Research Article

Insitu Mucoadhesive Nasal Gels of Metoclopramide Hydrochloride : Preformulation and Formulation Studies

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ABSTRACT

The prolonged residence of drug formulation in the nasal cavity is of utmost importance for intranasal drug delivery. The objective of the present investigation was to develop a mucoadhesive in situ gel with reduced nasal mucocilliary clearance in order to improve the bioavailability of the antiemetic drug, metoclopramide hydrochloride. The in situ gelation upon contact with nasal mucosa was conferred via the use of the thermogelling Pluronic flake 127 whereas mucoadhesion and drug release enhancement were modulated via the use of sodium alginate and polyethylene glycol polymers respectively. The results revealed that the mucoadhesive polymer increased the gel viscosity but reduced its sol gel transition temperatures and the drug release. The inclusion of polyethylene glycol polymer counteracted the effect of mucoadhesive polymer where by it decreased the gel consistency and increased the sol gel transition as well as in vitro drug diffusion. The in vitro tests performed for mucoadhesive strength and drug diffusion showed that nasal in situ gelling formulations prepared are having good mucoadhesive strength with nearly100% drug diffusion within 4 hours.

So this study points to the potential of mucoadhesive in situ nasal gel in terms of ease of administration, accuracy of dosing, prolonged nasal residence and improved nasal bioavailability.

Keywords: Nasal, Pluronic F 127, Mucoadhesive, PEG 6000.

INTRODUCTION

Metoclopramide hydrochloride (MTCH) is a potent antiemetic, effective in the treatment of nausea and vomiting associated with cancer therapy, pregnancy, and migraine etc [1]. The interest was renewed in this drug with its in vitro and in vivo radio- and chemosensetizing properties. However the oral bioavailability of MTCH is highly variable showing values between 32 and 98% due to extensive presystemic metabolism[1]. Oral forms of MTCH often get vomited before systemic absorption compelling parenteral or rectal administration where both methods result in low patient compliance. In these conditions, the intranasal delivery seems to be an attractive alternative.

However, low residence time of drug in nasal cavity is limitation of this route, which may affect absorption and in turn bioavailability of drug. Hence the design of nasal dosage forms has to consider the anatomic and physiologic characteristics of nasal mucosa and more particularly the rapid mucocilliary clearance (MCC) that limits the time available for drug absorption from the applied dosage form [2-3]. So the possible strategy to decrease rapid MCC is by the use of gel/ mucoadhesive formulations to prolong the residence time at the nasal absorption site and thereby facilitate the uptake of the drug. Ordinary gels are difficult to administer and an accurate drug dose cannot be measured while mucoadhesive powders are not highly favored products. They can cause irritation on the nasal mucosa and give a gritty feel to the tissues[4].

A nasal mucoadhesive in situ gel appears very

attractive since it is fluid like prior to nasal administration and can thus easily be administered as a drop allowing accurate drug dosing. Pluronic flake127(PF127) has excellent thermosensitive gelling properties[5], low toxicity and irritation [6], excellent water solubility, good drug release characteristics and compatibility with other chemicals [7]. It is an ABA triblock copolymer consisting of the hydrophilic polyethyleneoxide (PEO) and the hydrophobic polypropyleneoxide (PPO). 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 [6]. The objective of the present study was to develop a MTCH mucoadhesive in situ nasal gel with a modulated phase transition temperature which would enhance nasal residence time and absorption of drug across nasal mucosal membrane.

MATERIALS AND METHODS

Materials:

Metoclopramide hydrochloride was kindly gifted by Medi-Orals Pvt. Ltd., Satara India. PF127 was purchased from Hi Media Lab Pvt. Ltd., Pune, India. Sodium alginate (Na alginate), and polyethylene glycol 6000 (PEG 6000) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

Methods:

A. Preformulation Studies:

Preparation and optimization of thermoreversible PF127 gels:

The plain and drug loaded PF127 gels were prepared by cold method described by Schmolka et al [8]. For drug loaded PF127 gels, 10% of drug was stirred with sufficient quantity of double distilled water while for plain PF127 gels, only sufficient quantity of double distilled water without drug was kept overnight at 4^0C 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.

Optimization of plain and drug loaded PF127 gels were done by varying concentration of PF127 and evaluating them for gelation temperature.

Optimized concentration of PF127 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 0.1 to 0.5%. Also, optimized concentration of PF127 was used to study the effect of PEG 6000 on gelation temperature in the concentration range of 0.3-1%.

B. Formulation Studies:

Preparation of mucoadhesive thermoreversible nasal gels:

MTCH along with mucoadhesive polymer, and /or PEG 6000 and methyl paraben was dissolved in double distilled water by agitation at room temperature. After cooling the solution to 4^0C, PF127 was added slowly with agitation. The resulting dispersion was then kept overnight at 4^0C until clear and viscous transparent solution was formed. Finally volume was adjusted by using cold distilled water.

Evaluation of final formulations was done with respect to clarity, pH, content uniformity, gelation temperature, mucoadhesive force, diffusion through nasal mucosa and viscosity of formulation.

C. Evaluation of formulations:

1. Clarity:

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid: +, clear ++, very clear (glassy): +++.

2. pH of Formulation:

1ml quantity of each formulation was transferred to the 10ml volumetric flask and diluted by using distilled water to make 10ml. pH of resulting solution was determined by using pH meter. (Systronics ì pH System 362)

3. Content Uniformity:

1ml of formulation was taken in 100ml volumetric flask, added 50ml of distilled water with gentle shaking and final volume was adjusted to 100ml. 1ml quantity from this solution was transferred into the 100ml volumetric flask and final volume was made to 100ml by using distilled water to get 10mg/ml. Finally the absorbance of prepared solution was measured at 309nm by using UV visible spectrophotometer. (Shimadzu UV 1700, Japan)

4. Gelation Temperature:

The gelation temperature of aqueous solution of PF127 was measured by using procedures reported by Choi et al [9]. In brief, 10ml volume of solution was transferred to 20ml transparent vial containing a mag-

netic stirrer bar (1x5/16 inch octagonal). The vial was heated at an increasing rate of 1^0C/min with constant stirring at 100 rpm. The temperature at which rotation of bar stopped was taken as the gelation temperature.

5. Determination of Mucoadhesive Force:

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from sheep nasal mucosal tissue by using a modified chemical balance. A section of nasal mucosa was cut from the sheep's nasal cavity and instantly fixed with mucosal side out onto each glass vial using a rubber band. The vials with nasal mucosa were stored at 37°C for 5 minutes. Then next vial with a section of mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Fixed amount of sample of each formulation were placed onto the nasal mucosa of first vial. Then the height of second vial was adjusted so that mucosal surfaces of both vials come in intimate contact. Two minutes contact time was given to ensure intimate contact between tissues and the sample. Then weight was kept rising in the pan until vials get detached. 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 [10].

Detachment stress (dyne/cm^2) = m x g /A,

Where, m =Weight required for detachment of two vials in grams, g = Acceleration due to gravity [980cm/s^2], A = Area of tissue exposed

The nasal mucosa was changed for each measurement. Measurements were repeated six times for each of the gel preparations.

6. Spreadability

As evident from the theory of mucoadhesion [11], a mucoadhesive formulation that is having high spreadability and high surface tension will adhere strongly to the mucus membrane.

The spreadability in terms of flow ability of various mucoadhesive thermoreversible gels was determined. For assessing spreadability a rectangular, hollow, glass chamber (10x6x4cm) was used. Provision was made to the chamber for inlet and outlet of hot water.

The sheep nasal mucosa from serosal side was pasted on chamber. Then hot water was circulated for 15-20 minutes for acquiring temperature of mucosa to 34±2^{0}C. One drop of formulation was placed on mucosa at an angle of 120^0 and the distance traveled by drop before it gets converted into gel was determined. Measurements were repeated six times for each of the gel preparation.

7. In-vitro Permeation Studies:

Fresh nasal mucosa was carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. The mucosa was stored in normal saline with few drops of gentamycin sulphate injection to avoid bacterial growth. After the removal of blood and bony cartilage from the mucosal membrane it becomes ready for use.

Modified nasal diffusion cell[12]was used to study in vitro drug diffusion profile. 100ml of simulated nasal electrolyte solution [13](SNES) pH 5.5 at 34^{0}C was added to the acceptor chamber. The temperature within the chamber was maintained at 34^{0}C by circulating hot water. After a preincubation time of 20 minutes, formulation equivalent to 10mg of MTCH was placed in donor chamber. At predetermined time intervals, 1ml of sample was withdrawn from the acceptor compartment and replaced the sample volume with SNES pH 5.5 after each sampling, for a period of 4 hours. The samples withdrawn were diluted to 10ml by SNES, filtered and used for analysis. The amount of permeated drug was determined using UV-visible spectrophotometer ($?_{max}$= 309nm). In vitro drug permeation was carried out in triplicate.

8. Analysis of drug release data:

To understand the release mechanisms of various nasal in situ gelling formulations of MTCH, we attempted to describe the rate of release using the semi-empirical model of Korsmeyer et al. and more precisely the equation proposed by Peppas [14]:

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

$$\log (M_t / M_{inf}) = \log(k) + n \log(t)$$

Where, $(M_t / M_{inf})$is the fraction of released drug at time (t), (k) a characteristic constant of the dosage form and (n) the release exponent, indicative of the drug release mechanism. The kinetic parameters (n and k) were calculated from the plot of log (M_t / M_{inf}) 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.

9. Analysis of permeation coefficient:

The permeability coefficient was calculated for each set of formulation. Permeability coefficient was calculated at steady-state condition by using the following formula [15]:

Permeability Coefficient: J_{ss} / C_v

Where,

J_{ss} = slope of linear portion of graph of amount of drug permeated per unit area Vs time

C_v = Initial donor concentration

10. Viscosity measurement:

The viscosities of various formulations were measured with increase in temperature by using Cone and Plate viscometer (Brookfield viscometer Model Cap 2000 +2).

11. 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

A. Preformulation studies

Optimization of concentration of PF127:

Gelation temperatures for plain PF127 gels were observed for the concentration range of 16-20% w/v of PF127 and it was found that the gelation temperature of plane PF127 gels decreased with increasing concentration of PF127. When 10% of MTCH was added into PF127 gels, it was found that gelation temperature of formulations increased significantly ($P<0.05$). From Table II it was found that only 18% of PF127 gel with drug showed ability to form gel in the range of 25 to 32^0C. So, 18% w/v concentration of PF127 was used for further studies.

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

The optimization of Na alginate was done by considering optimum gelation temperature, mucoadhesive force and spreadability. As concentrations of mucoadhesive polymer increased, there was significant ($P<0.05$) decrease in gelation temperature and spreadability while increase in mucoadhesive strength of formulations. From Table III it was found that 0.2% of Na-alginate, with 18% w/v of PF127 showed optimum results in terms of mucoadhesion and spreadability. Hence that concentration of mucoadhesive polymer was selected for final formulations.

Effect of PEG on Gelation Temperature of PF127

Effects of PEG 6000 in the concentration range of 0.3 to 1% are shown in Table IV. As the concentration of PEG 6000 increased gelation temperature was found to be increased significantly ($P<0.05$). In the final formulations concentration of PEG 6000 was varied so as to maintain, gelation temperature in the range of 29 to 32^0C.

B. Formulation studies

Clarity, pH and Content Uniformity:

All the prepared sets of formulations were found to be clear. pH of all the formulations was found to be in the range of 4.5-6.5. The percentage drug content of all prepared nasal formulations were checked and found to be in the range of 93-104%. The percentage drug content of formulations from same batch was found to be uniform (See Table V).

Gelation Temperature:

Various excipients like mucoadhesive polymer as well as PEG 6000 had shown to have effect on the gelation temperature. Mucoadhesive polymer reduced the gelation temperature while PEG 6000 increased the $T_{sol\text{-}gel}$ of the corresponding mucoadhesive nasal insitu gelling formulations in a concentration dependent manner as described earlier (See Table V).

Determination of Mucoadhesive force

Preformulation studies have shown that mucoadhesive strength of formulations increases with increase in concentration of mucoadhesive polymer. From Table V it was found that as the concentration of PEG 6000 was increased there was significant ($P<0.05$) decrease in mucoadhesive strength.

In vitro drug diffusion studies

Diffusion of drug from various formulations was studied by using sheep nasal mucosa. 100% of drug diffusion was obtained at 240 minutes for formulation F9 (see table VI).

Drug permeation studies

Amount of drug permeated per unit area for various nasal insitu gelling formulations are shown in Figure I. Figure revealed that, formulations containing PEG 6000 permeate more quantity of drug per unit area and per unit time than the formulations without PEG 6000.

Viscosity studies

The plots of viscosity versus temperature for various nasal insitu gelling formulations are shown in figure II. There was no considerable change in viscosity upto the point of gelation temperature. Sharp rise in

viscosity was observed at the point of $T_{sol-gel}$ transition. From viscosity studies it was found that all formulations were in liquid state at room temperature and were converted into gel at nasal physiological temperature.

DISCUSSION

The physiological range of the nasal mucosal temperature lies between 32-34^{0}C [16]. So the thermoreversible nasal gels were prepared with the phase transition temperature in the range of 29-32 ^{0}C. From viscosity studies all formulations are in a liquid state at room temperature for ease of administration and accurate measurement of dose and would be converted into gel with increased residence time at the lower limit of the nasal physiological temperature range. The loading of MTCH in the different formulations was kept at 10%w/v such that 100ìl gel (the optimum volume for nasal administration) would contain 10mg, which is the adult dose [17].

The decrease in the gelation temperature with increase in PF127 concentration may be attributed 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. Incorporation of MTCH into insitu nasal gels increases the gelation temperature. This may be due to water soluble nature of metoclopramide hydrochloride which may cause modification of the process of micellar association of PF127 gels thereby increasing their $T_{sol-gel}$ [18].

$T_{sol-gel}$ lowering effect of mucoadhesive polymer could be explained by its 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[19]. The increase in $T_{sol-gel}$ observed upon addition of PEG 6000 could be attributed to its interference with the process of micellar association of PF127 chains[20]. The concentration of PEG 6000 was adjusted in such a manner that formulations may form gel at nasal physiological temperature. So its further addition was stopped when $T_{sol-gel}$ of the in situ nasal gels approaches to 32 ^{0}C.

Mucoadhesive strength increases with increasing concentration of mucoadhesive polymer. The mechanism of mucoadhesion may be related to hydrogen bonding between gel formulation and mucosal membrane via carboxyl groups of Na-alginate [21]. The addition of PEG 6000 to the formulations decreases the mucoadhesive strength in concentration dependent manner; this might be related to the decrease in viscosity caused by PEG 6000.

Assessment of spreadability in terms of distance traveled by in situ nasal gels reveals that spreadability may be related to the viscosity of in situ nasal gels. Increase in concentration of mucoadhesive polymer decreases the distance traveled by in situ nasal gels, since mucoadhesive polymer increase the viscosity of in situ nasal gels.

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 4.5-6.5.

The retardation of MTCH release with the mucoadhesive polymer could be explained by their ability to increase the overall gel product viscosity [22]. Also the molecular interactions between MTCH and mucoadhesive polymer appeared to be involved in the release retarding effect of the mucoadhesive polymer [23]. Also the release enhancing effect of PEG 6000 might be due to its higher water solubility and its viscosity lowering effect [22].

Permeability coefficient increases with increasing concentration of the PEG 6000, which proves its release enhancing effect. The Fickian (anomalous) release kinetics, revealed by (n) values between 0.45-0.57, might indicate that the release of drug followed by diffusion mechanism [14](See Table VI).

In the present study, effects of drug, mucoadhesive polymer and PEG 6000 on gelation temperature of PF127 were studied. Preformulation studies showed that drug and PEG 6000 increases the gelation temperature while mucoadhesive polymer was found to decrease the gelation temperature of PF127. Mucoadhesive strength increases with increase in concentration of mucoadhesive polymer. From the results of formulation studies it can be concluded that, PEG 6000 increased the drug diffusion and permeability while mucoadhesive strength gets decreased.

Table I Composition of various thermoreversible mucoadhesive nasal gel formulations

Composition	F1	F2	F3	F4	F5
Metoclopramide HCl	10	10	10	10	10
PF127	18	18	18	18	18
Na-Alginate	…	0.2	0.2	0.2	0.2
PEG 6000	…	…	0.4	0.5	0.6
Methyl Paraben	0.33	0.33	0.33	0.33	0.33
Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.

*All concentrations in percentage; batch size 20ml

Table II Gelation temperature of PF127 solutions

Composition	Gelation Temperature(^{0}C) Mean ± S.D. n=6	P value*
PF127 16%	42.25 ±0.30	0.30
PF127 16% + 10 % MTCH	44.00 ±0.62	
PF127 18%	25.58 ±0.23	0.0001
PF127 18% + 10 % MTCH	29.06 ±0.42	
PF127 20%	24.23 ±0.25	0.0068
PF127 20% + 10 % MTCH	24.70 ±0.48	

PF127- pluronic flake 127; MTCH–metoclopramide hydrochloride

Values are expressed as mean ± SD; n=6
$p < 0.05$ considered statistically significant

Table III Gelation temperature, mucoadhesive strength and spreadability of formulations containing sodium alginate

Composition	Gelation Temperature (^{0}C) Mean ± S.D.	Mucoadhesive Strength (dyne/cm^2) Mean ± S.D.	Distance Traveled (mm) Mean ± S.D.
PF127 18 % (control)	25.58 ±0.23	486±20	37.5±1.33
PF127 18%+0.1% Na alginate	24.92 ±0.15*	571 ±30*	23.33 ±1.53*
PF127 18%+0.2% Na alginate	24.53 ±0.16*	659 ±20*	17.67 ±1.15*
PF127 18%+0.3% Na alginate	23.93 ±0.12*	701 ±46*	13.33 ±1.15*
PF127 18%+0.4% Na alginate	23.52 ±0.12*	732 ±32*	10.33 ±1.53*
PF127 18%+0.5% Na alginate	23.1 ±0.11*	756±25*	8.4±1.25*

Values are expressed as mean ± S.D.; N=6; PF127- Pluronic Flake 127; Na alginate- sodium alginate
* $p < 0.05$ considered statistically significant

Table IV Effect of PEG on gelation temperature of PF127

Composition	Gelation Temperature ^{0}C Mean ± S.D.
PF127 18 % (control)	25.58 ± 0.23
PF127 18%+0.3% PEG 6000	26.52 ± 0.57*
PF127 18%+0.4% PEG 6000	27.05 ± 0.10*
PF127 18%+0.5% PEG 6000	27.4 ± 0.06*
PF127 18%+0.6% PEG 6000	27.62 ± 0.01*
PF127 18%+0.7% PEG 6000	27.93 ± 0.08*
PF127 18%+0.8% PEG 6000	28.26 ± 0.1*
PF127 18%+0.9% PEG 6000	28.48 ± 0.11*
PF127 18%+1.0% PEG 6000	28.7 ± 0.15*

Values are expressed as mean ± S.D.; N = 6. PEG 6000- polyethylene glycol 6000;
* $p < 0.05$ considered statistically Significant

Table V Clarity, pH, content uniformity, gelation temperature and mucoadhesive strength of mucoadhesive nasal insitu gelling formulations

Formulation	Clarity	pH	Content Uniformity n=3	Gelation Temperature(^{0}C) Mean ± S.D. n=6	Mucoadhesive Strength (dyne/cm^2) Mean ± S.D. n=6
F1	+ + +	5.7	104.31	29.06± 0.42	446±8.87
F2	+ +	5.2	102.47	28.96 ± 0.15	659±11.13
F3	+ +	5.1	94.16	30.9 ± 0.17	649.6±12.66*
F4	+ +	5	99.79	31.46 ± 0.21	633±12.49*
F5	+ +	4.9	98.31	31.86 ± 0.15	618.3±9.45*

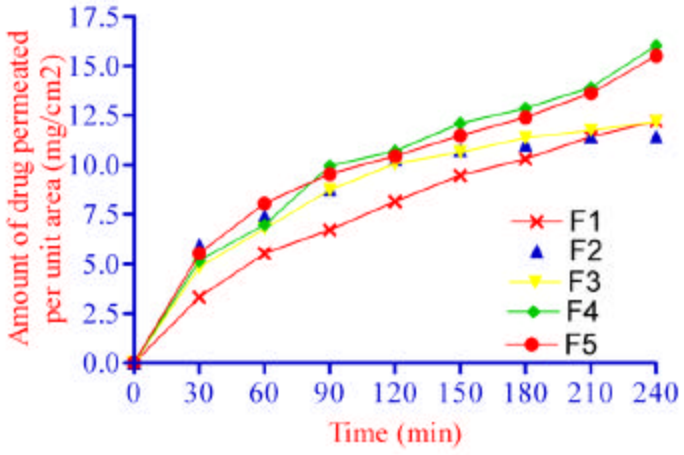
Values are expressed as mean ± S.D.
For mucoadhesive strength: F2 control. * $p < 0.05$ considered statistically significant

Table VI Release data of Metoclopramide HCl mucoadhesive nasal in situ gels

Formulation	% Drug diffused at 240 minutes (Mean± SD)	'n' value	'k' Value	Permeability Coefficient Mean ± SD (mg.cm/min)	Release Mechanism
F1(control)	77.6± 2.45	0.6275	2.5786	0.006562±0.00087	Non Fickian
F2	68.44± 8.93	0.3349	12.3178	0.0078005±0.00029	Fickian
F3	77.55± 6.53	0.4527	7.2959	0.0080107±0.00050	Fickian
F4	101.83± 1.86	0.5333	5.3368	0.0087238±0.00069*	Fickian
F5	98.67± 0.8	0.4618	7.5641	0.0083042±0.00062*	Fickian

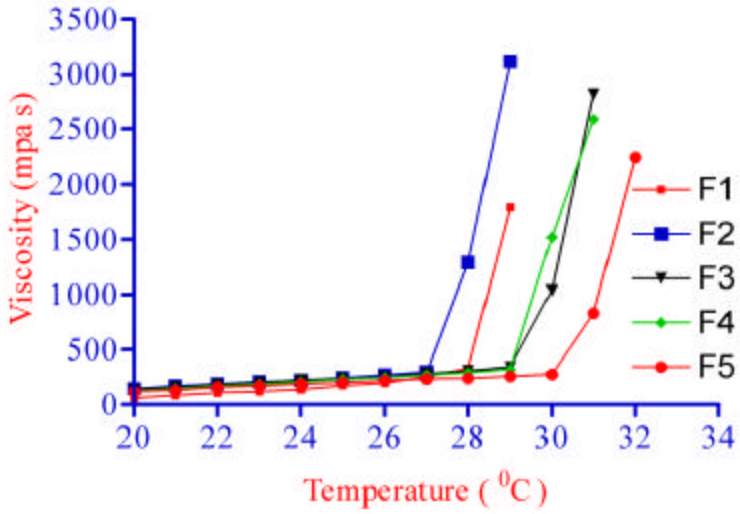
Values are mean ± S.D.; N = 3 $p < 0.05$ considered statistically significant

Figure I Amount of drug permeated per unit area against time for the in situ gelling formulations containing 0.2% Na alginate:



F1 contains PF127 with 10% MTCH without mucoadhesive polymer

Figure II Viscosity against temperature plot for the formulations containing 0.2% Na alginate:



F1 contains PF127 with 10% MTCH without mucoadhesive polymer

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