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Research Article

FORMULATION AND EVALUATION OF THERMOSENSITIVE IN SITU GEL OF SALBUTAMOL SULPHATE FOR NASAL DRUG DELIVERY SYSTEM

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ABSTRACT

This study aimed to formulate and evaluate Nasal drug delivery system containing Salbutamol Sulphate was prepared for improving the bioavailability & sustaining the drug release. Salbutamol sulphate is a selective β_2 adrenoreceptor agonist and rapidly absorbed from gastro intestinal tract but it is subjected to first pass metabolism. Thus oral bioavailability is only 50%. The main objective of present work is to enhance the bioavailability; reducing the dose. Thermoreversible, bioadhesive polymers such as poloxamer and Hydroxy Propyl Methyl Cellulose (HPMC) in the form of in situ gel by cold technique. The results revealed that as the increase of bioadhesive polymer HPMC concentration, decrease in gelation temperature (T1) and increase in gel melting temperature (T2). pH of all formulation were found to be within the range between 5.5 to 6. The drug content for all formulation was found to be 96%-100%. The mucoadhesive test indicates that the level of HPMC increases, the mucoadhesive strength also increases. The developed formulations had optimum viscosity. The optimized formulation shows the controlled drug release.

Keywords: In-situ gel, Salbutamol sulphate, Poloxamer 407, HPMC K4M, Nasal delivery.

INTRODUCTION

In situ forming systems are liquid aqueous solution before administration, but gel at physiological conditions as like solvent exchange, UV radiations, ionic cross linkage, pH[1,2] change, temperature modulations[3,4]. pH triggered system (e.g. carbopol [6, 8], cellulose acetate phthalate [9]), ion activated system. (e.g. gelrite [10, 11] sodium alginate [12]). In this system do not require any organic solvents, copolymerization agents or an externally applied triggered for gelation, have gained increasing attention, such as thermo sensitive nasal in situ gel[3].

Traditional drugs are administered by oral and parenteral routes. Oral administration is unsuitable for some drugs like the drugs undergoes significant degradation in gastrointestinal tract or metabolized via liver in high degree and gives undesirably slow effects[13]. For this parenteral route is preferred but it is undesirable or impractical if drug is intended for the treatment of chronic disease, so alternative route is preferred; also transdermal route is used for drug delivery but its use is limited due to low permeability of the skin to many drugs[14]. To overcome these problems nonparenteral routes are used includes nasal, buccal, pulmonary route. These nonparenteral routes have some advantages like self administration is possible in an ambulatory setting. The nasal route offers rapid onset of action, high absorption of small molecular weight hydrophobic drugs, high bioavailability, avoid the first metabolism, patient compliance[15]. Nasal route has been used for treatment of nasal congestion, allergy and infections. Nasal route mat be when rapid onset of action is required and small molecular weight polar drugs, peptide and proteins are not easily administered via other routes than by injection[16].

Salbutamol sulphate is a selective β_2 adrenoreceptor agonist and rapidly absorbed from gastro intestinal tract but it is subjected to first pass metabolism. Thus oral bioavailability is only 50%[17]. The main objective of present work is to enhance the bioavailability, reducing the dose and dosing frequency, gives patient compliance, increase the residence time so it gives the sustained drug release by using thermo sensitive polymers like poloxamer 407 and HPMC K4M. The unique characteristics of these copolymers are reverse thermal gelation; concentrated solution (15-20%) of polymer is fluid at refrigerator temperature (4-34º C) but is soft gel at body temperature. (37ºC)[18]. The reversal thermal gelation exhibited by Pluronic aqueous solution has been used as drug delivery system for ophthalmic[19], parenteral[20], rectal[21] and percutaneous route [22].

MATERIAL AND METHOD

Salbutamol sulphate was obtained as a gift sample from Cipla ltd. Mumbai. HPMC K4M, Benzalkonium chloride, sodium Meta bisulphate (All are LR grade) was purchased from S.D. fine chemicals Mumbai, Poloxamer 407 (Lutrol F 127) USF/NF was purchased from BASF Ltd. Mumbai

METHOD

Preparation of Standard curve of Salbutamol Sulphate

Accurately weighed 10 mg of Salbutamol Sulphate was dissolved in 100ml of distilled water to get stock solution of 100µg/ml. From this stock solution aliquots of 0.2, 0.4, 0.6, 0.8, 1 & 1.2 ml were withdrawn and further diluted to 10 ml with distilled water to obtain a concentrations range of 2 to 12 µl/ml. The absorbance of the solutions was measured at 276 nm by using UV-Vis spectrophotometer. A graph of concentration vs. Absorbance was plotted[23].

Preparation of Nasal gel formulations

Aqueous nasal gel was prepared by using the Cold method described by Schomolka (1972) et al. The weighed quantity of mucoadhesive polymer of HPMC at different ratios (0.1%, 0.2%, 0.3 % 0.4 %, & 0.5%) and drug Salbutamol sulphate (0.25% w/v) was dissolved in 10 ml of distilled water. To the above formulations the weighed quantity of thermosensative polymer, poloxamer 407 (18%w/v), was added slowly with constant stirring and kept at 4ºC over night until to form a clear gel[24].

Table 1: Shows Absorbance values of Salbutamol sulphate in distilled water at 276 nm for preparation of standard curve

S. No.	Conc.(µg/ml)	Absorbance
1	2	0.400
2	4	0.477
3	6	0.546
4	8	0.624
5	10	0.698

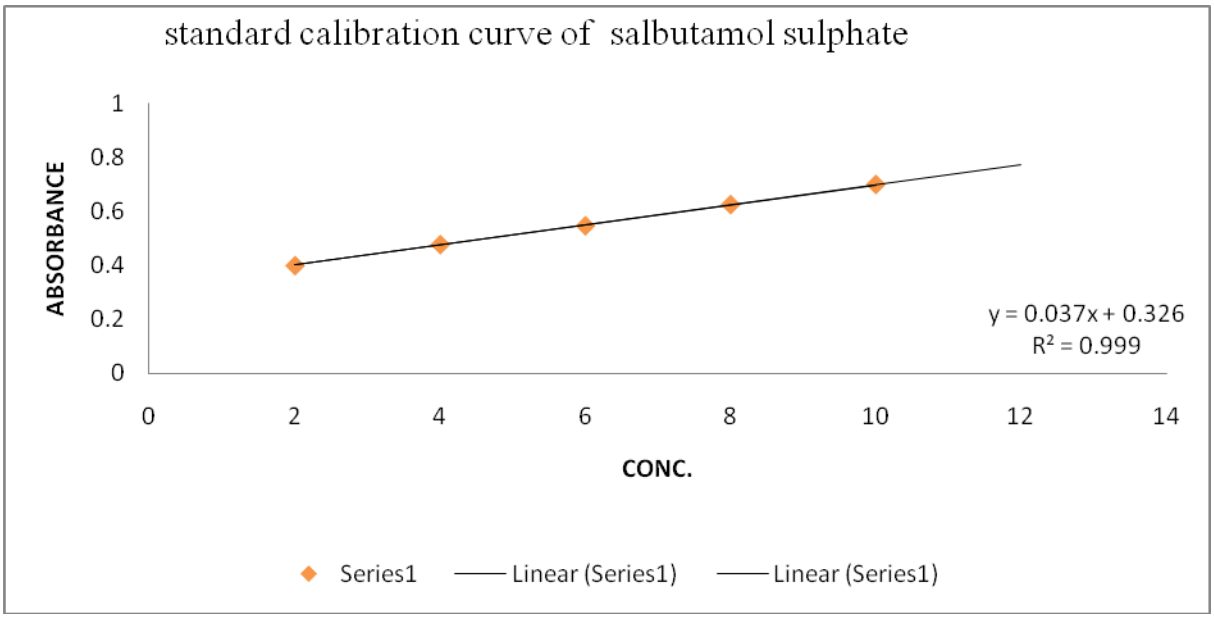

Fig. 1: Shows Calibration curve of Salbutamol Sulphate

Table 2: Shows Composition of Nasal in situ gel formulation of Salbutamol Sulphate

Ingredients (%w/v)	Formulations						
	F1	F2	F3	F4	F5	F6	F7
Salbutamol sulphate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Poloxamer 407	18	18	18	19	19	19	20
HPMC K4M	-	0.1	0.2	0.3	0.4	0.5	0.6
Sodium metabisulphate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Benzalkonium chloride(%v/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Characterization of Nasal in Situ Gel

Evaluation of in situ gel

Appearance

The developed formulations were inspected visually for clarity, colour in sol and gel form against white background and for particulate matter any if present[25].

pH of gel

Each formulated batch pH was measured using pH meter which was previously calibrated using standard buffers of pH 4 & pH 7.[26]

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

It was determined by using modified Miller and Donovan technique. A 2ml aliquot of gel was taken in to the test tubes, placed in water bath at 4^0 C. The temperature of water bath was increased in increment of 1^0 C. The samples were examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90^0. After attaining the temperature when the gel starts flowing upon tilting i.e. gel melting temperature. It is critical temperature when the gel starts flowing upon tilting test tube upon 90º .[27]

Drug content estimation

1ml of formulation was taken in 10ml volumetric flask, diluted using distilled water adjust to 10ml. 1ml quantity from this solution was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 276 nm against blank reagent using UV visible spectrophotometer (Shimadzu UV-1800)[28]. The concentration of the drug present in formulation was computed from the calibration curve using the equation:

$Y = mx + C$

Determination of Mucoadhesive Strength

The mucoadhesive strength of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosal was fixed on each of two glass slides using thread. 50mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with mucosal section were fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2 min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.[29]

Mucoadhesive strength (dynes/cm^2) = mg/A

Where, m = weight required for detachment in grams, g = Acceleration due to gravity (980cm/s^2). A = Area of mucosa exposed.

Spreadability

For the determination of spreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. Weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability.[30, 31]

S=ML/T

Where, M= weight tide to upper slide, L= length moved on the glass slide, T= time taken.

Measurement of gel strength

The gel strength was determined employing technique proposed by Choi. A sample of 50g of nasal gel was put in 100ml graduated cylinder and gelled in thermostatically controlled water bath at 37^0 C. A weight of 35g was placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5cm into gel.[32]

In Vitro diffusion studies

In vitro diffusion of gels was performed using jacked nasal diffusion cell sheep nasal mucosa. The receptor chamber was filled with 50ml distilled water (37^0 C± 2^0 C) and 0.2 ml test formulation was placed on the dorsal mucosa. Sample (1ml) at predetermined interval were transferred to test tubes and analysed spectrophotometrically at 276nm.[33]

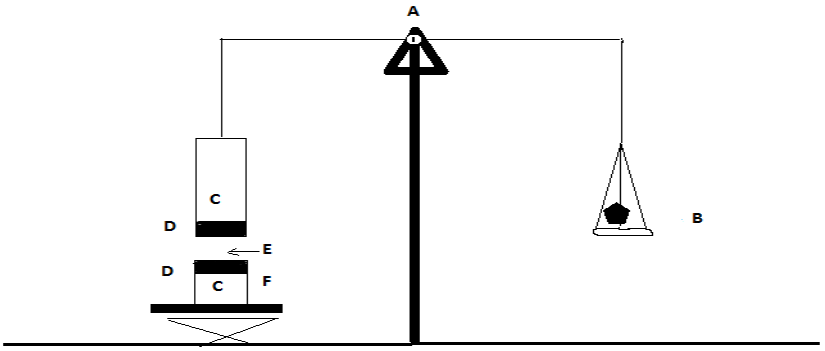

fig.Measurement of mucoadhesive force of in situ gel.
A:Modified balance, B: Weights, C: Glass vials, D: Poloxamer gel, E: Sheep nasal mucosa, F: Height adujestable Pan

Viscosity studies

Formulation with higher viscosity has a better contact time thus increases the absorption. At the same time, high viscosity enhanced the permeability of drugs. This has been observed during nasal delivery of insulin, acyclovir and metoprolol.. Zaki et al. Observed that the residence time enhanced as viscosity increased but drugabsorption diminished[34]. The rheological studies were carried out using the Brookfield LVDV- I model viscometer. The gel formulation under study was placed in the sample holder and the suitable spindle was selected lowered perpendicular into the sample. The spindle was rotated at constant optimum speed. Viscosity was measured at constant temperature 37±1^0 C. The viscosity determinations of formulation were carried out at different temperature from 5^0 C to 40^0 C[35].

In Vitro Release Studies

In vitro release study of the formulated in situ gel was carried out in two chamber diffusion cells through dialysis membrane-70 with molecular weight cut off 1200-1400 KDa. Diffusion of diameter 1.5 cm and 20 ml capacity consisted of upper cylindrical compartment open from above and diffusion membrane at its base. To prepare artificial membrane, pieces of dialysis membrane were soaked in PBS pH 7.4 for hrs before mounting on diffusion cell. Dialysis membrane was in a two chamber cells. In situ gels of poloxamer 407 loaded with drug were placed in the donor compartment. 20 ml of PBS 7.4 was placed in the receptor compartment. The temperature of receiver compartment was maintained at the 37^0 C ±1.0^{0}C during experimental and the content of the receiver compartment was stirred using magnetic stirrer. The position of the donor compartment was adjusted so that dialysis membrane just touches the diffusion medium. An aliquot of 1ml was withdrawn from receiver compartment initially after 15 and 30 min and then 1 hr interval and replaced with same amount of fresh medium. Aliquots withdrawn were suitably diluted and analysed using UV spectrophotometer at 276nmnm for drug. In vitro drug release was carried out for 8hrs [36,37]

In vitro permeation study[38]

Fresh nasal tissues were carefully removed from the nasal cavity of goat obtained from the local slaughterhouse. Tissue sample were inserted in Franz diffusion cell displaying a permeation area of 1.76 cm^2 7ml of 6.4 pH phosphate buffer saline was added to the acceptor chamber and agitated with magnetic stirrer at 34^0 C. After pre incubation time of 20 min, pure drug solution and formulation equivalent to 0.25%w/v of Salbutamol sulphate was placed in the donor chamber. From the acceptor compartment 0.2ml sample aliquots were withdrawn at predetermined time interval up to 6 hrs replacing the sample volume with 6.4 pH PBS after each sampling, filtered and analysed by UV spectrometer at 276nm.

RESULT AND DISCUSSION

Standard calibration curve of Salbutamol Sulphate was carried out in distilled water and absorbance measured at 276nm. Calibration curve is depicted in fig. No. 1.

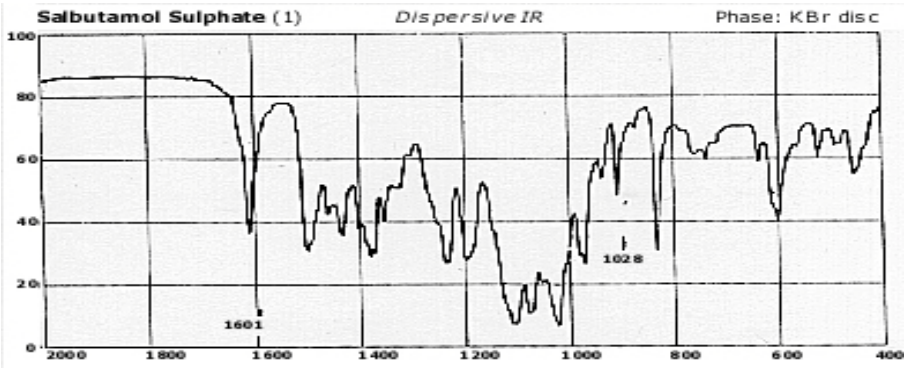

Fig. 2: Shows ir spectra of salbutamol sulphate

Appearance

All the formulations were found to clear. Terminal sterilization with autoclaving had no effect on physical, chemical properties of the formulations.

pH of formulation

The normal physiological pH of the nasal mucosa ranges from 5.5-6.. pH of All formulations were found to have pH value in between range 5-6. i.e. within the range of nasal mucosa. The results are presented in table 3.

Gelation time

The gelation time is defined as the time taken for the transition of liquid phase to a gel. In the present study, for the prepared formulation the gelation time was found to be within 2 min. Poloxamer 407, 19% w/v was used to produce the in situ gels with in 2 min.

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

At gelation temperature, liquid phase makes transition in to gel. Due to the addition of HPMC and Salbutamol sulphate there is change in T1 of gel formulation. Study shows that formulation F1, F2, F3, F4 having gelation temperature of 26, 30, 32, 33 ºC (Low level of HPMC 0.0, 0.1, 0.2, 0.3 w/v %) where as F6 & F7 has a T1 of 28, 26 ºC. which is having high level of HPMC K4M (0.5% & 0.6%). The result presented in table 2. This indicates that the mucoadhesive polymer, HPMC K4M has significant T1 lowering effect. The gelation temperature lowering effect might be caused due to increased viscosity after dissolution of mucoadhesive polymer. The gel melting temperature (T2) was also found to increase with increasing concentration of HPMC K4M.

Table 3: Shows Evaluation parameter of formulation

Formulation code	pH (mean ± S.D)	Drug content (mean ± S.D)	Mucoadhesive strength (Dynes/cm^2)	Gel strength (seconds) (mean ± S.D)
F1	5.4±0.01	96.03±1.26	2330±0.33	70±0.2
F2	5.6±0.03	97.36±1.01	2504±1.21	91±0.6
F3	5.3±0.1	98.89±0.12	3019±0.36	107±0.6
F4	5.1±0.11	99.32±0.60	3507±1.32	110±0.8
F5	5.7±0.14	99.77±0.21	3800±0.21	115±0.2
F6	6.0±0.2	98.21±0.33	4510±1.37	125±1.1
F7	6.0±0.1	98.00±0.91	4612±0.67	130±0.6

Table 4: Shows Evaluation parameter of formulation

Formulation Code	Gelation temperature (T1 ºC) (mean ± S.D)	Gel melting temperature (T2ºC) (mean ± S.D)
F1	26±0.6	47±0.32
F2	30±0.8	51±0.12
F3	32±0.12	50±0.1
F4	33±0.4	52±0.50
F5	36±0.6	52±0.20
F6	28±1.1	56±0.50
F7	26±0.65	54±0.45

Drug content estimation

Table no.2 shows the result of percentage drug for all the formulations. The drug content was found to be in acceptable range for all the formulations. % drug content was in the range 96- 99 % indicating uniform distribution of drug.

Mucoadhesive strength

Mucoadhesive strength was determined by measuring the force required to detach the formulation from mucosal surface i.e., detachment stress. Results reveal variable HPMC is having effect on mucoadhesive strength. It shows that as level of HPMC increases, mucoadhesive strength also increases. The results are presented in table 2. This was due to wetting and swelling of HPMC. Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose

Measurement of gel strength

It is very important that the nasal gel formulation must have suitable gel strength. The gel strength of nasal formulation at 37˚C, increased as the concentration of poloxamer 407 increased. Results are presented in table 3.

Spreadability

Formulation F5 showed good Spreadability as compare to other formulations.

Viscosity

Viscosity measurement of the formulation at various temperatures (25-40ºC), shows that there was increased in viscosity with increase in the conc. Of HPMC K4M viscosity profiles of formulation at 37ºC were represented in table no.4.

Table 5: Shows Viscosity of formulations at various temperature.

Formulation	Viscosity at 4^0 C	Viscosity at 34^0 C
F1	200	2000
F2	211	2810
F3	303	3221
F4	315	3590
F5	811	4890
F6	860	5420
F7	890	5602

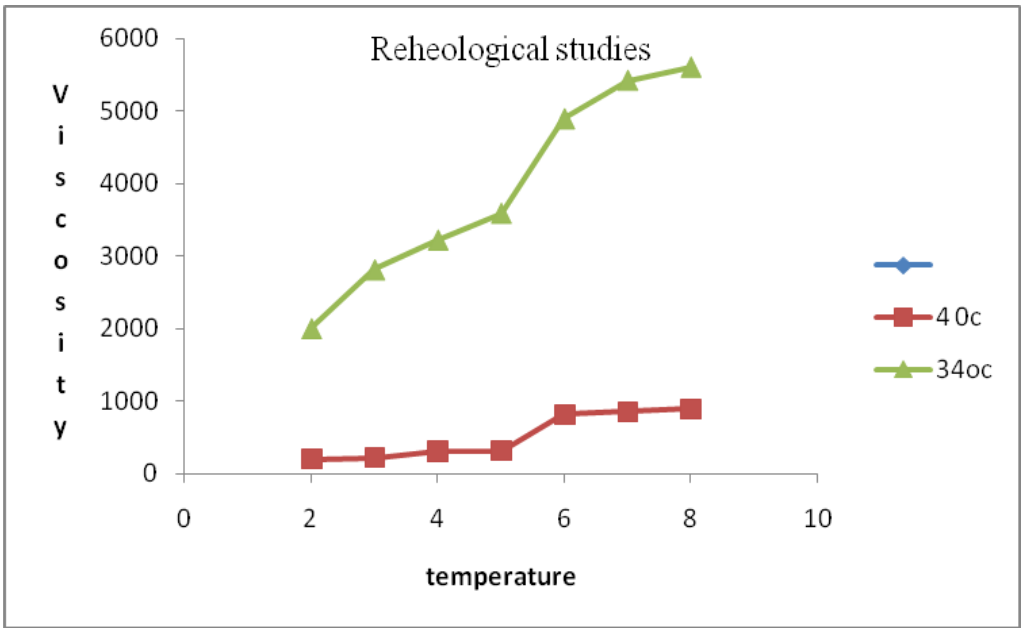

Fig. 3: Shows Rheological studies at various temperatures

In Vitro Release Studies

Diffusion studies were carried out using franz diffusion cell, F5 showed the sustained drug release. F3 showed drug release 79.76% at 8hrs. Concentration of HPMC increases leads to decrease the drug release. Poloxamer concentration affect on drug release.

Table 6: shows In vitro drug release

Time in min	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
30	14.89	15.45	12.85	12.96	13.96	14.96	13.07
60	22.97	25.97	26.08	29.89	21.88	20.86	23.96
120	31.97	34.86	37.97	36.47	32.89	35.97	34.08
180	47.89	44.96	46.97	49.96	49.91	48.93	47.95
240	56.97	52.89	59.96	57.94	51.74	51.97	59.96
300	69.78	63.83	65.85	62.95	60.04	60.95	64.95
360	78.84	72.95	73.94	71.95	72.85	69.96	70.84
420	82.97	81.86	83.94	82.94	83.95	73.95	74.09
480	99.98	97.75	93.97	91.75	90.76	80.75	79.06

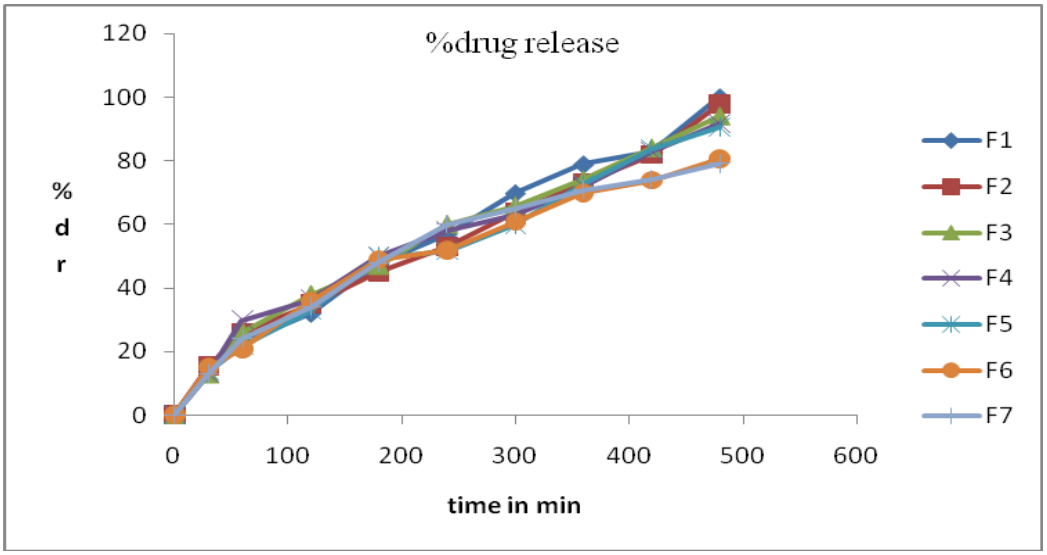

Fig. 4: Shows in vitro drug release

Table 7: Shows permeation drug release of selected batch with compare to drug solution.

Time in min	F5	Drug solution
0	0	0
30	13.96	19.67
60	21.88	25.96
120	32.89	31.85
180	49.91	39.86
240	51.74	42.72
300	60.04	51.95
360	72.85	67.96
420	83.95	86.94
480	90.76	98.64

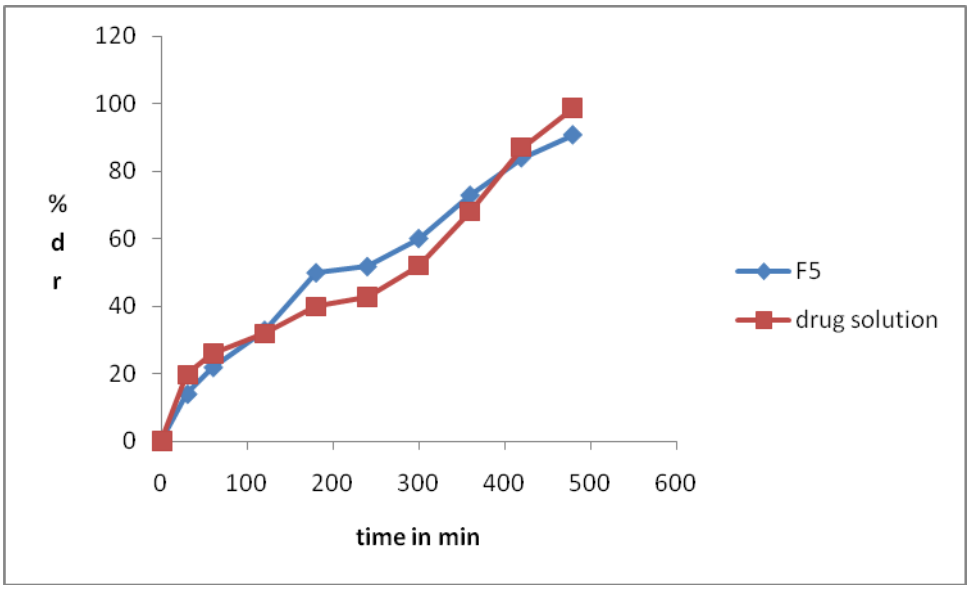

Fig. 5: Shows Ex vivo permeation of selected batch (F5) compare to drug solution.

Ex vivo permeation carried out by using nasal mucosa of goat. Permeation profile shown in figure no 4.

DISCUSSION

19% Poloxamer 407 (Pluronic F 127) gel formulation with 0.4 % HPMC K4M is a promising nasal drug delivery system for a antiasthmatic drug Salbutamol sulphate, which would enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore it also exhibited a good spreadability. In conclusion, this study demonstrated that the use of in situ gelling vehicle of PF 127 incorporating mucoadhesive polymer HPMC K4M could effective and safely improve the nasal residence time and absorption of Salbutamol sulphate.

Finally from this study it was concluded that mucoadhesive, thermosensative nasal in situ gel drug delivery was very beneficial in case of drug like Salbutamol sulphate.

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