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Formulation and evaluation of *in-situ* mucoadhesive nasal gel of montelukast sodium

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ABSTRACT

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 antiasthamatic drug, Montelukast Sodium. The in-situ gelation upon contact with nasal mucosa was conferred via the use of the thermo gelling Pluronic flake 127 whereas mucoadhesion and drug release enhancement were modulated via the use of Hydroxy Propyl Methyl Cellulose, Methyl Cellulose and polyethylene glycol 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 whereby it decreased the gel consistency and increased the sol-gel transition as well as in-vitro drug release. The in-vitro drug release performed through dialysis membrane and ex vivo studies performed by using sheep nasal mucosa. F10 and F2 formulations showed 88.33%±0.08 and 83.75%±0.12 of drug release at 12h respectively. 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.

Key words: Montelukast Sodium, Mucoadhesive, Pluronics P127, Sheep Nasal Mucosa.

INTRODUCTION

Montelukast Sodium is a leukotriene antagonist, effective in the treatment of asthma and allergic rhinitis [1-4]. The oral bioavailability of drug is variable showing values between 64 and 68% due to extensive presystemic metabolism [5]. 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. 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 [6]. Ordinary gels are difficult to administer and an accurate drug dose cannot be measured while mucoadhesive powders are not highly favoured products. They can cause irritation on the nasal mucosa and give a gritty feel to the tissues. 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 [7]. Pluronic flakes127(PF127) has excellent thermo sensitive gelling properties, low toxicity and irritation, excellent water solubility, good drug release characteristics and compatibility with other chemicals. It is an ABA triblock copolymer consisting of the hydrophilic polyethylene oxide (PEO) and the hydrophobic polypropylene oxide (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 [8]. The objective of the present study was to

develop a Montelukast Sodium 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:
Montelukast Sodium was obtained as a gift sample from Dr. Reddy's laboratory, Hyderabad, India. Pluronics F-127 from Matrix labs, Hyderabad, India. Hydroxy propyl methyl cellulose (HPMC), Methyl cellulose (MC), Methyl and Propyl Parabens was obtained from Hi- Media, Mumbai, India. Polyethylene glycol 400(PEG400) was obtained from Merck Ltd., Mumbai, India. All the chemicals and reagents purchased and used were of analytical grade.

Preparation of *in-situ* Mucoadhesive nasal gel:
Montelukast sodium *in-situ* gels were prepared by cold method. Small quantity of water dissolves various concentration ranges of pluronics separately such as 18, 20, 22, and 24% at cold conditions. The quantity of HPMC and MC was dissolved in that, according to the formulation chart. Later drug, PEG 400 and parabens were incorporated and stirred until clear solution was obtained. Finally make up the volume up to 5 ml with distilled water and kept it over night at (4-10°C) freezing conditions [9-10]. The compositions of gel were shown in table 1.

Drug and excipients compatibility studies:
Drug-excipients compatibility studies were carried out using FT-IR (Perkin Elmer). The spectra of samples were obtained for pure drug (Montelukast Sodium), polymers, pluronics, and one best formulation [11, 12].

EVALUATION STUDIES OF *IN-SITU* NASAL GELS:
Clarity Test:
The prepared formulations are visually examined for any foreign particles in it, if found any foreign particles is discarded [13].

pH Determination:
Each formulation 1 ml of sample was transferred to the 10 ml volumetric flask and diluted with distilled water. The pH of resulting solutions is determined using digital pH meter [14-15].

Gelation and gel meting point temperature:
Gelation temperature is the temperature at which the liquid tends to form as gel. Gel melting temperature is the temperature at which gel converts to liquid or temperature at which gel tends to flow.

2 ml of prepared formulation was transferred into a test tube and placed in a water bath at 4°C and sealed with aluminium foil. The temperature of water bath was increased for 1°C and left to equilibrate for a minute; at each increment in temperature till gel formation is examined. Gelation temperature (T1°C) is confirmed when the meniscus of preparation in test tube would no longer move on tilting the tube at 90°C angle. After gelation, again the temperature is increased by 1°C till the stiff mass starts flowing. This temperature is noted as gel melting temperature (T2°C) [16].

Drug content estimation:
Each formulation 1 ml of sample was taken in a 100 ml volumetric flask and diluted with phosphate buffer (PBS) pH 6.2 and shaken to dissolve the drug [17-19]. The solution was filtered through 0.45µ pore size (whatmann) filter paper. The content of the drug was estimated using UV-Spectrophotometer (Shimadzu UV – 2201, Japan) at 357nm.

***In-vitro* drug release studies through dialysis membrane:**
The *In-vitro* release studies of the formulated *in situ* gel were carried out using Franz diffusion cell. The pre treated dialysis membrane was mounted in between reservoir compartment and donor compartment with help of clam and the dose equivalent amount of gel was placed in donor compartment. The reservoir compartment was filled with 20 ml phosphate buffer pH 6.2 and the set was arranged on magnetic stirrer for uniform mixing of diffusion medium. The study was carried out at 37 ± 1°C. 5 ml of samples were withdrawn from reservoir compartment at predetermined time interval and absorbance was measured spectrophotometrically at 357 nm. Each time the reservoir compartment was replenished with the same quantity of fresh phosphate buffer pH 6.2[20-21].

***Ex-vivo* drug permeation studies through sheep nasal mucosa:**
The *Ex-vivo* drug permeation studies were carried out for best formulations form above studies using Franz diffusion cell. The excised sheep nasal mucosa was mounted in between reservoir compartment and donor compartment with help of clam and the amount of gel was placed in donor compartment. The reservoir

compartment was filled with 20 ml phosphate buffer pH 6.2 and the set was arranged on magnetic stirrer for uniform mixing of diffusion medium. The study was carried out at 37 ± 1°C. 5 ml of samples were withdrawn from reservoir compartment at predetermined time interval and absorbance was measured spectrophotometrically at 357 nm. Each time the reservoir compartment was replenished with the same quantity of fresh phosphate buffer pH 6.2 [22-26].

Drug Release Kinetic Studies:
Drug release kinetic studies were calculated to predict the order and mechanism of drug release. Release data were fitted to various mathematical models like zero order, first order, Higuchi, Hixson Crowell, and Korsmeyer Peppas [27].

Based on 'n' value the mechanism of drug release is characterized.

Stability Studies:
Stability studies were conducted to test the physical and chemical stability of the developed nasal gel [15]. A sufficient quantity F2 and F10 gel, in screw capped vials, is stored at different temperature conditions as 4±3°C, 25±3°C, 40±3°C for 1 month. Later the physical stability including appearance, drug content and drug release were studied.

RESULTS AND DISCUSSION

The prepared in situ nasal gel found to thick, viscous and clear.

Drug and Excipients compatibility studies:
The obtained FTIR spectrums were shown in Figure 1-4. The pure form of Montelukast Sodium has shown characteristic an absorption peak at 3420.21 cm^{-1} due to OH-stretching (Aromatic), 2573.49 cm^{-1} due to CN-stretching (Aliphatic), 1663.86 cm^{-1} due to C=C stretching and 3420.10 cm^{-1} due to OH stretching. The absorption peaks found in pure drug spectra were compared with peaks in optimised formulation Figure 4, this depicts that there is no incompatibility between the drug and excipients used in formulation.

pH estimation:
pH of human nasal mucosa is found to be in the range of 5-6.5. But it can tolerate about 4-7.5. Prepared nasal formulations should be within the range the nasal mucosa can tolerate in order to reduce nasal irritation. pH of all formulations was tabulated in Table 2. The results indicated that in all formulations pH is found to be tolerable. They are in the range of 5.8 to 6.9.

Table 1. Formulation of Montelukast Sodium nasal gel

Formulation	Montelukast Sodium (mg)	Pluronics P127 (%)	HPMC (%)	MC (%)	Water (ml)
F1	8	18	-	-	q.s
F2	8	20	-	-	q.s
F3	8	22	-	-	q.s
F4	8	24	-	-	q.s
F5	8	18	0.5	-	q.s
F6	8	20	1	-	q.s
F7	8	22	1.5	-	q.s
F8	8	24	2	-	q.s
F9	8	18	-	1	q.s
F10	8	20	-	2	q.s
F11	8	22	-	3	q.s
F12	8	24	-	4	q.s
F13	8	18	1	2	q.s
F14	8	20	2	1	q.s
F15	8	22	2	4	q.s

PEG 400 0.5 ml; Parabens 0.05 ml added to all formulations

Gelation and Gel Melting Temperature:
All the formulations tested for this study. Individually studied effect of excipients on gelation (T1) and gel m.p temperature (T2). The resulted values were shown in Table 3. Formulations F1 to F4 found to be as the concentration of pluronics increased, the T1 decreased. This may be due to higher number and volume occupied by the micelles at low temperature. Whereas the T2 found to be an increased. Therefore gel range broadens with concentration of polymer. For F5 to F8 formulation observed that the HPMC was used as gelling or viscosity enhancing agent. On its concentration an increased to T1 decreased and T2 an increased. For F9 to F12 formulations

MC used as gelling or viscosity enhancing agent it also exhibits same effect as that of HPMC on T1 and T2 but to a lower extent as compared to HPMC. For F13 to F15 Combination of both polymers with pluronics shows synergistic effect on lowering the T1 and enhancing the T2 values.

Table 2. Drug Content Estimation and pH values

Formulation	% Assay	pH value	*In-vitro* % of drug released
F1	93.57±0.30	6.4	66.69±3.38
F2	96.70±0.30	6.8	86.59±0.15
F3	97.56±0.30	5.9	86.33±1.92
F4	92.88±0.30	6.3	69.90±0.12
F5	97.91±0.52	6.1	67.77±0.67
F6	97.39±0.52	6.2	79.03±0.04
F7	90.27±0.30	5.8	76.94±0.33
F8	93.92±0.30	6.3	65.55±0.08
F9	89.58±0.52	6.2	66.60±0.23
F10	98.43±0.52	6.4	92.09±0.57
F11	94.09±0.30	6.4	88.40±0.29
F12	96.35±0.52	6.2	63.30±0.13
F13	89.58±0.52	6.9	70.89±0.12
F14	91.84±0.60	6.2	71.01±0.96
F15	90.27±0.30	6.1	61.70±0.16

Table 3. Gelation and Gel Melting point Temperature

Formulation	T1$^{\circ C}$Gelation temp Mean ± SD; n=3	T2$^{\circ C}$ Gel M.P temp Mean ± SD; n=3
F1	31.00±0.50	67.96±0.45
F2	28.66±0.28	71.80±0.32
F3	28.16±1.25	75.83±0.76
F4	22.73±0.64	78.90±0.60
F5	28.60±0.65	68.80±0.72
F6	25.66±0.76	74.46±0.50
F7	22.03±0.55	76.66±0.28
F8	17.66±0.57	78.83±0.28
F9	30.38±0.53	69.46±0.55
F10	25.83±0.28	72.76±0.25
F11	23.90±0.36	75.60±0.52
F12	19.33±0.57	77.33±0.57
F13	22.16±0.76	71.16±0.28
F14	20.16±0.28	76.43±0.51
F15	16.50±0.50	81.66±0.28

Table 4. Release kinetics for F10 formulation

Drug release kinetics	R^2
Zero order	0.996
First order	0.928
Higuchi	0.954
Korsmeyer Peppas	0.987

Table 5. Stability Studies of F2 and F10

Formulation	Drug Content		*In-Vitro* Drug Release	
	Before	After	Before	After
F2	96.70±0.30	95.81±0.12	86.59±0.15	85.67±0.05
F10	98.43±0.52	97.92±0.26	92.09±0.57	91.55±0.13

Drug content estimation:

The drug content estimation was performed to ensure the accurate distribution of drug. The results were tabulated in Table 2. The results indicated that in all the formulations the drug content was uniform. The percentage of drug content was varied in between 89.58±0.52 to 98.43±0.52.

***In-vitro* drug release studies through dialysis membrane:**

For all prepared gels drug release studies were carried and results were showed in Table 2 and Figure 5 to 7. Among them formulations, F2, F3, F6, F10 and F11 showed better percentage of drug release at 12h in the varied from 86.59±0.15, 86.33±1.92, 79.03±0.04, 92.09±0.57, 88.40±0.29 respectively. The formulations containing pluronics in

the concentration of 20 and 22% showed better drug release. The order of release of drug from formulations found to be F10> F11> F2> F3> F6> F7> F14> F13> F4> F5> F1> F9> F8> F12> F15.

Figure1. FTIR spectrum of pure drug Montelukast sodium

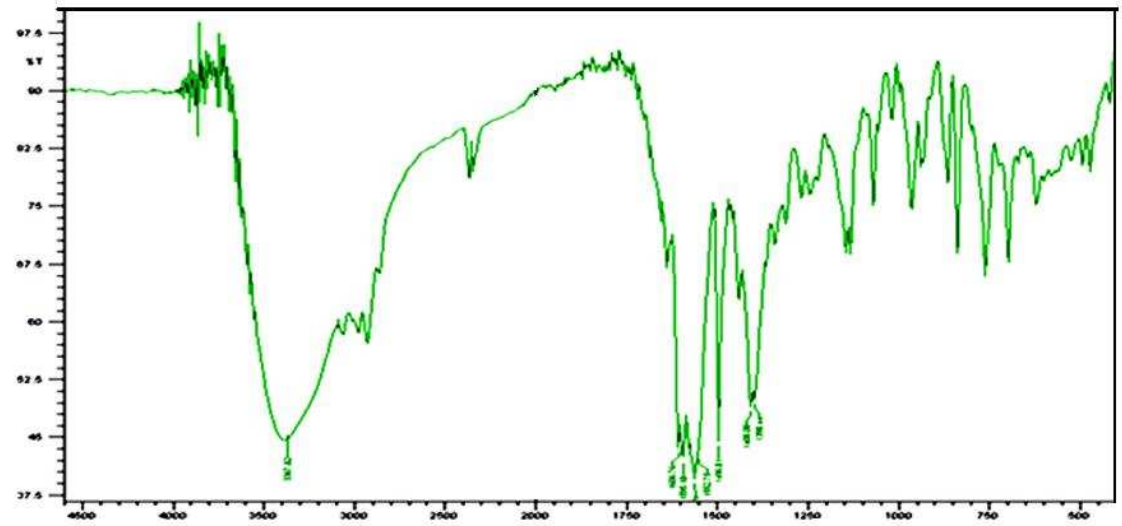
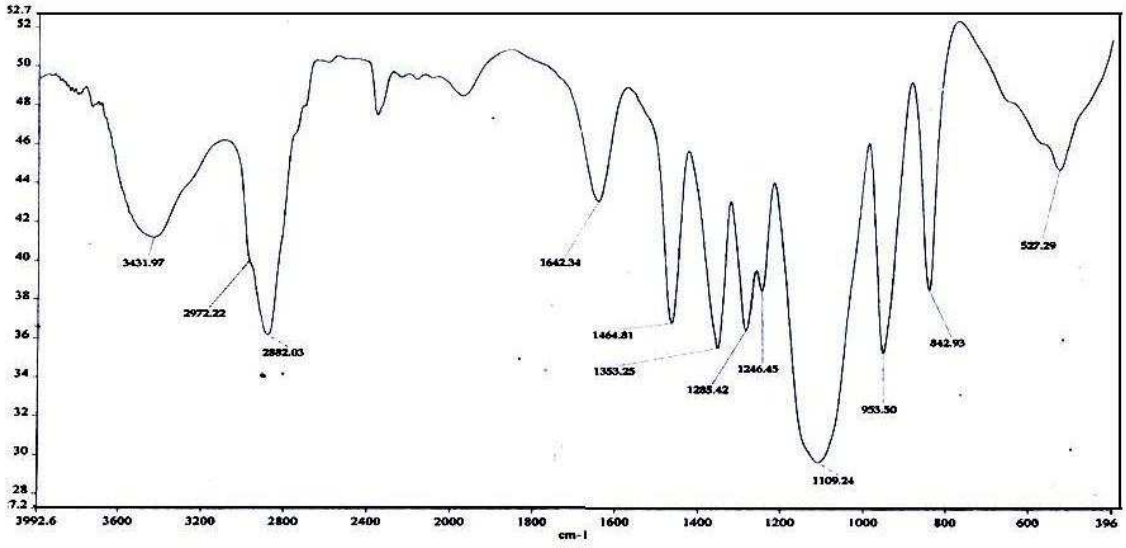


Figure 2. FTIR spectrum of pluronics P127



***Ex-vivo* drug permeation studies through sheep nasal mucosa:**

The *Ex- vivo* studies were conducted for F2, F3, F10 and F11using sheep nasal mucosa because of these are shown good in vitro drug release profile. The Figure 8 shows that *ex vivo* drug permeation through nasal mucosa of sheep. Among them, F10 and F2 showed sustained release of Montelukast Sodium for 12 h, 88.33±0.08 and 83.75±0.12 respectively these studies show that with 20 % of PF127 was good for 12h studies.

Kinetics of Optimised Formulation F10:

Kinetics studies were performed for optimized formulation F10 (Table 4). These studies are done in order to estimate the type and order of its release. Based on r^2 values from the above table, it explains that the drug release is concentration independent i.e., it follows zero order mechanism. According to Korsmeyer peppas equation; release exponent 'n' value is found to be 0.76. This depicts that the formulation follows non-fickian type of diffusion.

Stability studies:

Formulations F2 and F10 was found to be good stability (Table 5) because of before and after storage the estimated drug content and in vitro drug release profile was no change significantly.

Figure 3. FTIR spectra of HPMC polymer

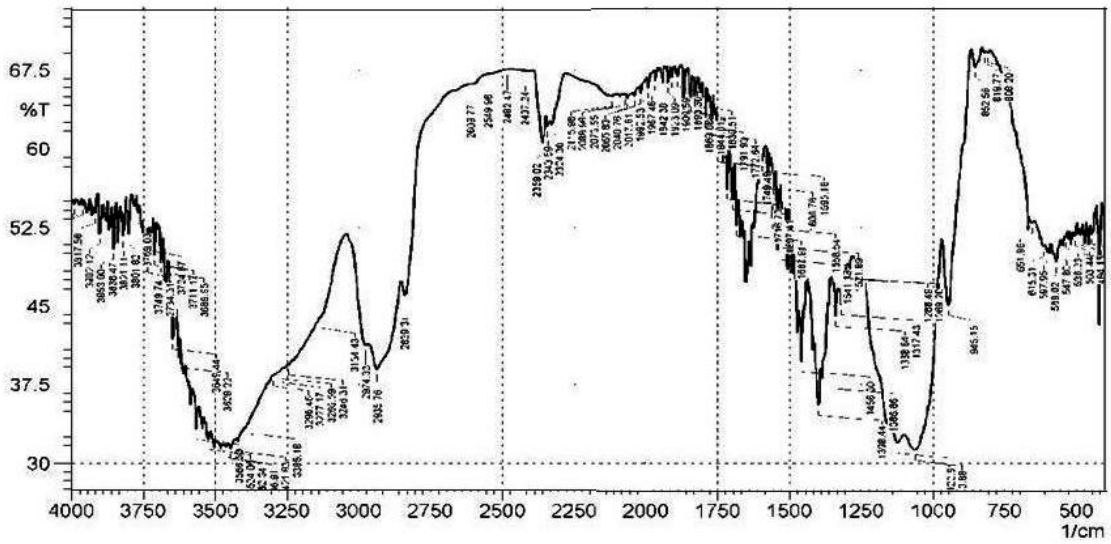


Figure 4. FTIR spectrum of formulation F10

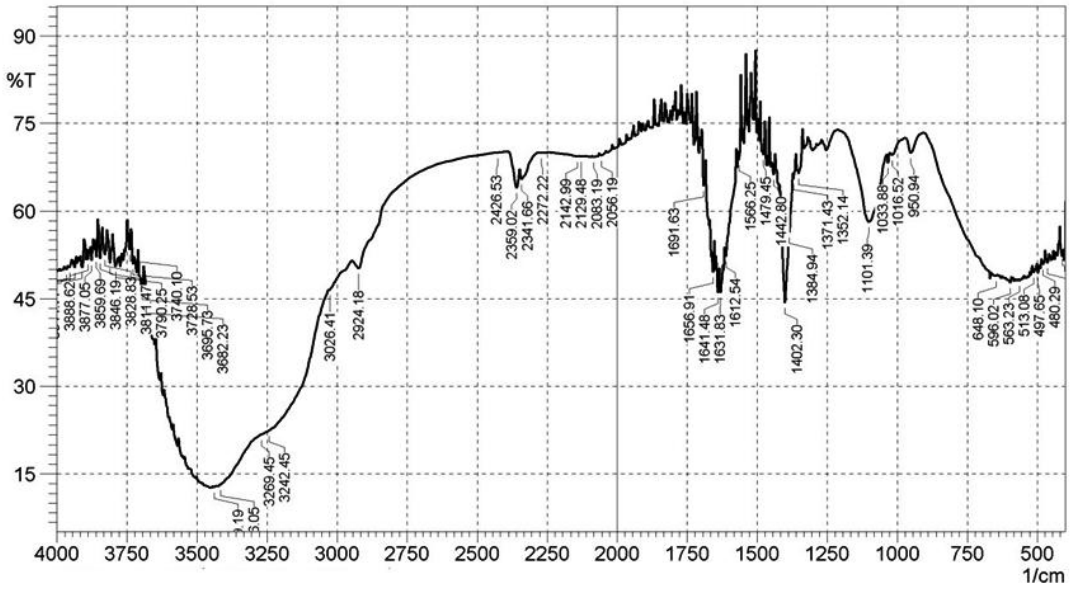


Figure 5. *In-vitro* drug release studies through dialysis membrane for F1 to F5

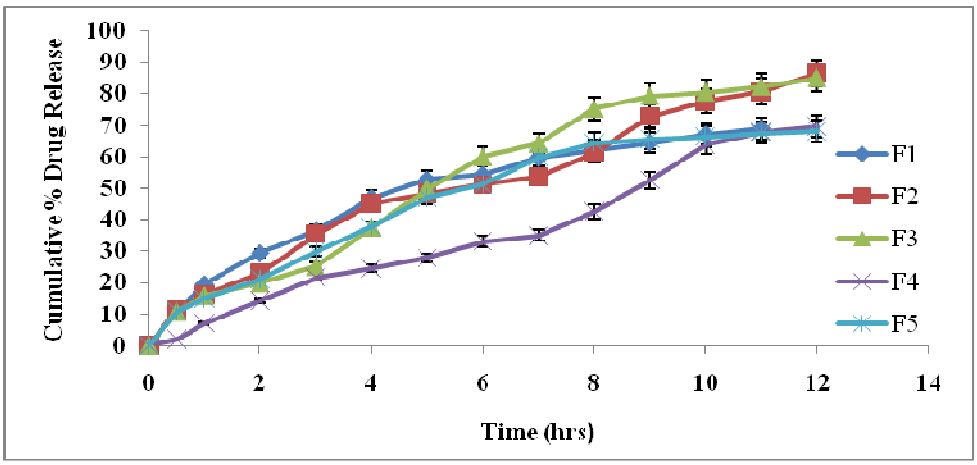


Figure 6. *In-vitro* drug release studies through dialysis membrane for F6 to F10

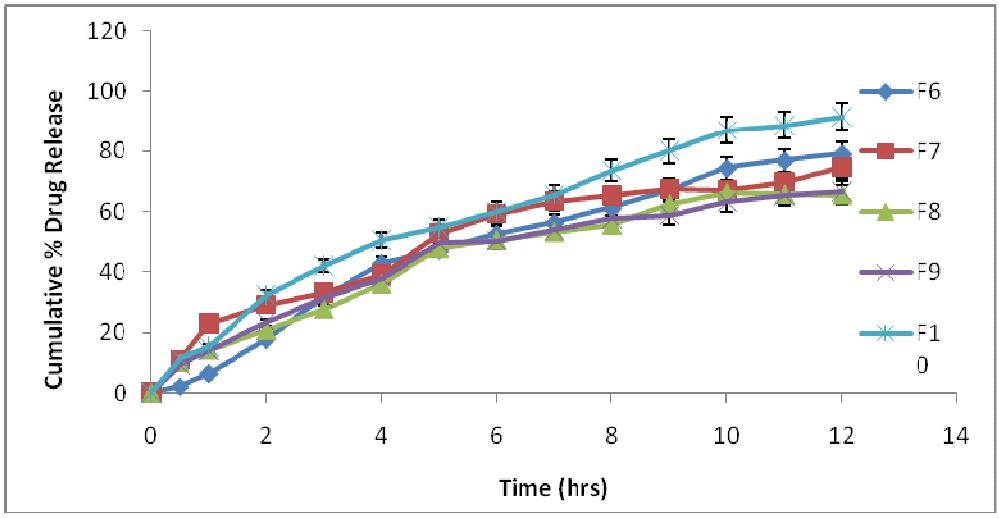


Figure 7. *In-vitro* drug release studies through dialysis membrane for F11 to F15

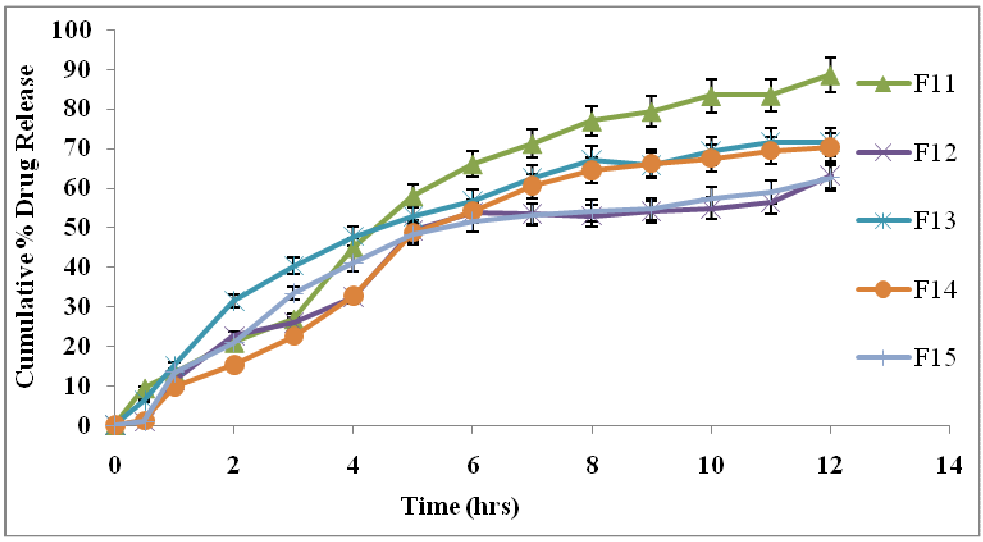
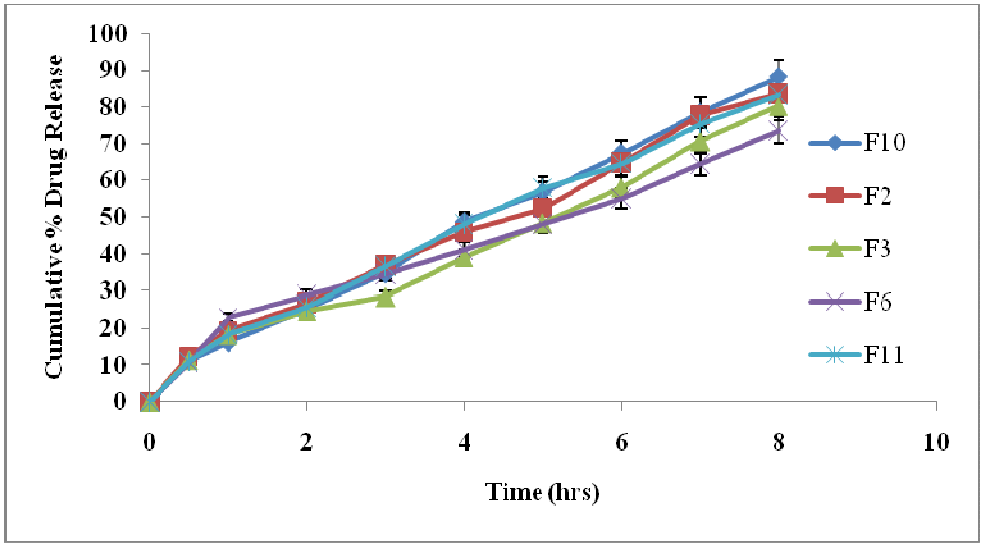


Figure 8. *Ex-vivo* drug permeation studies through sheep nasal mucosa forF2, F3, F6, F10 and F11



CONCLUSION

The *in-situ* nasal gels of Montelukast sodium were prepared using a thermo reversible of pluronics and mucoadhesive polymers HPMC, MC by varying their concentrations. Among all developed formulations, Montelukast Sodium *in-situ* gels formulated using 20% of pluronics shown good *in-vitro* and *in-vivo* drug release. Further, this type of delivery is pleasant and painless alternative to other delivery systems.

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