

## CONCLUSION

Thermoreversible nasal *insitu* gel of granisetron HCl was formulated by using PF 127 as a thermo gelling polymer and mucoadhesion to increase nasal residence was achieved by using optimized concentration of MG, CMTG and SA. Preformulation studies showed that drug increases gelation temperature while mucoadhesive polymers were found to decrease the gelation

temperature of PF127. Mucoadhesive strength was found to be increased with increase in concentration of mucoadhesive polymers. PF127 gel formulation with CMTG was found to be optimum formulation in terms of gelation temperature, mucoadhesive strength, in vitro drug diffusion and permeation. Further safety of formulation was confirmed by histopathological study. Developed CMTG based formulation could be used effectively to increase residence time of formulation by mucoadhesion in nasal tract thereby enhancing the bioavailability of formulation and alternative to other polymers for nasal delivery of drug.

## CONFLICT OF INTERESTS

The authors declare that they do not have any financial or personal relationships with other people or any other organizations that could inappropriately influence this research work.

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